Global Health Impacts of Vector-Borne Diseases: Workshop Summary

DETAILS

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GLOBAL HEALTH IMPACTS OF VECTOR-BORNE DISEASES

WORKSHOP SUMMARY

Alison Mack, *Rapporteur*

Forum on Microbial Threats

Board on Global Health

Health and Medicine Division

The National Academies of
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This workshop summary has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published workshop summary as sound as possible and to ensure that the workshop summary meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this workshop summary:

Jacqueline Fletcher, Oklahoma State University
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Dirk Pfeiffer, The Royal Veterinary College
David Rizzo, University of California, Davis

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before its release. The review of this workshop summary was overseen by **Melvin Worth.** He was responsible for making certain that an independent examination of this workshop summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this workshop summary rests entirely with the rapporteur and the institution.

Preface

The Forum on Emerging Infections was created in 1996 in response to a request from the Centers for Disease Control and Prevention and the National Institutes of Health. The purpose of the forum is to provide structured opportunities for leaders from government, academia, and industry to regularly meet and examine issues of shared concern regarding research, prevention, detection, and management of emerging, reemerging, and novel infectious diseases in humans, plants, and animals. In pursuing this task, the forum provides a venue to foster the exchange of information and ideas, identify areas in need of greater attention, clarify policy issues by enhancing knowledge and identifying points of agreement, and inform decision makers about science and policy issues. The forum seeks to illuminate issues rather than resolve them. For this reason, it does not provide advice or recommendations on any specific policy initiative pending before any agency or organization. Its value derives instead from the diversity of its membership and from the contributions that individual members make throughout the activities of the forum. In September 2003, the forum changed its name to the Forum on Microbial Threats.

Acknowledgments

The Forum on Microbial Threats wishes to express its sincere appreciation to the individuals and organizations who contributed their valuable time to provide information and advice to the forum. Their participation in the planning and execution of this workshop made it greater than the sum of its parts. A full list of presenters, and their biographical information, may be found in Appendix E.

The forum gratefully acknowledges the contributions of the members of the planning committee. We would also like to thank the following Academies staff—past and present—and consultants for their invaluable contributions to this activity: Clyde Behney, Chelsea Frakes, Greta Gorman, Faye Hillman, Alison Mack, Khaki McClure, and Bettina Ritter, among others.

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Workshop Overview

GLOBAL HEALTH IMPACTS OF VECTOR-BORNE DISEASES[1]

Pathogens transmitted among humans, animals, or plants by insects and arthropod vectors have been responsible for significant morbidity and mortality throughout recorded history. Such vector-borne diseases—including malaria, dengue, yellow fever, plague, trypanosomiasis, and leishmaniasis—together accounted for more human disease and death in the 17th through early 20th centuries than all other causes combined (Gubler, 1998). By the mid-20th century, implementation of strategies to reduce populations of the mosquitoes that spread malaria, yellow fever, and dengue effectively reduced the impact of these diseases on human health—albeit temporarily.

Over the past three decades, previously controlled vector-borne diseases have resurged or reemerged in new geographic locations, and several newly identified pathogens and vectors have triggered disease outbreaks in plants and animals, including humans. A variety of factors underlie this trend among emerging vector-borne diseases, including

- The rapid expansion of global travel and trade, enabling the geographic spread of pathogens, vectors, and animals that serve as so-called reservoirs[2] of disease;

[1] The planning committee's role was limited to planning the workshop, and the workshop summary has been prepared by the workshop rapporteur as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants, and are not necessarily endorsed or verified by the National Academies of Sciences, Engineering, and Medicine, and they should not be construed as reflecting any group consensus.

[2] Defined in glossary, Appendix D.

- Recent, unprecedented, population growth associated with rampant and unplanned urbanization in the tropics, and the resulting increased juxtaposition of humans, animal reservoirs of pathogens, and vector species in geographically constrained environments;
- Societal, cultural, and behavioral practices that encourage disease transmission; and
- Decreased support for and deterioration of the public health surveillance and control infrastructure for infectious diseases in general, and specifically for vector-borne and zoonotic diseases.

Domestic and international capabilities to detect, identify, and effectively respond to vector-borne diseases are limited. Few vaccines have been developed against vector-borne pathogens. At the same time, drug resistance has increased in vector-borne pathogens while their vectors are increasingly resistant to insecticide controls. Furthermore, the ranks of scientists trained to conduct research in key fields including medical entomology, vector ecology, and tropical medicine have dwindled, threatening prospects for addressing vector-borne diseases now and in the future.

In June 2007, as these circumstances became alarmingly apparent, the Forum on Microbial Threats hosted a workshop to explore the dynamic relationships among host, pathogen(s), vector(s), and ecosystems that characterize vector-borne diseases. Revisiting this topic in September 2014, the forum examined trends and patterns in the incidence and prevalence of vector-borne diseases in an increasingly interconnected and ecologically disturbed world, as well as recent developments to meet these dynamic threats. This public workshop featured invited presentations and discussions that described the emergence and global movement of vector-borne diseases, considered research priorities for understanding their biology and ecology, and assessed global preparedness for and progress toward their prevention, control, and mitigation.

WORKSHOP CONTEXT

Disease Burden

Vector-borne diseases[3] have long been associated with significant human illness and death. Over half the world's human populations are currently at risk from vector-borne infections, which collectively account for 17 percent of the human global infectious disease burden (CDC, 2014d). In April 2014, the World Health Organization (WHO) devoted its annual World Health Day to vector-borne illnesses, issuing a global brief that profiled key diseases and their individual and collective impacts (WHO, 2014a). In her foreword to this report, WHO Director-General Margaret Chan noted that vector-borne illnesses caused more than one million deaths each year, but that "death counts, though alarming, vastly underestimate the human misery and hardship caused by these diseases, as many people who survive infection are left permanently debilitated, disfigured, maimed, or blind." These burdens are borne most heavily by the world's poorest people, communities, and countries.

Explosive epidemics have marked the recent resurgence of several previously controlled vector-borne diseases, including plague, dengue, and yellow fever. Less sensational—but equally destructive—infectious, vector-borne disease outbreaks in plants, domestic animals, and wildlife have disrupted ecosystems and reduced agricultural productivity. In addition to these acute impacts, persistent vector-borne diseases impose a significant burden on plant, animal, and human health and are an impediment to socioeconomic development (IOM, 2008). This is exacerbated by the chronic or long-term effects of diseases including West Nile viral fever, dengue, chikungunya, and Chagas disease, which have also been associated with chronic or long-term sequelae (Garcia et al., 2011, 2014; Montgomery et al., 2014; Murray et al., 2014; Schilte et al., 2013).

[3] A disease that is transmitted to humans, plants, or animals by an insect or other arthropod (see next footnote) is called a vector-borne disease. (Plant pathologists refer to these as vector-associated diseases.) From the perspective of infectious diseases, vectors—which can be either living (biological) or nonliving (mechanical)—are the transmitters of disease-causing organisms; that is, they carry pathogens from one host to another. By common usage, vectors are considered to be invertebrate animals, usually arthropods. A broader definition of vector-borne disease recognizes that other animals can serve in the role of infectious disease vector by harboring pathogens that cause disease only in susceptible populations. These include invertebrates other than arthropods (e.g., snails, in the case of schistosomiasis), rodents (which spread a variety of viral diseases, including hantavirus pulmonary syndrome), fungi, plants, and even humans (in the case of sudden oak death), who may also serve as vectors for a variety of plant diseases (IOM, 2008).

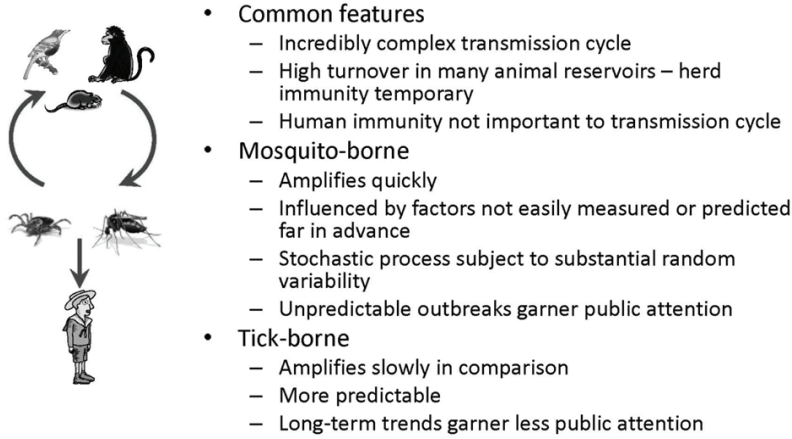

FIGURE WO-1 Vector-borne disease transmission: Humans as incidental hosts.
SOURCE: As presented by Lyle Petersen on September 16, 2014.

Vectors and Pathogens

As illustrated in Figure WO-1, many vector-borne pathogens (viruses, bacteria, fungi, and parasites) are transmitted among and between their primary and incidental hosts by arthropods such as mosquitoes, ticks, biting flies, and aphids.[4]

These pathogens include the mosquito-borne protozoans (*Plasmodium* spp.) that cause malaria[5] and the tick-borne parasite that causes babesiosis, *Babesia microti*; the newly described beetle-borne fungus, *Geosmithia morbida,* that causes thousand canker disease of black walnut trees[6]; the tick-borne bacterium

[4] Arthropods (members of the phylum Arthropoda) are invertebrates with jointed limbs, segmented bodies, and exoskeletons made of chitin. They include insects, spiders, crustaceans (e.g., shrimp, lobsters), and centipedes.

[5] There are many different types of plasmodium parasite, but only five types cause malaria in humans. These are

- *Plasmodium falciparum*—Mainly found in Africa, it is the most common type of malaria parasite and is responsible for most malaria deaths worldwide.
- *Plasmodium vivax*—Mainly found in Asia and South America, this parasite causes milder symptoms than *P. falciparum*, but it can stay in the liver for up to 3 years, which can result in relapses.
- *Plasmodium ovale*—Fairly uncommon and usually found in West Africa, it can remain in the liver for several years without producing symptoms.
- *Plasmodium malariae*—This rare species is usually found only in Africa.
- *Plasmodium knowlesi*—This very rare species is found in parts of South East Asia.

Source: http://www.nhs.uk/Conditions/Malaria/Pages/Causes.aspx (accessed March 25, 2016).

[6] See http://www.fs.fed.us/psw/publications/seybold/psw_2010_seybold008(tisserat).pdf (accessed March 25, 2016).

that causes Lyme disease, *Borrelia burgdorferi*; and the mosquito-borne West Nile and dengue viruses.

The arthropod-borne viruses, or arboviruses, are the largest class of vector-borne human pathogens. More than 500 arboviruses have been described, of which about 100 are known to cause diseases that include dengue, chikungunya, and several types of encephalitis (Gray and Banerjee, 1999; Gubler, 1998; Weaver and Reisen, 2010). Arboviruses circulate among wild animals, and many can be transmitted to humans and agriculturally important domestic animals through a process known as spillover (Weaver and Reisen, 2010). Infectious disease outbreaks resulting from such spillover events include epidemics of West Nile viral fever in the United States and of Rift Valley fever in Africa and the Middle East. Arthropod vectors also transmit most identified plant viruses (Hogenhout et al., 2008), as well as several important fungal and bacterial pathogens of plants (Fletcher and Wayadande, 2002; Gergerich and Dolja, 2006; Weintraub and Beanland, 2006).

Vector–pathogen relationships are central to the epidemiologies of many important plant diseases (Gergerich and Dolja, 2006; Purcell, 1982; Weintraub and Beanland, 2006). While only certain bacterial pathogens of plants require a vector for transmission, most plant viruses are spread from infected to healthy plants via a plant-feeding arthropod, nematode, or plant-parasitic fungus. Even humans appear to serve as vectors of plant disease. Sudden oak death, an emergent pathogen that has caused widespread dieback of several tree species in West Coast forests, has been spread to new areas by hikers, mountain bikers, and equestrians (COMTF, 2013). With sudden oak death, asymptomatic plants are actually the more important vectors.

Several important bacterial pathogens are delivered directly into plants' sugar-transporting phloem or water-transporting xylem networks by insects that feed on plant vascular fluids (Fletcher and Wayadande, 2002). These unusual pathogens and their multiple hosts provide fascinating examples of complex webs of organismal interactions. They include

- Spiroplasmas and phytoplasmas, which are tiny bacteria transmitted mainly by leafhopper insects. Pathogenic strains cause more than 700 distinct plant diseases, including corn stunt, coconut lethal yellowing, and pear decline (ARS, 2013; Fletcher and Wayadande, 2002; Weintraub and Beanland, 2006).
- Fastidious phloem-colonizing bacteria, so called because they cannot be consistently cultivated from infected hosts (which include species of herbaceous plants, trees, vegetables, fruits, grains, and ornamental plants) (Fletcher and Wayadande, 2002). Diseases caused by this group include citrus greening, which causes major losses in Asia and Africa and has been introduced recently into the United States, and cucurbit yellow vine.

- Fastidious xylem-limited bacteria, transmitted by xylem-feeding sharpshooter insects and spittle bugs. The best studied among these pathogens, *Xylella fastidiosa*, causes economically important damage in a wide range of plant hosts. In grapevines, it causes Pierce's disease, a significant threat to California's table grape and wine industries (Fletcher and Wayadande, 2002; NRC, 2004).

Viral infections of plants, such as the Citrus tristeza virus, stunt growth, lower yield, reduce fruit quality, and thereby diminish agricultural productivity (Gergerich and Dolja, 2006). Aphids transmit Barley yellow dwarf virus, the most widely distributed viral disease of cereals, among oats, wheat, maize, triticale, and rice (Miller and Rascochova, 1997). Aphids also spread plum pox, a severe disease of stone fruit trees that is easily spread from orchard to orchard (Damsteegt et al., 2007).

As winters become warmer in northern latitudes, more bacterial and fungal pathogens will likely survive through the winter, which may lead to more severe plant diseases, and increases in their geographic range. A shift in climate may also influence host resistance and growth, resulting in lowered resistance to fungal and viral diseases in plants (Harvell et al., 2002). Figure WO-2 illustrates major taxonomic pathogen groups causing emerging infectious disease in plants. According to Harvell et al. (2002), if climate change modifies host or pathogen geographic ranges, formerly separate species could converge, resulting in more severe disease outbreaks.

Ecology and Evolution

Vector-borne pathogen transmission occurs when host, vector, and pathogen interact in space and time within a permissive environment, as illustrated in Figure WO-3. Several environmental components (e.g., vegetation, climate, geology) may define the geographic area within which transmission takes place for a particular vector–host–pathogen system (Reisen, 2010).

Speaker Rodrigo Almeida (see Appendix A1), of the University of California, Berkeley, dissected the ecological complexity of vector-borne diseases into the following layers: the environment; the individual ecologies of pathogen, vector, and host; the outcome of their various interactions; and the effects of disease management. Local variation in the interplay among ecological forces shaping vector-borne diseases may produce dramatic shifts in disease transmission dynamics.

Similarly, several speakers described the effects of pathogen, vector, and host evolution on the transmission of West Nile viral fever, dengue, and chikungunya, among other vector-borne diseases.

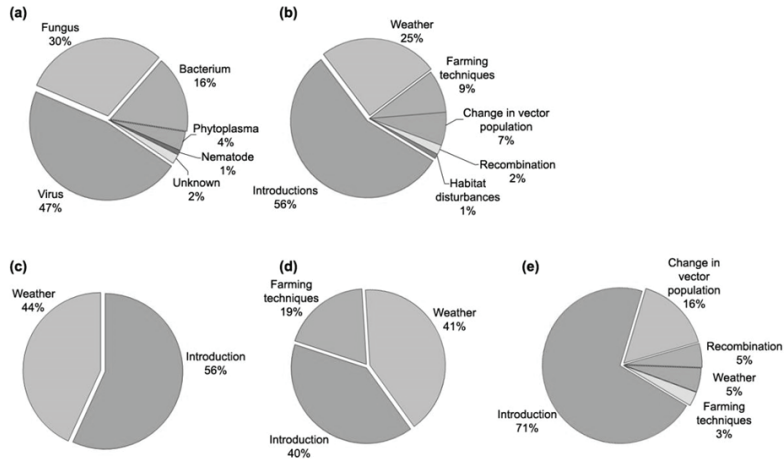

FIGURE WO-2 Major taxonomic groups of pathogens causing plant emerging infectious diseases: (a) viruses, fungi, and bacteria cause the most emerging infectious diseases in plants; (b) introduction of pathogens causes the most plant emerging infectious diseases; (c, d, and e) factors cited as the cause of disease emergence for bacteria (c), fungi (d), and viruses (e). The percentage of plant emerging infectious disease driven by introduction declines proportionately with the size of the pathogen (highest for viruses and lowest for fungi). Weather conditions, although major drivers of bacterial and fungal plant diseases, do not have as much impact on diseases caused by viruses.
SOURCE: Anderson et al., 2004. Reproduced with permission from Elsevier.

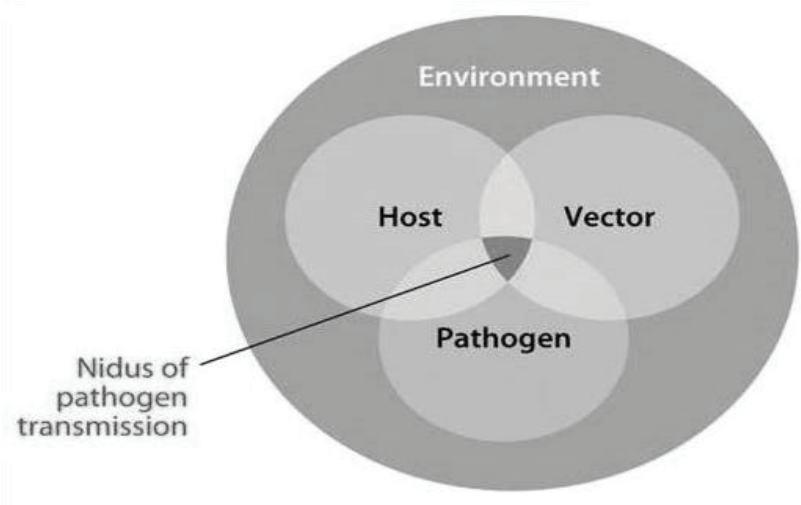

FIGURE WO-3 Key influences on vector-borne plant diseases.
SOURCE: Reisen, 2010. Reproduced with permission of *Annual Review of Entomology*, Volume 55, © by Annual Reviews, http://www.annualreviews.org.

Epidemics and Emergence

Emerging infectious diseases are caused by pathogens that (1) have increased in incidence, geographic, or host range (Funk et al., 2013); (2) have altered capabilities for pathogenesis; (3) have newly evolved; or (4) have been discovered or newly recognized (Anderson et al., 2004; Daszak et al., 2000; IOM, 1992). Recent epidemics of vector-borne disease have arisen from specific conditions occurring within the context of the large-scale drivers of infectious disease emergence listed in Box WO-1. Local surges in vector density, as well as increased vector competence—a measure of a given vector's intrinsic capacity to be infected by a pathogen, to replicate it, and to transmit it—fuel outbreaks (see Kilpatrick and Randolph in Appendix A6). Epidemics have also arisen in naïve host populations, whose exposure to vector-borne diseases has increased with the globalization of travel and trade, and with the decline of vector control efforts.

For viruses such as the West Nile virus (WNV) and dengue virus (DENV) that have recently expanded their geographic range, increased transmission has driven selection for strains with greater epidemic potential, while increased gene flow among vector populations has been associated with higher viral transmission rates. Figure WO-4 depicts the confluence of multiple drivers of vector-borne disease emergence in humans, all of which were explored in detail in the forum's initial workshop on vector-borne diseases (IOM, 2008).

Many of these same factors, in particular the global expansion of travel and trade, have driven the emergence of vector-borne plant diseases. Speaker Anna Whitfield, of Kansas State University, noted many similarities among vector-borne diseases of plants, animals, and humans, and in the health and research challenges

BOX WO-1
Drivers of Emergence for Vector-Borne Pathogens

- Globalization
  - Pathogen introduction
  - Vector introduction
  - Host introduction
- Land use change
  - Agriculture and urbanization
  - Community ecology and transmission dynamics
- Climate and climate change
- Evolution
  - Pathogens, vectors, hosts

SOURCE: As presented by Marm Kilpatrick on September 17, 2014.

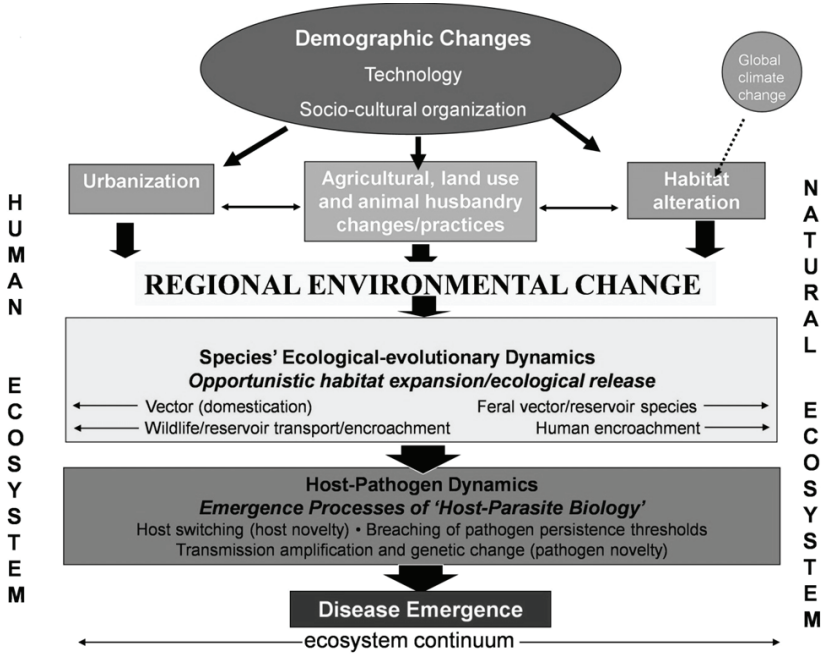

FIGURE WO-4 Epidemiological effects of urbanization and environmental change. SOURCE: Adapted from Wilcox and Gubler (2005) with permission from the Japanese Society for Hygiene.

they present (see Appendix A12). She described several emerging vector-borne plant diseases that threaten U.S. agriculture and horticulture, including

- Citrus greening disease, also known as huanglongbing, is caused by the bacterium *Candidatus Liberibacter asiaticus*, which is transmitted by an invasive insect, the Asian citrus psyllid (*Diaphorina citri*). Since its first appearance in Florida in 1998, it has become a major threat to that state's citrus crop, and it has spread across the southern continental United States to California, as well as to Hawaii and Mexico (University of California Agriculture and Natural Resources, 2013).
- An entirely new genus of viruses, *Emaravirus*, of which all known members are transmitted by eriophyid mites, infects a broad variety of plants including roses typically thought to be pest resistant, raspberries, pigeonpea, and the European mountain ash (Mielke-Ehret and Muhlbach, 2012).
- The soybean vein necrosis virus, transmitted by thrips, was first reported in Arkansas and Tennessee in 2008. Soybean vein necrosis is now the most widespread viral disease of soybeans in the United States (Zhou and Tzanetakis, 2013).

Recent Developments

The vast and complex challenges identified in the forum's 2007 workshop on vector-borne diseases continue to preoccupy researchers and policy makers (IOM, 2008). However, the field has undergone considerable change in the intervening years. In its fall 2014 workshop, the forum chose to highlight recent developments in the identification, emergence, and transmission of vector-borne diseases, as well as the public health response to vector-borne infections; advances in our understanding of the epidemiology and ecology of vector-borne diseases; and new insights on mitigating their effects. All of these topics, of course, raised further questions to be explored.

OVERVIEWS: VECTOR-BORNE DISEASE IN HUMANS, PLANTS, AND ANIMALS

The workshop opened with three presentations examining vector-borne disease systems that affect humans, plants, and animals. Speakers described dynamic interactions among pathogens, vectors, hosts, and their ecosystems, relating them to historic patterns of disease and patterns of emergence. Speakers also reviewed efforts to halt vector-borne diseases, considered possible future initiatives, and predicted possible future transmission patterns.

Emerging Human Mosquito- and Tick-Borne Diseases

Speaker Lyle Petersen from the Centers for Disease Control and Prevention (CDC) focused on mosquito- and tick-borne diseases that posed the greatest threat to the health of the U.S. population. Many of these pathogens are transmitted primarily among other animal species (reservoir hosts). For example, primates are the natural reservoir hosts of DENV (Bean et al., 2013) and chikungunya virus (CHIKV), and there is evidence that some animals, including nonprimates, such as rodents, birds, and small mammals, may also act as reservoirs for CHIKV (WHO, 2014b).

Vector-Borne Diseases of Concern in the United States

Humans are incidental hosts for most mosquito-borne viruses including WNV, as well as for tick-borne pathogens, which include *Borrelia burgdorferi* (the bacterial cause of Lyme disease), the recently discovered Heartland virus,[7] and parasites of the genus *Babesia* (the agents of babesiosis). All such diseases

[7] Heartland virus belongs to a family of viruses called Phleboviruses. Viruses in this family are found all over the world. Some of these viruses can cause people to get sick. Most of the phleboviruses that cause people to become ill are passed through the bite of a mosquito, tick, or sandfly. http://www.cdc.gov/ncezid/dvbd/heartland/ (accessed on October 5, 2015).

feature "incredibly complex" transmission cycles, and when herd immunity[8] within the animal reservoirs becomes important in slowing transmission, it is often transient, Petersen observed. Mosquito-borne diseases that incidentally infect humans may amplify quickly in response to any of a wide spectrum of factors that are difficult to anticipate far in advance, he continued (see Petersen et al. in Appendix A9). By contrast, pathogens amplify gradually in the longer-lived tick, producing more predictable transmission patterns. Such "slow burn" epidemics tend to garner little public attention in comparison to dramatic, sporadic outbreaks of mosquito-borne illness, he pointed out.

WNV, which first emerged in the United States as a human pathogen in 1999, is a prototypic arbovirus for which people serve as incidental hosts, according to Petersen. "It had to be brought in [to this country] by man, probably by importation of an infected animal," he said. "I think it is also important that it emerged during a heat wave," he added, because heat has been shown to increase the transmission efficiency of WNV by increasing concentrations of WNV in vector mosquitoes and shortening the time between an infected blood meal and when they become infectious (extrinsic incubation period). Evolution has also played a significant role: the emergent strain featured a key mutation that increased transmissibility, which was further improved by a second mutation in a replacement strain that arose in 2002. In addition, he explained, "There is continued coevolution both in birds [the reservoir host for WNV] and in the virus, where birds are becoming less susceptible to illness and death following infection, but at the same time, the viruses may be becoming more virulent by creating higher viremia in birds. So, in essence, it is an arms race between the host and the pathogen."

To date, it is possible that more than five million WNV infections have occurred in the United States. The vast majority have been asymptomatic, Petersen stated, but a fraction of a percent of them has progressed to severe neuroinvasive disease, of which more than 17,000 cases have been reported. Many of these cases occurred during three major outbreaks, which took place in 2002, 2003, and 2012—all during heat waves, he added. As illustrated in Figure WO-5, certain geographic regions of the United States seem to be at higher ecological risk for West Nile viral disease compared to other regions of the United States. "When the virus came across the U.S., there [was] no way we could have predicted that South Dakota would become the highest-incidence state for a tropical virus," he observed.

Some tick species serve as vectors for several different human diseases, while some pathogens can be spread by several different tick species. Petersen noted that ticks may be carriers of viruses, bacteria, and parasites that incidentally infect humans. Over the past century, land use changes, combined with

[8] Herd immunity occurs when a sufficient percentage of a population is immune to a pathogen to prevent its transmission. The more efficiently a pathogen can spread between members of a "herd," the greater the percentage that must acquire immunity to stop its transmission. Human immunity is inconsequential for pathogens that infect humans incidentally.

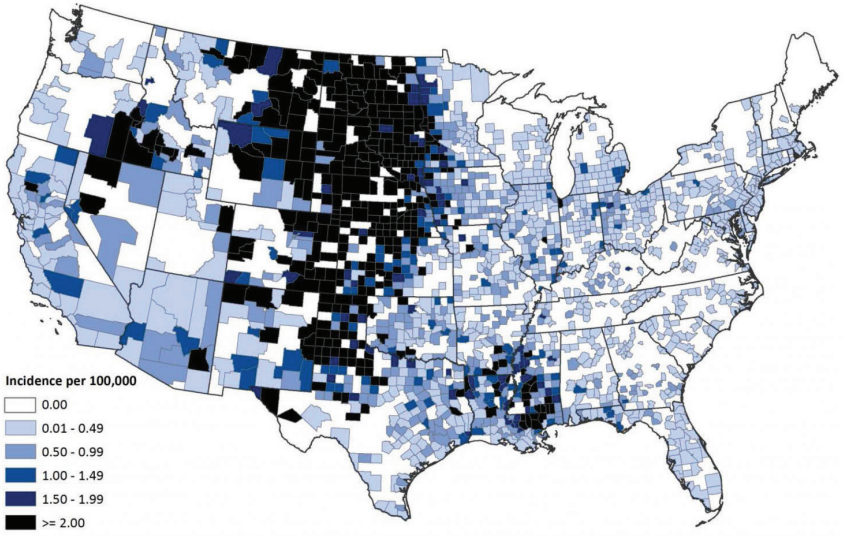

FIGURE WO-5 Average annual incidence of WNV severe neurological disease by county, United States, 1999–2013.
SOURCE: ArboNET, Arboviral Diseases Branch, CDC.

favorable environmental conditions, have enabled several tick species to increase in numbers and expand their geographic ranges, according to Petersen (see Figure WO-6). As a result, he observed, the incidence of essentially all tick-borne human diseases reported in the United States has increased. In addition, several novel tick-borne human pathogens have recently been identified, including relatives of known bacterial disease agents, as well as the Heartland virus, which Petersen characterized as a potential cause of hundreds to thousands of severely debilitating (and occasionally lethal) cases of illness per year (see later discussion in the section, "Changing Paradigms for Tick-Borne Diseases in the Americas").

Humans serve as primary hosts for the mosquito-borne dengue and chikungunya viruses, both of which are on the rise in the Americas. According to the CDC, dengue is caused by any one of four related viruses transmitted by mosquitoes (CDC, 2015). Infection with any of the four *Flaviviruses* can cause a painful febrile illness, dengue fever, or the life-threatening dengue hemorrhagic fever. Dengue virus now infects about 400 million people each year, having resurged after DDT-based vector control efforts were halted in the 1970s, according to Petersen. Dengue's expansion has also been abetted by the introduction of an additional vector species, *Aedes albopictus*, from Asia to the United States in 1985 (in a shipment of used tires). Before this introduction 30 years ago, dengue's geographic "footprint" was limited to a more range-restricted vector, *Aedes*

aegypti, in the Americas. Figure WO-7 illustrates the dramatic increase of the incidence of dengue in the Americas, which accelerated after reaching an apparent turning point around 2000. "The ecological factors all sort of aligned. The creation of megacities in the tropical world and all of the problems that trended with them have suddenly caused this incidence of dengue to go up and up and up," he observed.

Today, in tropical locations such as Puerto Rico, over 90 percent of residents have already been infected by DENV, Petersen stated. Can dengue fever—once a common illness in the southern United States—reemerge in this country? There is certainly reason to worry that it might, he observed. *Ae. aegypti* is endemic in the South and has expanded its geographic range to new areas, such as California, while *Ae. albopictus* has spread throughout the East. At the same time, increasing numbers of dengue-infected travelers are entering the United States. Yet, since 2009, only 10 dengue outbreaks (8 in Texas and 2 in Florida) have occurred in the United States, and each involved limited numbers of cases within restricted areas. "The U.S.–Mexico border is like the Berlin Wall of dengue," he quipped. "You

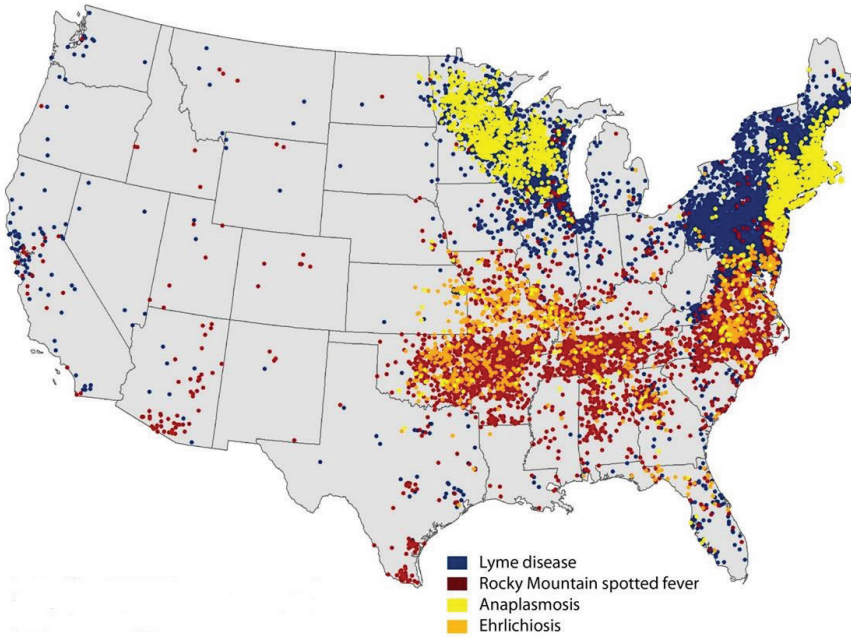

FIGURE WO-6 Distribution of key tick-borne diseases, 2012.
NOTE: In 2012, no cases of tick-borne illness were reported from Hawaii. Alaska reported 10 travel-related cases of Lyme disease.
SOURCE: CDC.

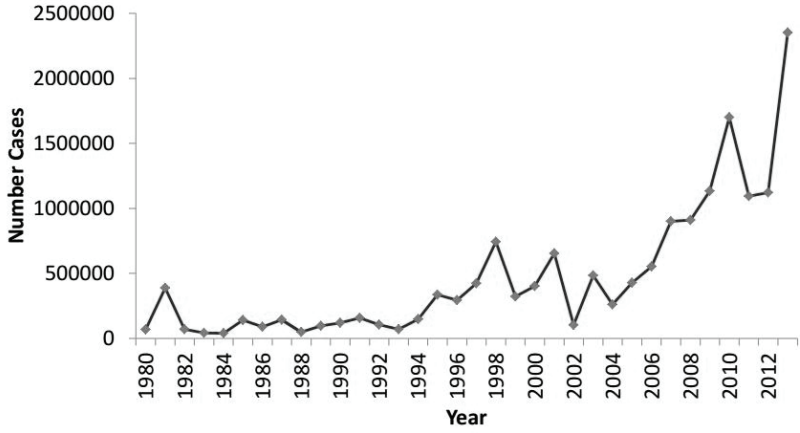

FIGURE WO-7 Dengue incidence is rapidly increasing in the Americas.
SOURCES: As presented by Lyle Petersen on September 16, 2014. Data from Pan American Health Organization.

find these huge outbreaks on the Mexican side of the border, just right across the Rio Grande River." His group's investigation of this paradox revealed vastly different human behaviors and environments in adjacent towns on either side of the border, and suggested that the lack of air conditioning and more crowded living conditions in Matamoros, Mexico, resulted in much higher rates of dengue transmission in comparison to Brownsville, Texas (Ramos et al., 2008). For now, it appears, lifestyle and living conditions help to protect the United States from dengue becoming endemic.

CHIKV is an *Alphavirus* that, like DENV, is transmitted between humans by both *Ae. aegypti* (its traditional urban vector) and *Ae. albopictus*, Petersen stated. Yet, unlike dengue, chikungunya infection is usually symptomatic, causing fever, debilitating joint pain, and often a rash. The virus emerged in the Americas in late 2013, on the island of St. Martin, and quickly spread across the Caribbean, as illustrated in Figure WO-8.

In May 2014, the Caribbean Public Health Authority declared that chikungunya had reached epidemic status (Carribean 360, 2014). By September 2014, more than 700,000 cases had been reported to the Pan American Health Organization (PAHO), with 113 deaths. As with dengue, these included only a few isolated cases of locally acquired chikungunya in the contiguous United States, all of them in Florida.

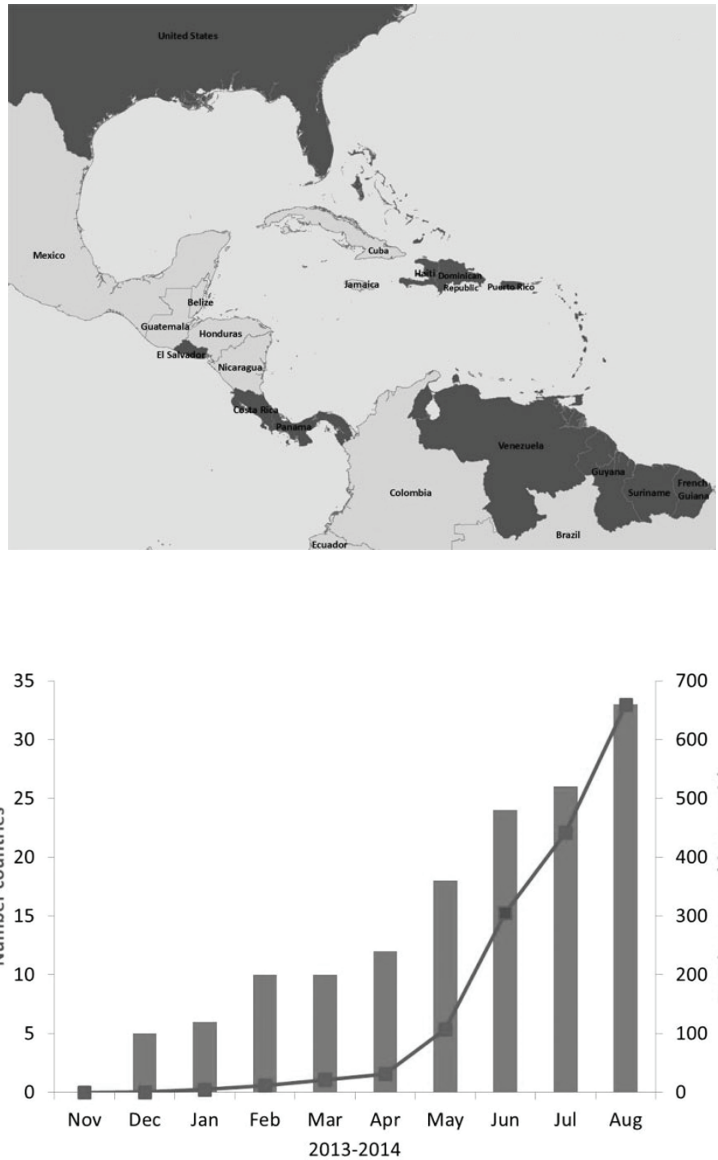

FIGURE WO-8 Chikungunya in the Americas and in the Western Hemisphere. Top: Countries and territories in the Americas where autochthonous chikungunya cases have been reported in the Western Hemisphere as of September 9, 2014; bottom: Chikungunya cases in the Americas reported to PAHO as of September 12, 2014. There were 706,093 cases and 113 deaths reported.
SOURCES: Top, CDC; bottom, as presented by Lyle Petersen on September 16, 2014. Data from Pan American Health Organization.

U.S. Outlook and Preparedness

Petersen offered the following general predictions of vector-borne disease activity in the United States and its territories:

- Continued focal and regional outbreaks of West Nile viral disease;
- Significant public health effects of dengue (ongoing) and chikungunya (until herd immunity is established) in U.S. territories;
- Increased incidence and distribution of tick-borne diseases;
- Discovery of additional novel tick-borne pathogens, some of public health significance; and
- Importation and emergence of nonendemic pathogens, such as the Zika virus.[9]

Are we prepared to meet these challenges? "In some instances, I think the glass is reasonably full," Petersen concluded. The ArboNET surveillance system, developed to track WNV, is the only system in the world that simultaneously collects human, animal, and vector data, he said. It can and has—in about a dozen cases—been adapted to monitor additional emergent and endemic arboviruses. Advanced molecular detection systems have proved extremely valuable in detecting imported and novel vector-borne pathogens. Another important legacy of the response to WNV is greatly improved communication capacity among physicians, public health agencies, and medical centers regarding actual or potential vector-borne disease outbreaks, he added. On the other hand, he warned that the existing system for tick-borne disease surveillance is becoming overwhelmed by the rising numbers of cases. More broadly, he observed, ecological parameters of pathogen transmission remain largely unknown, which limits the usefulness of disease models—as does the fact that many communities are not prepared to respond to vector-borne disease threats owing to inadequate surveillance and/or mosquito control capacity.

For prevention and treatment, "The glass is only half full," Petersen noted. While screening has—at great expense—nearly eliminated the risk of acquiring blood-borne WNV or *Trypanosoma cruzi* (the protozoan agent of Chagas disease), the U.S. blood transfusion system cannot currently detect the pathogens that cause dengue, babesiosis, chikungunya, ehrlichiosis, or anaplasmosis, or the next novel or imported vector-borne pathogen, Petersen pointed out (see "Blood Donation Screening for Vector-Borne Diseases"). Effective treatment regimens are available for the vector-borne bacterial diseases, but these conditions often go unrecognized, undiagnosed, or improperly treated, he observed. No such

[9] Zika virus is a *Flavivirus* related to yellow fever, dengue, West Nile, and Japanese encephalitis viruses. In 2007, it caused an outbreak of relatively mild disease characterized by rash, arthralgia, and conjunctivitis on Yap Island in the southwestern Pacific Ocean. This was the first time that Zika virus was detected outside of Africa and Asia (Hayes, 2009).

therapeutics exist for viruses, and while promising vaccines are in development against DENV and WNV—and also against *Borrelia burgdorferi*, the bacterial agent of Lyme disease—it is unclear when and if these will become commercially available, in part, because they may lack a robust commercial domestic market (see "Outlook for West Nile and Chikungunya Vaccines").

Much the same can be said about promising pesticides in development for vector control, Petersen stated. Of particular concern, he noted that no effective, scalable, vector control method exists for *Ixodes scapuarlis* or *Ae. aegypti*, each of which represents a major threat to public health throughout the Americas.

Lessons from a Model Plant Disease

As previously alluded to, after describing the ecological "layers" within which vector-borne plant diseases occur and the multiple factors that influence their transmission dynamics (see Figure WO-1), Almeida used the example of the vector-borne bacterium *Xylella fastidiosa* to illustrate these concepts (see Appendix A1). Typically a benign colonist of more than 300 species of plant species, *X. fastidiosa* is transmitted by insects that feed on the liquids transported within the xylem of host plants.[10] The bacterium can, under certain circumstances, grow so profusely that it blocks the upward flow of fluids in the plant, resulting in scorched leaves and shriveled fruit. This condition affects several important woody crop plants and trees, most notably grapevines in the United States (called Pierce's disease of grapevines). Pierce's disease escalated from a low-level problem into a major threat to California's viticultural industries following the arrival of an alternate vector—the glassy-winged sharpshooter (*Homalodisca vitripennis*)—to California in the late 1980s (Fletcher and Wayadande, 2002). Since then, the geographic and host plant range—and the economic, political, and social significance—of *X. fastidiosa* have expanded in the United States and internationally.

The emergence of Pierce's disease has paralleled significant changes in the study of vector-borne plant diseases, as well as in their dynamics, Almeida pointed out. Around the time that the glassy-winged sharpshooter invaded California, plant pathologists relied on studies of pathogen host range, epidemiological surveys, and the outcomes of vector control measures attempted in the field to inform mitigation efforts. These ecologically based methods were sufficient to manage diseases within a limited geographic area and time frame—a sensible approach, prior to the global transmission of economically important diseases, he said. Similarly, research priorities were short to medium term and were directed toward managing disease, not toward understanding patterns of transmission or factors of emergence.

[10] Xylem is the conductive tissue in vascular plants through which water and nutrients flow upward from the roots.

"The present is a little more complicated," Almeida continued. Today, significant vector-borne plant disease threats frequently involve pathogens and/or vectors introduced to new ecosystems via international travel and trade, and sequence-based identification methods allow researchers to trace the origins of outbreaks. By these means, researchers determined that pathogenic subspecies of *X. fastidiosa* occur with a distinct range within the Americas, and specific subspecies now threaten olives and other important crops in Europe, as well as grapes and the Asian pear in Taiwan.

Before it was identified in southern Italy for the first time in October 2013, *X. fastidiosa* was not believed to be able to cause disease in olives, Almeida noted. Given the olives' economic and cultural significance in this region (as both a source of oil and a tourism attraction), as well as the potential for pathogen spillover to grapes and citrus, it is perhaps no wonder that the European agricultural community is extremely concerned about it. Plant scientists are often not trained to handle such situations where the disease has large economic, trade, and social consequences at the international sphere; in this case it also included the generation of conspiracy theories suggesting that Almeida himself had spread the disease to Italy, as an agent of developers or agribusiness, or that *X. fastidiosa* itself does not exist. "It is really an interesting problem that goes way beyond a plant disease and how to manage it."

How do plant diseases actually move to new ecosystems? In many cases it occurs on ships, according to Almeida, as contrasted to their human counterparts, which move readily by air travel. Current data strongly suggest that the epidemic in Italy originated from ornamental coffee plants imported from Costa Rica. Pest and diseases also travel with "suitcase plant material," such as the anecdotal case of a grapevine cutting of Israeli origin smuggled into California by a grower (which is supported by molecular data), along with what has become a major insect pest, he noted. Vector introductions provide a second important path to plant disease outbreaks, if they increase transmission of an existing pathogen. This occurred in California when the glassy-winged sharpshooter quickly achieved large populations, which permitted more frequent encounters with *X. fastidiosa*, more successful infections of various plant species, and, ultimately, higher incidence of disease, he explained. New associations between the novel vector and *X. fastidiosa*, coupled with the vector's ability to transmit additional pathogen strains among a broader range of host plants, may also have contributed to the recent emergence of Pierce's disease and other *X. fastidiosa* diseases in California, he suggested.

In addition, complex insect–pathogen–plant interactions must be understood at the molecular level in order to address vector-borne plant disease threats. Almeida observed that plant pathogen transmission hinges on complex interactions among surface proteins and receptors in pathogens and vectors. Having identified some of such moieties in *X. fastidiosa*, Almeida and coworkers are attempting to inhibit bacterial attachment to insect tissues, which might be achieved by

genetically modifying host plants to produce molecules that block key interactions. This approach has recently been shown to work for an insect-transmitted plant virus (Whitfield and Rotenberg, 2015, reprinted in Appendix A12). Host plants could also be genetically manipulated to produce molecules that kill specific insect vectors that feed on them, he added.

Another active area of current research examines how, over the course of evolution, pathogens manipulate vector behavior directly, or through their effects on host plants, so as to increase transmission efficiency. Many such interactions have been identified, employing a broad range of mechanisms and strategies, Almeida reported.

Vector-Borne Disease in Animals

"We are just barely getting a grasp on patterns of vector-borne diseases in animals," observed speaker William Karesh, of the EcoHealth Alliance, as he introduced this topic. However, he continued, the importance of vector-borne animal diseases is increasingly apparent. Vector-borne animal pathogens are included in priority pathogen categories by the National Institute of Allergy and Infectious Diseases, in the catalog of significant trade-related animal diseases by the World Organisation for Animal Health (OIE), and among novel pathogens listed by the United States Agency for International Development's PREDICT project, he reported. Nearly one-third of all known viruses that infect mammals are vector borne, and a recent analysis of 86 emerging zoonotic viruses determined that, among those transmitted from wild animals to humans, 40 percent were vector borne, including all viruses for which wild birds served as reservoir species.

Recent studies of transmission patterns reveal potential strategies for addressing vector-borne animal diseases, Karesh noted (see Appendix A5). For example, researchers have shown that while vector-borne mammalian viruses tend to have a broad host range, they are generally transmitted among these hosts by a single vector. In some cases where that is true, he suggested, controlling vector populations and their ability to spread pathogens to humans or animals offers relatively simple routes to reducing disease transmission, as compared with pathogens that follow multiple transmission routes between animals and people, and are therefore more prone to spillover. The identification of land use change and international travel and trade as primary drivers of emergence of vector-borne animal diseases should guide disease surveillance and prevention efforts, he added.

Disease Patterns in Emerging Pathogens

Karesh described the state of knowledge regarding patterns of disease for the notable emerging vector-borne animal pathogens in the following subsections.

Schmallenberg virus emerged suddenly in sheep in Germany in 2011—related viruses had previously been identified only in Africa, the Middle East, and Asia—and from there spread across Europe much as the West Nile virus moved across the United States upon its emergence in 1999, Karesh recalled. The biting midge (*Culicoides* species), considered the major vector of Schmallenberg virus, is "extremely efficient in transmitting the virus to sheep," he said. However, he added, "Sheep are not so efficient in transmitting the virus back into midges." Nevertheless, the virus is highly contagious, with each infected animal producing as many as seven infections (European Food Safety Authority, 2014). These infections—which last a few days and cause fever, reduced milk yield, diarrhea, and abortion—have primarily been reported among ruminants (none have been reported in humans).

Schmallenberg virus infection is not listed as a reportable disease by the OIE, Karesh noted. "Currently, the disease does not meet the criteria for OIE listing," he explained. "If an animal is positive it means they are protected for life. If they are negative it means they are free of the disease." On the other hand, he continued, the disease can be disastrous for individual farmers, who operate on small economic margins. Vector control might seem a reasonable way to reduce disease transmission, but it would not be an easy route to take, given our limited understanding of the ecology of the widespread midge, he observed. Vaccines are available but have not been widely used, he remarked, perhaps because the transient infection is not perceived as sufficiently burdensome to warrant prevention.

WNV, as Petersen noted, infects a wide range of bird species. Elucidating the resulting patterns of disease has been difficult, according to Karesh, because "there are so many variables at play." While climate and weather likely influence disease incidence, many additional factors vary across the geographic range of the virus, he noted (Crowder et al., 2013). The number of avian WNV cases reported to the CDC has varied widely from year to year, he stated (Lindsey et al., 2014). Some of this variation may reflect the 60 percent decline in dead bird surveillance that occurred between 2004 and 2012, and which occurred simultaneously with a significant decrease of pathogen surveillance in trapped mosquitoes, Karesh noted (Hadler et al., 2014). Thus, "It is very hard to say whether we are having a changing pattern when we are changing the tools we are using to monitor patterns . . . [and] we are investing less in surveillance," he concluded.

Tick-borne pathogens threaten animal and human health worldwide. Ten percent of tick species carry such pathogens, Karesh reported (Jongejan and Uilenberg, 2004). The resulting diseases affect 80 percent of the world's cattle, at a cost of up to $19 billion per year, which is borne disproportionately by resource-limited countries in the tropics and subtropics (Minjauw and McLeod, 2003).

Once again, vector control is not a likely solution to this problem, Karesh observed. Effective vector control to address tick-borne disease in livestock

would need to extend to wild animals that are part of transmission cycles, making it prohibitively expensive, particularly for people earning less than 1USD per day, which is common in India and Africa (Minjauw and McLeod, 2003). In such circumstances, it can cost more to control disease in cattle than it does to raise cows, he pointed out.

Multidisciplinary Research on Rift Valley Fever Virus

"We can't really lump the vector-borne diseases together and say they are all headed in the same direction," Karesh concluded (Kilpatrick and Randolph, 2012, reprinted in Appendix A6). Pathogen introductions, ecological shifts, and changes in host immunity all affect patterns of disease, he noted. Thus, rather than tackle vector-borne diseases as a whole, he proposed that researchers undertake multidisciplinary, long-term, broad-based studies of individual vector-borne diseases.

The EcoHealth Alliance and a large group of collaborating agencies are currently attempting such a study of Rift Valley fever[11] in South Africa. There, according to Karesh, rainfall patterns are predictable as much as 3 months in advance. This would in theory provide adequate warning to vaccinate animals against the spread of Rift Valley fever virus (RVFV). But, according to Karesh, partly because of sociological reasons that may be difficult to counteract, this has not happened. Therefore, it may be equally important to develop a targeted, effective approach to disease control that will enlist the support of people and governments. A better strategy against Rift Valley fever in South Africa might take advantage of herd immunity, which also appears to influence outbreak patterns there, he observed.

To investigate this possibility in detail, Karesh and coworkers have embarked on a plan to monitor changes in immunity to RVFV in individual animals, flocks, herds, mixed-species populations, and mixed populations of wildlife and domestic animals within a 40,000 km^2 area to identify factors that influence immunity at a population scale. The project, which began around the time of the workshop, is expected to last 5 years, he said. It will track antibody levels in local domestic animals (including cattle, goats, and sheep), free-ranging wildlife (including several antelope species) and those on game ranches, mosquitoes, and people. Those measurements will be integrated with data on vegetation and weather, in order to accomplish the following series of objectives:

[11] Rift Valley fever is a viral zoonosis that primarily affects animals but also has the capacity to infect humans. Infection can cause severe disease in both animals and humans. The disease also results in significant economic losses due to death and abortion among RVF-infected livestock. The virus was first identified in 1931 after an epidemic struck sheep on a farm in the Rift Valley of Kenya. Since then, outbreaks have been reported in sub-Saharan and North Africa. In 1997–1998, a major outbreak occurred in Kenya, Somalia, and Tanzania, and in September 2000, cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent and raising concerns that it could extend to other parts of Asia and Europe. Source: http://www.who.int/mediacentre/factsheets/fs207/en (accessed March 25, 2016).

- To compare how immunity to RVFV changes over time in vaccinated and unvaccinated sheep and antelope;
- To determine herd immunity in wildlife and domestic animals;
- To investigate the ecology of RVFV and its mosquito vector, with reference to soil types, vegetation, and climate; and
- To evaluate human behavioral practices and measure immunity among people working on farms within the study area and detect new infections.

By collecting data at the individual, population, and meta-population levels, among both domesticated and wild animals, the researchers expect to discover useful distinctions in herd immunity to RVFV among different populations. Karesh noted, for example, that herd immunity in cattle kept by pastoral farmers—which tend to live longer than those raised in commercial feedlot systems—is likely to be more persistent. Likewise, he said, long-lived wildlife such as buffalo might also have high immunity to RVFV. Such information should allow these investigators to better anticipate when particular animal populations are susceptible to outbreaks of disease that could, in turn, spill over into humans—knowledge that would support more efficient vaccination programs or other disease control measures, he concluded. Implementation will require social engagement which, he said, means "making sure [the program] . . . is cost-effective, convincing people that [it] is worth doing, and really understanding where they are coming from so we can come up with some solutions that make sense."

Common Ground

In her introduction to this workshop session, moderator Mary Wilson of the Harvard School of Public Health (now at the University of California, San Francisco), encouraged participants to recognize commonalities among the pathogen–vector–host–environment systems described by the three speakers, and to consider research and policy issues that lie at these points of intersection. In the course of their presentations, the speakers raised several such ideas that were further explored in discussion immediately afterward, and throughout the workshop.

Need for Consistent, Comprehensive Surveillance

Both Petersen and Karesh noted that initially robust support for WNV surveillance has declined with disease incidence following the 2002–2003 outbreaks. Limited resources were available to respond to another peak outbreak in 2012. While it is now possible to make accurate local predictions of WNV outbreaks in time to prevent their occurrence, Petersen said, communities are not investing in local surveillance, nor are they willing to implement vector control measures in advance of human cases of the disease.

"By the time an effective response is mounted at a local level, the outbreak is often well on its way and possibly on the downhill slope," Petersen stated. "That is exactly what we saw in the big outbreak in Dallas. By the time a widespread response was mounted, three-quarters of the cases had already occurred. We were able to show quite nicely that it did stop the outbreak, but it was done too late. So there is really a problem with the intensity of surveillance and getting people to actually respond effectively in a timely way." Speaker James Hadler, of Yale University, further explored this dilemma (see subsequent section, "Loss of Arbovirus Disease Surveillance Capacity in the United States").

Surveillance for most other vector-borne pathogens—including novel, emerging ones—is less thorough than for WNV. Vector-borne plant diseases pose an especially difficult problem for surveillance, according to several participants. Until a plant disease becomes epidemic, it is perceived as a problem only to the farmers whose crops are infected, Almeida noted. "For a plant disease to come to the radar you need thousands if not hundreds of thousands of plants to be sick," he said.

On the other hand, forum member David Rizzo of the University of California, Davis, noted that many plant pathogens are emerging as the result of ecosystem disturbance, much as are animal pathogens. "I can think of a half a dozen in the United States right now with the potential to spill over into agriculture," he stated, such as laurel wilt, which now threatens avocado crops. "By the time you see millions of dead plants, then it is too late to really do something," he warned. Unfortunately, he added, little such surveillance is occurring, especially in natural ecosystems.

Gaps in Training and Their Consequences

All three speakers and several discussants expressed concerns regarding the limited opportunities for the education and training of the next generation of vector-borne disease researchers, coupled with the decline of certain key disciplines. Training of scientists working with vector-borne diseases in plant pathology fails to emphasize either quantitative work or field ecology, Almeida noted. Petersen similarly criticized the public health community for its neglect of research and training in ecology—as well as medical entomology—and warned that insufficient funding and career opportunities for scientists in these disciplines would diminish future capacity to address vector-borne disease threats. In a later presentation, Christopher Paddock, of the CDC, added cartography to the list of endangered core disciplines for vector-borne disease research. When mosquito vectors of emerging pathogens cannot readily be identified by species owing to a lack of skilled medical entomologists, Karesh observed, "it is going to be an ugly world."

Today's investigators also lack the ability to connect the study of vector-borne diseases—and emerging diseases in general—with their social, political,

and economical consequences. "I think there will be a push for incorporating social sciences into what we do," Almeida predicted, and offered as an example the work of a postdoctoral researcher in his laboratory (Dr. Adam Zeilinger), shown in Figure WO-9.

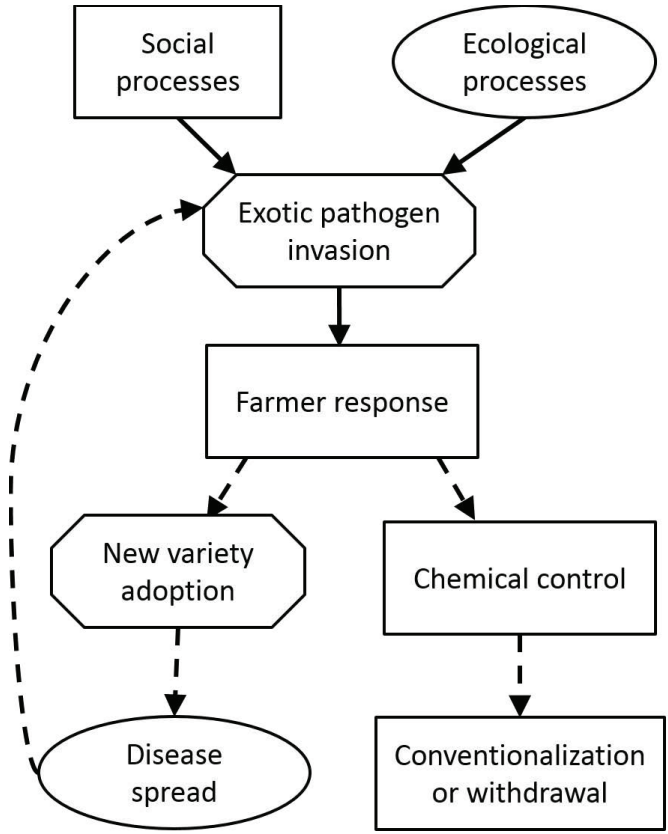

FIGURE WO-9 Conceptual model of the socioecological relationships between invasive species, farmer responses, pathogen spread, and conventionalization of organic agriculture. Traditional social entities are represented by squares, traditional ecological entities are represented by ovals, and co-constructed entities represented by octagons. Dashed lines remain as poorly described, hypothetical links.
SOURCE: Figure created by Dr. Adam Zeilinger, University of California, Berkeley. Reproduced with permission.

Advantages of the One Health Approach

The narrow training of investigators both reflects and drives a specialized, technology-centric approach to the study of vector-borne diseases. Speakers and participants alike noted that as the field has become increasingly focused on molecular-level interactions between pathogen, host, and vector, it has lost sight of the ecological contexts of these interactions, as well the many benefits of interdisciplinarity.

In plant pathology, Almeida observed, basic questions are being overlooked. For example, in the case of citrus greening—a disease that threatens to decimate Florida's citrus industry—some of the most fundamental experiments have yet to be performed. If "we still don't know how long it takes for a plant to express symptoms once it is infected by an insect," inferences from epidemiological models will suffer from lack of information, according to Almeida.

Moreover, solutions to specific disease problems are being studied in isolation and in ignorance of the drivers of disease emergence, which might productively be addressed interdisciplinarily. "Is plant pathology, as well as other disciplines, willing to diversify and view agricultural systems in a more holistic manner?" Almeida wondered. "That is not clear," because there is limited research of that kind currently going on—nor is it at all clear, he observed, whether or how funding agencies would support such an approach to transdisciplinary research.

Almeida, along with several other workshop participants, decried a funding bias toward research in molecular biology in general, and specifically in support of the use of transgenics to address vector-borne disease. In addition, lack of funding has made programs highly competitive. "USDA funding rates for fundamental science [i.e., single Principal Investigator grants] are currently less than 10 percent," Almeida observed. He also noted, however, that the National Science Foundation recently revived the dwindling science of systematics through a targeted training grant program. Petersen described a similar effort by the CDC to boost medical entomology following the emergence of WNV in the early 2000s that has since been discontinued.

Increasing recognition of the value of the One Health paradigm—defined as "the collaborative effort of multiple disciplines—working locally, nationally, and globally—to attain optimal health for people, animals, and the environment" (AVMA, 2008)—offers hope that these trends may be reversed. Forum member Kevin Russell of the Armed Forces Health Surveillance Center pointed out that the Global Health Security Agenda emphasized a multisectorial approach to global health security, which depends in part upon the economic ramifications of plant, animal, and human diseases. Karesh noted that the Department of Defense is funding the described long-term, broad-based RVFV project he discussed in his presentation, which aligns with Global Health Security Agenda mandates for a One Health approach to disease control encompassing humans, wildlife, and livestock. "I think there are some cost-savings and efficiencies when we start to

pull together . . . thinking on animal and human and plant vector-borne diseases," Karesh predicted. "We have to really start interacting more at that level and using that to leverage reducing budgets."

Ecological Complexity

Underpinning the concept of One Health and influencing patterns of disease is an understanding of the essential role of the environment to address vector-borne diseases, both individually and collectively. Petersen observed, for example, that WNV outbreaks cannot be predicted beyond the local scale because "the ecology is incredibly complicated. It varies from place to place." Some common denominators, such as heat waves, are generally predictive of outbreaks, he continued, but ultimately, "If you start narrowing down the ecology, Phoenix is not like Chicago." Even within parts of Phoenix—which, in the middle of the desert, is an unlikely but raging hot spot for WNV—there is variation in transmission of the virus, he added. "The ecology of the whole United States is amazingly complicated and varied," Petersen observed. This point was illustrated again and again over the course of the workshop, through the lens of various vector-borne diseases in natural, agricultural, urban, and suburban contexts.

CHANGING DISEASE PATTERNS

Four speakers illustrated the dynamic nature of vector-borne diseases in presentations describing the evolution and epidemiology of dengue and chikungunya, shifting patterns of insect-borne parasitic infections closely associated with poverty, leishmaniasis and Chagas disease, and the recent range expansion of multiple tick-borne diseases in the United States.

Arbovirus Evolution in Humans and Mosquitoes

Speaker Rebecca Rico-Hesse, of Baylor College of Medicine, used the examples of DENV and CHIKV to illustrate how evolution influences dynamic relationships among pathogens, vectors, and hosts (see Appendix A10). Consisting of little more than a strand of RNA that encodes 8 to 10 proteins, these highly mutable and adaptable arboviruses represent "the smallest, most simple organisms that we know of that have changed history of humankind," she pointed out. DENV and CHIKV are transmitted to humans by the mosquito vectors *Ae. aegypti* and *Ae. albopictus*, which have quite distinct habitats and biting habits.

Global Spread of a More Virulent Dengue Virus

Most human dengue infections produce the flulike illness known as dengue fever, but some cases progress to the life-threatening dengue hemorrhagic fever

(DHF), with massive internal bleeding. Any of the four serotypes of DENV can cause dengue fever, and it has been known for decades that the risk of DHF increases if a person is serially infected by two different viral serotypes (Rico-Hesse, 2009). The majority of dengue epidemics to date, and most cases of DHF, have been linked to serotype 2 (DENV-2), which has been isolated in Asia, Africa, and the Americas, Rico-Hesse reported (Cologna et al., 2005). A single genotype within DENV-2, once limited to Southeast Asia, has been detected in the majority of isolates from patients with DHF—including, in recent years, patients in the Americas and West Africa, as well as in Southeast Asia. "This genotype, the one that is more severe and more virulent, has displaced all of the other ones," she concluded. A similar displacement of less virulent genotypes worldwide by a more virulent virus has occurred within the DENV-3 serotype as well, she added.

How did the Southeast Asian genotype of DENV-2 outcompete native genotypes? Comparative infection experiments in cultured human dendritic cells—the cell type targeted by DENV—reveal that while the American genotype infects a larger number of cells, the Southeast Asian virus replicates much more efficiently, generating a larger number of viruses per cell infected, according to Rico-Hesse. Experiments in mosquitoes showed that both genotypes were equally capable of binding the insect's midgut, but that the Southeast Asian virus was more prevalent in the salivary glands, and therefore more available for transmission to humans, she added. Both features contribute to the 60-fold-higher transmission efficiency of the Southeast Asian genotype, which in turn explains its ability to displace the American genotype, she concluded.

To investigate the source of heightened virulence in the Southeast Asian genotype of DENV-2, Rico-Hesse and colleagues created "humanized" mice, in which up to 80 percent of their white blood cells are of human origin (Brehm et al., 2013). "What we have is a mouse that gets infected, gets viremic and gets a rash, gets thrombocytopenic, and does all of the things just like humans do with dengue fever," she explained. By infecting these mice, via mosquito bite, with selected viruses, the researchers were able to determine that the Southeast Asian genotype remained longer in the bloodstream and achieved higher titers than other genotypes. Subsequent theoretical studies of nucleotide folding among genotypes of DENV-2 suggest that subtle structural differences may determine virulence, she said; they plan to test these ideas in experiments with chimeric viruses.

The researchers also discovered that infecting via mosquito, rather than by inoculation, significantly increased DENV viremia in humanized mice, Rico-Hesse reported (Cox et al., 2012). This, she said, likely resulted from immune deficiencies that limit the mice's ability to respond to infection as a human would. Interestingly, the humanized mice also made antibodies to mosquito saliva alone, and these, too, were very long lived. "We can't say that mosquito saliva is not important," she insisted. "We have to start including this in any studies

of pathogenesis, control of vaccination strategies. The mosquito saliva is doing things very unexpectedly in the human immune system."

Chikungunya Expansion and Adaptation

Urban epidemics of chikungunya are characterized by rapid spread and high infection rates, leading in most cases to symptoms that resemble those of dengue: acute fever and debilitating joint pain (Nasci, 2014). These typically resolve within a week; however, joint pain and fatigue may persist for 2 years or longer in some individuals. In contrast to DENV, CHIKV infects endothelial cells and fibroblasts, but how it interacts with the human immune system and the mechanisms by which pathogenesis manifests remain a mystery, according to Rico-Hesse. She and coworkers plan to investigate these processes—as well as dengue pathogenesis—with a recently developed mouse model that better mimics the human immune system.

CHIKV was first identified in Tanzania in 1952, and subsequently found throughout Africa and Asia, where it caused periodic small outbreaks (Nasci, 2014). In June 2004, a chikungunya epidemic on Lamu Island, Kenya, spread to other islands in the Indian Ocean. This epidemic produced nearly half a million cases of chikungunya. A later epidemic resulted in more than 1.5 million cases in India, which then continued on through Southeast Asia to islands in the Pacific Ocean. Since then, CHIKV has been recognized as an important emerging vector-borne pathogen. The first locally transmitted cases of chikungunya in the Western Hemisphere were reported in October 2013, on the island of St. Martin; others have since been reported on several more islands in the Caribbean.

The recent range expansion of CHIKV resulted from separate advances by two of the three known viral genotypes, Rico-Hesse explained (Thiberville et al., 2013). The East Central South African genotype of CHIKV, which caused the first wave of epidemics, has not yet reached the Americas, but the Asian genotype has, she said. Recent evidence shows that the Asian genotype can be transmitted with equal efficiency by both mosquito vectors, one of which—*Aedes albopictus*—is well adapted to temperate climates (Vega-Rua et al., 2014). She concluded by observing that, "We [now] have a chikungunya virus that can be spread in *Aedes albopictus*—which, by the way, is everywhere in Houston."

Dengue and Chikungunya in the Americas

Building on Petersen's update on dengue and chikungunya emergence, Harold Margolis, of the CDC, described how rapid increases in their incidence in the Americas has prompted changes in diagnostic methods and protocols for all febrile disease syndromes.

Dengue

Since around 2000, as Petersen noted, dengue case reports have risen rapidly in the Americas. Margolis pointed out, however, that when considered on a country-by-country basis, this trend has been far from uniform. Rather, he observed, case rates have increased sharply in countries most affected by urbanization and migration. "Part of this increase is how we are recognizing the disease and what we are measuring and what we are diagnosing," he suggested.

The symptoms of dengue fever can resolve within a week, or during the same period it can progress to severe hemorrhagic disease or death, Margolis noted. Many of dengue's symptoms resemble those of several other febrile diseases, such as leptospirosis. In Puerto Rico, for instance, the diagnostic testing of patients meeting WHO criteria for suspect dengue typically finds that only about half of them are actually infected with the virus, he reported. On the other hand, researchers in Thailand determined that among a group of nearly 400 schoolchildren who tested positive for DENV infection, only half exhibited symptoms that met WHO criteria for clinical diagnosis (Sabchareon et al., 2012). Clearly, he concluded, "The only way you know if somebody has dengue is diagnostic testing."

Margolis reported that major changes in dengue diagnostics in recent years are improving this situation. Once a slow and complicated process requiring both acute and convalescent samples for immunoglobins (IgM) testing, diagnosis by specific DENV subtype or by IgM can now be performed quickly on a single acute-phase sample through rRT-PCR, he explained. It is now possible to detect about 90 percent of cases that will seroconvert through molecular diagnostic testing. In the United States, where most molecular diagnostic tests like these are performed commercially, routine testing for DENV occurs only in public health laboratories, he said, although guidelines promoting DENV testing are under development by the CDC and the Association of Public Health Laboratories. Meanwhile, PAHO has established a network of dengue diagnostic laboratories, where the disease is endemic, throughout tropical and subtropical South and Central America and the Caribbean (PAHO, 2014a).

Because most of the United States is nonendemic for dengue, the majority of current cases involve returning travelers, Margolis noted. Dengue is the leading cause of acute febrile illness in travelers returning from the Americas, the Caribbean, and Asia (Freedman et al., 2006). Between 2000 and 2007, the number of such cases requiring hospitalization tripled in the United States (Streit et al., 2011). With thousands of travelers returning from dengue-endemic areas, coupled with the presence of *Ae. aegypti* in Florida, Texas, and Arizona, it is not surprising that a few episodes of limited local transmission have recently occurred in the United States, he remarked. The potential for more widespread local transmission, particularly involving *Ae. albopictus,* remains to be determined (Eisen and Moore, 2013).

Chikungunya

Like dengue fever, chikungunya is an acute febrile illness that can be reliably diagnosed only through molecular diagnostic testing, preferably by polymerase chain reaction (PCR),[12] according to Margolis. Only supportive treatment (typically with nonsteroidal anti-inflammatory drugs) is available for chikungunya, and it is important to rule out dengue before proceeding, given the risk of hemorrhage, he pointed out.

The rapid geographic expansion and rise in chikungunya cases in the Americas, since its emergence in 2013, will continue, Margolis predicted, echoing Petersen's earlier observations. Locally acquired cases have been confirmed in many countries throughout the Americas (CDC, 2014c), with the first such case in the United States reported in Florida in July 2014 (CDC, 2014b). This development was anticipated, and is expected to be repeated, given the large numbers of U.S. travelers returning from locations where major outbreaks have occurred (including Puerto Rico, as Margolis noted), coupled with the presence of *both* competent mosquito vector species—*Ae. albopictus* and *Ae. aegypti*—in this country (CDC, 2014a; Fischer et al., 2014; Khan et al., 2014).

At the time of the workshop, about 1,400 cases of chikungunya per week were being reported in Puerto Rico, mainly in the metropolitan San Juan area, Margolis stated.

The introduced virus has been traced to the Dominican Republic, he said, and many of these early cases have arisen in the city's Dominican community. For such a "virgin soil" epidemic, it is difficult to predict how many epidemic cycles will occur before herd immunity is established. CHIKV's rapid expansion clearly demonstrates that its mosquito vector, *Ae. aegypti*, is not controlled, he observed.

Dynamics of Leishmaniasis and Chagas Disease

Two vector-borne parasitic diseases, leishmaniasis and Chagas disease, are strongly associated with poor living conditions that expose people to the insect vectors that carry them. "These are diseases of poverty," observed speaker James Maguire, of the Harvard Medical School. "In some senses the vector is poor. I think the poor vectors are picking on poor people." This is also true of African sleeping sickness, which, like leishmaniasis and Chagas disease, is caused by a member of a group of flagellated protozoa known as kinetoplastids.[13] Members of this group parasitize a broad range of animals and plants (Wiser, 2013).

[12] PCR is a laboratory technique used to amplify DNA sequences. The method involves using short DNA sequences called primers to select the portion of the genome to be amplified. The temperature of the sample is repeatedly raised and lowered to help a DNA replication enzyme copy the target DNA sequence. The technique can produce a billion copies of the target sequence in just a few hours (http://www.genome.gov/Glossary/?id=159).

[13] The major distinguishing feature of this group is a subcellular structure known as the kinetoplast, a distinct region of the mitochondria (Wiser, 2013).

Leishmaniasis

The more than 20 species of *Leishmania* capable of causing leishmaniasis can be vectored by nearly 100 species of sand flies, according to Maguire. Cases have been reported on every continent except Antarctica and Australia, and about 1.5 million new infections occur each year. All except two species of *Leishmania* that infect humans are zoonotic, and most species tend to cause subclinical disease. Clinical leishmaniasis presents in three main forms: cutaneous, mucosal, and visceral. All three are treatable to some degree, he said (Antinori et al., 2012).

While *Leishmania* have existed for at least 80 million years, they have only coexisted with humans for several millennia—an association that has produced tremendous diversity, he continued. "This is still a very dynamic set of organisms," he observed. "They are emerging. They are reemerging. They are expanding their geographic range. This is a parasite that is definitely on the move."

Maguire presented the following examples to illustrate the spectrum of drivers that influence leishmaniasis' transmission patterns and geographic range:

- Cutaneous leishmaniasis, present in the Americas prior to human arrival, has recently exhibited increased incidence and broadening geographic range in response to human incursions into the forest (e.g., chicle harvesting) and the expansion of human settlement to formerly forested areas. Its complex transmission patterns involve multiple parasite and sand fly species, reservoir hosts, and varied ecology.
- Cutaneous leishmaniasis caused by a parasite that was once apparently sylvatic has become adapted exclusively to the domestic environment in a Brazilian community, with dogs and humans serving as its sole hosts.
- The range of locally transmitted cutaneous leishmaniasis in Oklahoma and Texas has moved northeast with increasing temperature, as predicted by models (Clarke et al., 2013). Similar predictions of expanded range with increasing temperature have also been borne out for visceral leishmaniasis in Europe.
- Most cases of cutaneous leishmaniasis in the United States have occurred among travelers, including significant numbers of military personnel. As a result, that sector has greatly advanced the prevention, diagnosis, and treatment of leishmaniasis, Maguire said.
- An apparently non-vector-borne outbreak of visceral leishmaniasis in a kennel of fox hounds in New York state spread to 18 other states as infected dogs traveled to participate in hunts. No human cases resulted, despite the presence in the area of a competent vector species.
- Visceral leishmaniasis in Brazil, long a rural disease, shifted around 1980 to a primarily urban or peri-urban disease, coincident with drought-induced mass migration from rural to urban areas. Epidemics occurred in several major cities, and incidence and geographic range increased, unabated by reactive spraying and campaigns to cull infected dogs (current

strategies under investigation include insecticide-impregnated collars for dogs, and a canine vaccine).

- At the same time, as HIV has moved into more rural regions of Brazil, co-infection with visceral leishmaniasis—which tends to produce severe disease—is on the increase. Moreover, co-infected individuals were found to be highly infectious to sand flies.
- In Europe, co-infection with HIV and leishmaniasis has occurred most frequently via shared needles, rather than insect bite.
- A recent large outbreak of leishmaniasis in Madrid was probably related to the development of a park within the city, which altered the ecology of hares, which served as a reservoir for the parasite (Carrillo et al., 2013).
- An explosive and lethal outbreak of visceral leishmaniasis occurred in East Africa during the early years of a civil war in southern Sudan, after refugees migrated through a region with high concentrations of sand flies.
- Eighty percent of visceral leishmaniasis cases occur in Bangladesh, India, Nepal, and South Asia, where humans are the sole hosts. Nearly extinguished by indoor insecticide spraying in the 1960s, leishmaniasis resurged in this region after malaria eradication efforts were abandoned.

Chagas Disease

Several species of blood-sucking triatomine bugs native to the American continents (but not the Caribbean) transmit *Trypanosoma cruzi*, which is known to infect over 100 species of mammals, Maguire stated. The first human hosts encountered and displaced the bug when clearing forests several thousand years ago. Some species adapted to the domestic environment and are today responsible for most human infections, he explained. About eight million people are permanently infected with this parasite that, decades after infection, can provoke life-threatening heart or gastrointestinal disease. Available treatments are "not satisfactory" and "toxic," and it is uncertain whether they prevent the development of heart disease, he noted (Rassi et al., 2010).

Maguire described several settings in South and Central America where he and coworkers had investigated transmission of Chagas disease since the 1960s. Each illustrated one or more factors that supported disease transmission. In one community, only about half of the population was infected—those who could not afford a house with plaster walls and a tile roof to prevent colonization by bugs. Other outbreaks coincided with the introduction of a new vector species as roads were built, and as religious pilgrims visited the area. Researchers accidentally introduced a domesticated vector prevalent in one region into El Salvador. The introduced vector spread through Central America along the Pan American highway and beyond, becoming more important in this new territory than the native vector for Chagas disease. Maguire recalled that when poor migrants from rural

areas to cities supported themselves by selling their blood, a major outbreak of transfusion-associated cases of Chagas occurred.

In the early 1990s, the six southernmost countries in South America—which accounted for the majority of cases in the hemisphere—collaborated in an effort to knock out parasite transmission by the major vector in the Southern Cone region, *Triatoma infestans*, and to end blood-borne transmission of Chagas disease. "It cost $30 million to $50 million dollars a year versus billions of dollars of economic losses from the disease," Maguire reported, and "it was incredibly successful." As a result, Uruguay and Chile are now free of parasite transmission, Brazil is free of transmission by *T. infestans*, and the other countries have only low transmission rates. Additional initiatives in the Andean and Central American regions used similar tactics to target different vectors of Chagas disease. Together, these efforts have lowered the prevalence of infection from as high as 18 million in the 1980s to its current level of about 8 million.

A number of obstacles stand in the way of eliminating Chagas disease altogether in these regions, Maguire noted:

- the re-infestation of houses with sylvatic *T. infestans* or other sylvatic triatomine bugs that can adapt to domestic environments;
- passive transport of alternative vectors from other regions;
- the development of insecticide resistance by vector species; and
- ongoing migration from rural to urban areas.

In the Amazon region, which is particularly rich in reservoir and vector species, outbreaks of acute Chagas disease have been traced to the ingestion of juice contaminated with vector feces, he noted.

Maguire observed that in the United States, there are as many people infected with *T. cruzi*—about 300,000—as in 8 of the other 20 countries where Chagas disease is endemic (Montgomery et al., 2014). An estimated 30,000 cases of chronic Chagas-related heart disease and hundreds of cases of congenital disease remain undiagnosed, he added. Nearly half the states in the United States are inhabited by several species of the triatomine bug vector and mammals—including dogs—that are heavily infected with *T. cruzi*. Both acute and asymptomatic disease have been reported, mostly among immigrants from South and Central America, but there have also been 23 locally transmitted cases, 5 transfusion-associated cases, and a single case of congenital disease, he reported.

A similar situation exists in Europe and Japan, where such nonvectorial routes of transmission have outstripped vector-borne Chagas disease, Maguire observed. This is increasingly true in South and Central America as well. To control transmission through this diversity of routes will require infected people to be identified, served by the health care system, and treated with effective drugs, he concluded.

Changing Paradigms for Tick-Borne Diseases in the Americas

Ticks rank second only to mosquitoes as arthropod vectors of medical importance, according to speaker Christopher Paddock of the CDC. In the United States, tick species in four genera—*Amblyomma*, *Ixodes*, *Dermacentor*, and *Rhipicephalus*—transmit the majority of human pathogens. Each of these has three different feeding stages and wide host ranges, so their potential to transmit zoonotic disease is "really tremendous," he said—and it has been increasingly realized in this country over the past century, during which nearly every recognized tick-borne disease emerged (see Paddock et al. in Appendix A8). "It has been a century of discovery and change," he observed. "There have been lots of newly recognized diseases. There have also been dramatic shifts in the incidence and distribution of certain historically recognized disease like Rocky Mountain spotted fever . . . [as well as] paradigm changes in terms of vector and ecology associations with these pathogens." In particular, he noted, human activity has profoundly affected tick-borne infections, which continue to shift in scope and magnitude.

As an example of this trend, Paddock described recent changes in the epidemiology of Rocky Mountain spotted fever (RMSF), recognized as the first tick-borne disease of humans in this country more than 100 years ago. Until a decade ago, only two vectors were believed to transmit the bacterial pathogen, *Rickettsia rickettsii*, the Rocky Mountain wood tick in the East, and the American dog tick—*Dermacentor variabilis*—in the West. Neither species inhabits Arizona, yet in 2003 a boy there died of RMSF, and soon afterward, 14 members of his small mountain community were found to be infected. Upon investigation, the brown dog tick—which typically does not bite humans but is present throughout the Americas and on other continents as well—was identified as the vector in these cases. The local population of those ticks had exploded, driving them to expand their host range.

This situation has been duplicated in other Arizona communities such that Paddock described it at this meeting as the "new normal" of RMSF epidemiology in the western United States. In 2011, for example, 77 cases of RMSF were reported in Arizona, including 6 deaths. Tragically, he added, children less than 10 years of age comprise the majority of RMSF cases vectored by the brown dog tick, because young children typically share dogs' habitats more than do other age groups. More severe outbreaks involving this vector have occurred in Northern Mexico, he added, resulting in significant mortality. "This may become a border health issue," he warned. Increasing numbers of cases are being reported from cities like Calexico, California, and Nogales, Arizona, involving people who traveled to endemic regions within Mexico and then returned to the United States, where their symptoms caused them to be hospitalized.

Over the last decade, additional rickettsial diseases have been identified as distinct from RMSF, Paddock added. Cases of milder *R. parkeri* rickettsiosis

(inferred to exist in 1948 by the eponymous R. R. Parker) have been confirmed in several states since 2004 (Cragun et al., 2010; Paddock et al., 2008). In California, discovery of the first cases of 364D rickettsiosis has led to the suggestion that this pathogen is behind most cases of "RMSF" in that state (Johnston et al., 2013). Another relatively mild—and distinct—rickettsiosis was recently identified in Brazil where RMSF is also present (Angerami et al., 2009). All of these rickettsioses respond to treatment with doxycycline, Paddock noted, but it is nevertheless important to distinguish one from another. "If you are going to accurately describe the clinical features and the epidemiology of these diseases you really have to know . . . what is causing them," he insisted.

An analogous situation exists with *Borrelia miyamotoi*, a tick-borne pathogen closely related to the Lyme disease bacterium, *Borrelia burgdorferi*, Paddock continued. Patients infected with *B. miyamotoi* can have antibodies that cross-react with *B. burgdorferi* antigens. *B. miyamotoi* was first associated with human disease in 2011, and has been detected in ticks in areas where Lyme disease is endemic in both the northeastern and western United States. The seroprevalence of *B. miyamotoi* infection among residents of New England, for example, has been determined to be as high as 4 percent. Its clinical spectrum remains to be determined, but the few cases that have been evaluated range from fever to very severe meningoencephalitis, he said. Meanwhile, novel clinical manifestations of Lyme disease have been identified, including sudden cardiac death among patients in their 20s and 30s (CDC, 2013).

The Heartland virus, previously described by Petersen as an emerging tick-borne disease of newly recognized importance, was first isolated in 2009 from two patients in Missouri who were initially suspected to have ehrlichiosis (McMullan et al., 2012). All confirmed infections with this virus that have occurred were in men older than 50 years of age, in whom it is a life-threatening infection, Paddock stated. Cases have been sporadically identified throughout the range of its vector, the Lone Star tick, he noted, so there are probably many more unrecognized infections. "It is going to be wherever this tick exists in the United States, which is also expanding in its range," he observed.

Explosive population growth among white-tailed deer has surely driven the expansion and emergence of tick-borne disease in the United States, Paddock remarked (Paddock and Yabsley, 2007). As many as 30 million deer now inhabit this country, as compared with an estimated 300,000 animals at the beginning of the 20th century. Deer are keystone hosts for the two most important vector species of tick-borne pathogens in the United States, namely black-legged ticks (*Ixodes scapularis*), known to transmit at least seven different human diseases, including Lyme, and Lone Star ticks (*Amblyomma americanum*), which carry Heartland virus and two species of *Ehrlichia* known to cause human disease.

THE PUBLIC HEALTH RESPONSE

Four speakers described the considerable challenges encountered by public health organizations in the Americas and Europe as they attempt to identify and adapt to changing patterns of vector-borne diseases.

Vector-Borne Disease Surveillance and Response in Latin America and the Caribbean

Many countries in the Americas are endemic to both vector-borne and neglected tropical diseases, which present similar public health challenges, according to speaker Luis Gerardo Castellanos of PAHO. PAHO, founded with the First General International Sanitary Convention of the American Republics in 1902, is the world's oldest continually functioning international public health agency. Today PAHO represents 35 countries that make up nearly one-third of the world's land mass, and 14 percent of its population. The organization also includes associate members, observer states, and participating states representing territories in the region. PAHO's initial mission of controlling epidemic diseases has broadened to include noncommunicable disease control, health education, and environmental improvements designed to help all people, especially those in need, he explained.

Yellow fever was the first vector-borne disease to be battled in the Americas, Castellanos noted. PAHO coordinated 11 countries in an attempt to eliminate yellow fever and malaria from the Panama Canal Zone, following identification of their common vector, *Ae. aegypti* (see section on "History and Current Challenges of Dengue Vector Control" for a detailed account of this effort and its aftermath). Currently malaria is endemic in 21 countries represented by PAHO, and more than 430,000 cases and 82 deaths were reported in 2013—a 64 percent reduction in cases since 2000, he reported. Fourteen member states are free of local malaria transmission today, he added, and according the WHO, seven additional countries may soon qualify as malaria free. PAHO has supported this progress by preparing strategic plans of action that, once approved by a country's minister of health, become binding and are documented by annual progress reports, he explained.

All four serotypes of DENV, also spread by *Ae. aegypti*, are present and causing disease throughout Latin America and the Caribbean, according to Castellanos. In 2003, PAHO undertook an integrated management strategy[14] to control the spread of dengue which, in turn, informed the WHO's Global Strategy for Dengue Prevention and Control, launched in 2012. That the Americas currently report more dengue cases, but lower case fatality, than any other global region he

[14] See http://www.paho.org/hq/index.php?option=com_content&view=article&id=4501&Itemid=41038&lang=en (accessed March 25, 2016).

attributed to "a very robust surveillance system across all countries" (see Castellanos in Appendix A4).

The emergence of chikungunya in the Americas in late 2013 was anticipated by PAHO which, in 2010, began developing preparedness plans for the Caribbean region in collaboration with partners including the CDC and the Institut Pasteur, Castellanos said. Although these plans were in place by 2012, as of November 2014, more than 900,000 suspected and nearly 16,000 confirmed locally transmitted cases of chikungunya have been reported in the Americas (PAHO, 2014b). PAHO and its partners have established a network of referral laboratories located in Argentina, Brazil, Cuba, French Guyana, and the United States to support the entire region in responding to this challenge.

In the meantime, PAHO continues to pursue elimination of several infectious diseases throughout the Americas, including onchocerciasis (river blindness) in Ecuador, which is soon to be declared free of the disease by WHO, according to Castellanos; Mexico and Guatemala are expected to gain that designation by 2016. He also noted the following vector-borne diseases expected to achieve elimination: trachoma in Mexico; lymphatic filariasis in Brazil; schistosomiasis in Suriname, the Dominican Republic, and St. Lucia; malaria in Argentina and Paraguay; and Chagas disease within several cities, provinces, or departments of Argentina, Columbia, Mexico, and Peru. Despite these gains, he observed, "Vector-borne diseases will continue to be a dynamic public health threat to countries in the Americas." Governments and international stakeholders must therefore commit themselves to preventing the further spread of these diseases, he concluded.

Vector-Borne Disease Emergence and Spread in Europe

The European Union is a hot spot for infectious disease emergence, and it is highly interconnected with other hot spots internationally, according to speaker Jan Semenza of the European Centre for Disease Prevention and Control (ECDC). Based on a foresight study conducted by ECDC in 2008, Europe is at risk of vector-borne disease threats owing to anticipated changes in drivers of infectious diseases by 2020. These predictions were validated in 2013 with an analysis of the infectious disease threats (and their contributing drivers) that occurred over the preceding 5 years that were identified by epidemic intelligence at ECDC. Semenza described these studies and their results in more detail (see Appendix A11) and elaborated on the ECDC's strategy to tackle these threats from vector-borne disease emergence.

For their prospective study of infectious disease threats to Europe through 2020, the ECDC first assembled expert panels to identify major drivers of infectious disease in the region, Semenza reported. These were determined to fall into one of three broad categories: globalization and environmental change; social and demographic change; and public health systems (Suk and Semenza, 2011). Based

on the results of an extensive literature review structured around these disease drivers, the researchers created scenarios to anticipate their influence on eight likely, significant near-term infectious disease threats to Europe as illustrated in Figure WO-10. Among them, introductions of vector-borne diseases or shifts in their transmission were predicted to be driven primarily by trends subsumed under the category of globalization, migration, and environmental change, as well as by social inequality (Suk and Semenza, 2011).

To validate the conclusions of this "thought experiment," as Semenza described it, the investigators conducted a retrospective analysis of ECDC epidemic intelligence data on infectious disease threats to European member states of the ECDC between July 2008 and December 2013. Among the 116 qualifying public health events they analyzed, nearly one-quarter involved vector-borne diseases, Semenza reported. Sixty-one percent of these vector-borne disease threats were attributed to global and environmental change, he added.

Given the disproportionate contribution of global and environmental change drivers to vector-borne disease threats in Europe, the ECDC turned to infectious disease experts for advice in crafting strategies to meet these challenges. Many of these experts considered climate change to be of particular concern as a driver of selected vector-borne disease such as Lyme disease, West Nile viral fever, tick-borne encephalitis, and leishmaniasis, Semenza stated (Semenza et al., 2012). The arrival and dispersal of tropical pathogens commonly associated with warmer temperatures is a potential threat to the safety of the blood supply. A ranking of emerging infectious diseases that can be a threat to substances of human origin (such as blood cells, tissues, or organs) in the European Union was compiled, based on an assessment of experts in the field (Semenza and Domanovic, 2013).

The majority of these infectious disease experts also expressed concern that their national disease surveillance and health systems were unprepared to deal with the effects of climate change on infectious disease dynamics, Semenza noted (Semenza et al., 2012). To prioritize surveillance improvements in light of these public health challenges, ECDC evaluated both notifiable and non-notifiable infectious diseases in terms of the strength of their link with climate change and the potential severity of their consequences to society, he said (Lindgren et al., 2012). Top-ranked diseases, in need of more surveillance activities, included Lyme disease, dengue fever, tick-borne encephalitis (TBE), Rift Valley fever, chikungunya, and leishmaniasis, he reported.

To address gaps in surveillance and preparedness, ECDC has built the European Environment Epidemiology (E3) Network. The E3 Network is built to monitor environmental precursors of epidemic events, including vector-borne disease outbreaks, to facilitate a more effective public health response, Semenza said. "We have compiled and processed a large number of environmental data that are now available for epidemiologic analysis such as prediction modeling," he explained. These data and prediction models are hosted at the E3 Geoportal (Semenza et al., 2013). The following examples illustrate how data from the

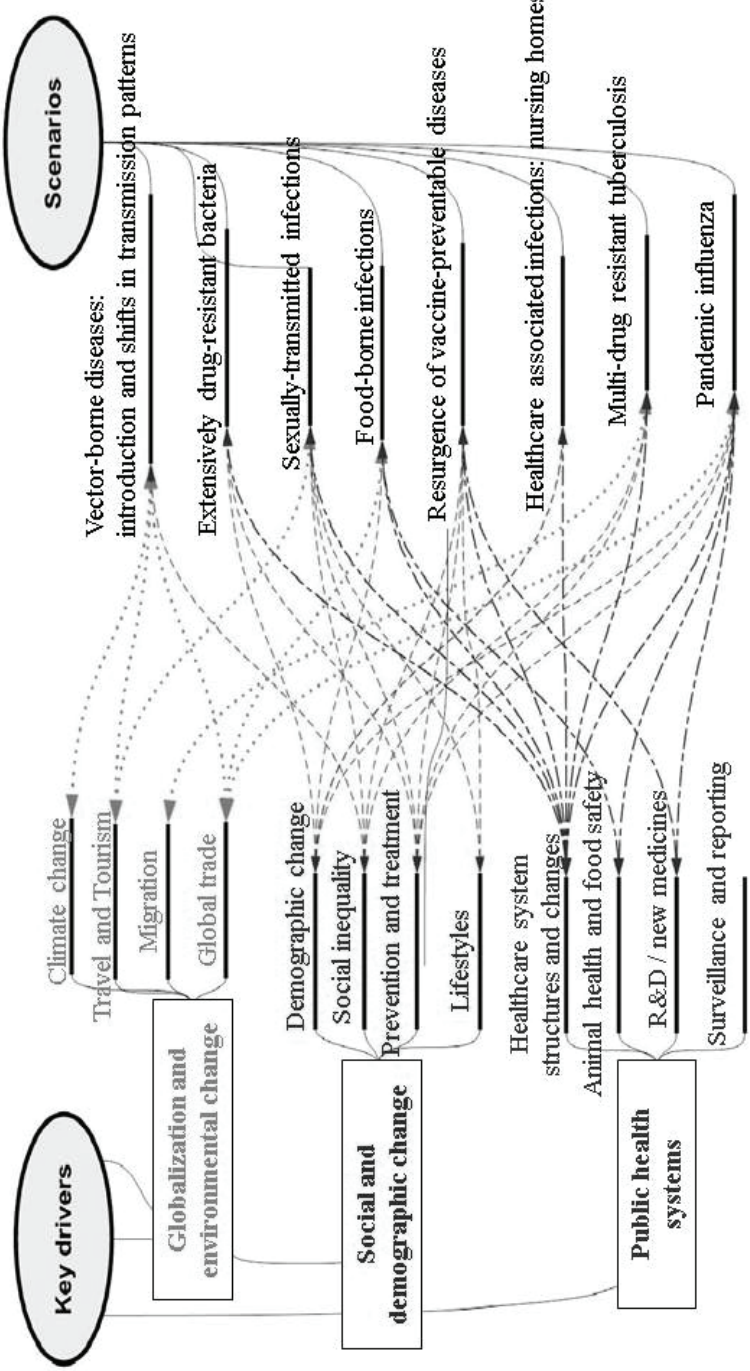

FIGURE WO-10 EID drivers and plausible scenarios.
NOTE: R&D = research and development.
SOURCE: Suk and Semenza, 2011. Reproduced with permission from the Sheridan Press, on behalf of the American Public Health Association.

E3 Geoportal have been used to inform effective public health action to address vector-borne diseases:

- Following the reintroduction and autochthonous transmission of malaria to Greece in 2009–2012, environmental data from the E3 Geoportal were used to develop a multivariate model to identify areas at risk for transmission based on environmental and climatic conditions. These insights were used to enable targeted pesticide spraying, dedicated surveillance, and public outreach. Disease transmission was subsequently interrupted, ending the outbreak in 2013 (Sudre et al., 2013).
- The risk for transmission of TBE in certain areas of southern Sweden was characterized with environmental data from the E3 Geoportal. By delineating these areas environmentally suitable for transmission, residents and tourists can be alerted to the risks and targeted with vaccination campaigns; the same approach could potentially be expanded to other areas at risk for TBE in northern Europe.
- The relationship between temperature deviations from the mean and WNV infections were assessed during the WNV outbreaks in Europe in 2010. Environmental and meteorological data (July temperature) from the E3 Geoportal were used to develop a predictive model of WNV that can now be used to predict outbreak areas in the future (Paz et al., 2013; Tran et al., 2014).
- The risk for dengue importation into Europe was modeled for 2010 with air passenger volume from dengue active areas internationally. These analyses can be used in the future to predict the airports most at risk and the timing of potential onward transmission in the destination country (Semenza et al., 2014).
- Similarly, these data were also used to identify spatiotemporal risk parameters for chikungunya importation into Europe from the Americas.

In addition to these applications of the E3 Network, the ECDC has compiled a handbook with practical climate change adaptation measures for infectious diseases: *Climate Change and Communicable Diseases in the EU Member States*[15] (Ebi et al., 2013). "By monitoring the environmental precursors of disease we hope to be able to help forecasting and predicting these patterns of disease emergence in order to enhance preparedness and reduce human and economic costs, particularly in resource strapped regions in Europe," Semenza concluded.

[15] See http://www.ecdc.europa.eu/en/publications/Publications/1003_TED_handbook_climatechange.pdf (accessed March 25, 2016).

Loss of Arbovirus Disease Surveillance Capacity in the United States

Before discussing the findings of a recent assessment of national capacity for arbovirus surveillance, speaker James Hadler of Yale University described the structure of public health surveillance in the United States. His review emphasized that surveillance for diseases of public health importance is a state function. Even in the case of nationally notifiable conditions, states collect and transmit information to the CDC as they see fit. He also noted that 80 percent of all surveillance by state health departments is federally funded (mainly through the CDC) and that, as a condition of funding, the CDC can require standardized surveillance methods and reporting.

Hadler noted that arboviruses such as WNV are of particular interest to public health—and therefore candidates for surveillance—for several reasons. Some cause severe morbidity and death; they are associated with large, rapidly developing outbreaks with the potential to overwhelm the health care system; they can be transmitted through the blood supply (or via organ transplant) as well as by insects; and both the infections and outbreaks they cause are potentially preventable if we know which arboviruses are present and the level of threat associated with them.

Before the emergence of WNV in the United States in 1999, no federal funding supported state or local surveillance for arboviral infections, which was limited largely to the voluntary reporting of human and animal cases of several types of encephalitis—and in several states was nonexistent, Hadler stated. That year federal funding for WNV surveillance was distributed from the CDC to the affected states through a cooperative agreement program known as Epidemiology and Laboratory Capacity (ELC). By 2004, WNV had reached every state except Washington, Alaska, and Hawaii, and ELC funding and guidance were extended to all 50 states and six major cities or counties. Also, by 2004, the ArboNET electronic national reporting system (previously discussed by Petersen) was collecting information from every state on avian mortality and surveillance of sentinel birds, horses, mosquitoes, and human infections. A 2005 Council of State and Territorial Epidemiologists (CSTE) survey of state and selected local health departments found that federal funding had enabled the development of broad-based, multisectorial WNV surveillance capacity in all states and recommended that states be permitted to expand the use of ELC funding to more broadly address vector-borne disease surveillance (CDC, 2006).

This recommendation was implemented, Hadler said, but as neuroinvasive cases declined rapidly after peaking in 2003, annual ELC funding for WNV surveillance gradually shrunk from $24 million in 2004 to $9 million in 2012, as shown in Figure WO-11. In 2012, however, neuroinvasive West Nile viral disease cases spiked to levels not seen in nearly a decade. “It was clear that WNV still had the potential to cause large-scale outbreaks, measurable not just locally, but nationwide,” he observed. In response to this development, and also to the threat of other emerging arboviruses such as DENV and CHIKV, CSTE—in partnership

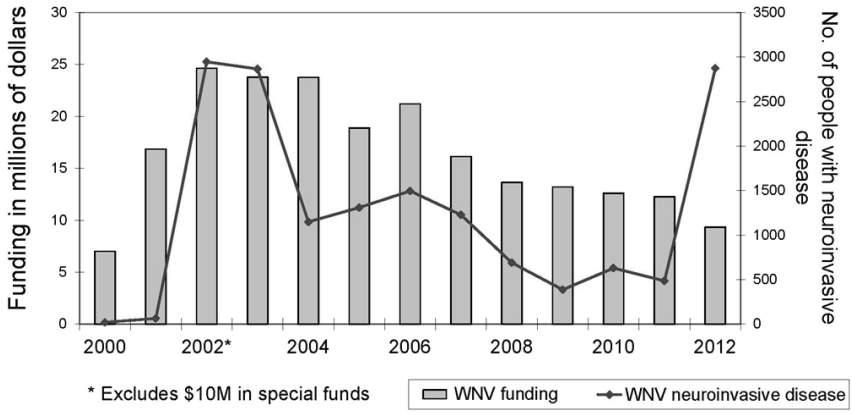

FIGURE WO-11 ELC funding support for West Nile virus surveillance and number of people with West Nile virus neuroinvasive disease, 2000–2012.
NOTE: WNV = West Nile virus.
SOURCE: Hadler et al., 2014 (CDC).

with state health departments and additional public health organizations—set out to examine how arbovirus surveillance capacity had changed through years of lean ELC funding. Their follow-up assessment found that since 2004, while nearly all states continued to conduct at least passive human disease surveillance, 22 percent of jurisdictions had stopped conducting active human surveillance, 13 percent had stopped mosquito surveillance, 70 percent had reduced mosquito trapping and testing, and 64 percent had eliminated avian mortality surveillance (Hadler et al., 2014).

Hadler reported that state surveillance capacity has decreased substantially since 2004, particularly with respect to conducting active human surveillance (including offering testing), mosquito surveillance, and laboratory resources. "From our perspective this has really reached a tipping point in some states," he said. Due in part to decreases in ELC funding, he observed, "The ability to rapidly detect emerging and outbreak level threats and thus initiate prevention activities has clearly been compromised." The CSTE report recommends that the CDC examine national arboviral surveillance capacity and secure additional support as needed to ensure mosquito surveillance occurs in all metropolitan areas with historically high West Nile viral disease burden (Hadler et al., 2014).

Hadler characterized current national capacity to conduct surveillance for vector-borne pathogens other than WNV as "very patchy," partly because of limited laboratory capacity at the state and local levels. CSTE estimates that a 50 to 60 percent increase in full-time employees will be required to enable state

and local health departments to meet their criteria for "full capacity" arbovirus surveillance (Hadler et al., 2014). According to Hadler, public health jurisdictions at "full capacity" for arbovirus surveillance meet the following three criteria:

1. They have the ability to complete a standard case report form on every suspected/confirmed case and report it to ArboNET.
2. They have the ability to test by IgM for all relevant arboviruses on any cerebrospinal fluid (CSF)/serum specimen submitted on a suspected case of arboviral disease.
3. They have a surveillance system that includes mosquito surveillance to routinely monitor both larval and adult arboviral activity in all parts of the jurisdiction in which there is the potential for human outbreaks of arboviral disease based on past experience.

In subsequent discussions, workshop participants considered how to address the issue of inadequate surveillance for mosquito-borne diseases in particular. Both Petersen and Margolis noted that while mosquito surveillance proved a good early indicator of outbreaks of West Nile fever, that is not the case for dengue, nor might it be for chikungunya. Petersen noted that surveillance for mosquito-borne diseases in the continental United States is limited largely to *Culex* mosquitoes, which are very different from the *Aedes* species.

Moreover, Petersen said, mosquito-based surveillance is useful for preventing or controlling outbreaks only if it generates a speedy response. "Really, we want to aim to build local capacity for mosquito-based surveillance. They are the ones making the decisions. They need to make them quickly." To this end, forum member Roger Breeze of Lawrence Livermore National Laboratory recommended that health departments take advantage of technological advances, such as multiplex PCR, to expedite pathogen analysis. "We are still stuck in a very 1990s paradigm, and bemoaning the fact that we don't have lots of people doing 1990s technology that you can do with a machine," he observed. He also noted that the Department of Defense is attempting to develop "a completely autonomous system to analyze what [pathogens are] . . . flying around in the mosquito and report to you."

Blood Donation Screening for Vector-Borne Diseases

Beginning with syphilis testing in 1938, and increasingly since the emergence of HIV in the early 1980s, blood donations in the United States have been screened for a growing number of pathogens, according to speaker Susan Stramer, of the American Red Cross. Many vector-borne disease agents have been shown to be, or suspected to be capable of, transfusion transmission, which is important because of the large, explosive nature of outbreaks caused by these agents. Few interventions for such agents are available, and treatments can be

costly with the development of new therapeutics likely to be slow, she observed. Thus, vector-borne diseases figure prominently among those infectious diseases deemed threats to the U.S. blood supply,[16] as determined by the AABB (formerly the American Association of Blood Banks). To estimate the magnitude of such a threat, she explained, researchers attempt to answer a series of key questions about the pathogen and the disease it causes, including

- Does the disease have an asymptomatic blood-borne phase, enabling donors who feel and appear well to transmit it?
- Does the pathogen survive through blood component preparation, distribution, and storage?
- How severe is the disease? What is the outcome in those who are immunosuppressed?
- Is the disease treatable?
- Is the pathogen present in the donor population? If so, is it increasing or decreasing?
- Does the public fear this pathogen (whether or not that fear is justified)?
- What intervention(s) would effectively protect the blood supply from this pathogen? (Dodd, 2012)

Such questions have been incorporated into models such as one Stramer described called the European Upfront Risk Analysis Tool,[17] which estimates transmission risk in blood. In her presentation to the workshop, she described recent and current attempts to assess and address the transfusion-associated transmission risk posed by several emerging vector-borne pathogens. Each of these pathogens, she noted, has been addressed on a case-by-case basis. Collectively, they illustrate the need to create decision-making processes for protecting the blood supply from the wide range of vector-borne pathogens.

Lessons from WNV

The response to WNV serves as a model of success in recognizing and preventing transfusion-associated transmission of a vector-borne disease, Stramer said. Although most WNV infections are asymptomatic, interventions introduced less than 1 year after the first transfusion-associated cases were identified greatly

[16] The August 2009 issue of *Transfusion* included a supplement on emerging infectious disease (Stramer et al.) agents and their potential threat to transfusion safety. Members of AABB's Transfusion Transmitted Diseases Committee identified 68 infectious agents and described them in detail, providing background information about each agent, along with a variety of assessments such as the clinical features of the agent and those characteristics specifically related to transfusion transmission. New fact sheets on emerging threats and updates to previously published fact sheets are also available. See http://www.aabb.org/tm/eid/Pages/default.aspx for details (accessed March 25, 2016).

[17] See http://eufrattool.ecdc.europa.eu.

reduced exposure to the virus, she stated. Continued refinement of testing procedures has further decreased "breakthrough" transmission. To date, more than 3,700 WNV-positive donations have been removed from the blood supply, she reported.

WNV taught stewards of the U.S. blood supply several important lessons, Stramer noted. It was their first experience in dealing with a transfusion-transmissible infection that was an acute, rather than chronic infection, like HIV, or hepatitis B or C viruses. While recognizing the potential of nucleic acid testing to provide rapid results, they also discovered that testing pooled blood samples—which can save both time and cost—may be insufficiently sensitive to low levels of virus, she said.

The DENV Conundrum

Like WNV, DENV frequently produces asymptomatic infections, Stramer observed. There is as yet no Food and Drug Administration (FDA)-licensed screening test for DENV. Investigational testing under way in Puerto Rico since 2010, however, has produced comparable results to established WNV protocols, she reported. In retrospective tests of more than 15,000 blood donations acquired at the peak of the Puerto Rican dengue epidemic, about 1 in 500 samples tested positive for DENV (Stramer et al., 2012).

On the other hand, only three clusters of transfusion-associated DENV transmission have been reported, in Hong Kong, Puerto Rico, and Singapore (updated data, however, indicate seven clusters). This does not appear to be an artifact of inadequate surveillance, Stramer said. She noted, however, that it is often difficult to distinguish mosquito-borne from blood-borne cases of dengue in developing countries, where even hospitalized patients may be significantly exposed to mosquito bites. It is also possible that either immunosuppression or the simultaneous receipt of antibodies to DENV in transfused blood may reduce apparent transfusion-associated infections, she added. Lastly, recognition of dengue symptoms in severely ill patients may be difficult against the background of underlying disease in the recipient. Alternatively, as Rico-Hesse suggested, amplifying factors in mosquito saliva may significantly increase the effectiveness of vector-borne transmission.

Responses to CHIKV

Like DENV, blood donated within the United States is not presently being routinely screened for CHIKV RNA, Stramer reported. However, unlike DENV infection, which CHIKV resembles in terms of the progress of viremia and antibody development, approximately three in four cases of chikungunya infection are symptomatic, she noted. This would tend to reduce the number of infected donors, and also theoretically make it possible to intercept donations from people

who report postdonation symptoms within a few days. To date, there have been no documented CHIKV transmissions associated with blood transfusions, but this may also be caused by the limitations noted above for DENV, she reported.

Blood screening and other preventive measures have been taken in response to epidemic chikungunya elsewhere in the world, most notably after an explosive outbreak on the Indian Ocean island of Réunion between 2005 and 2007, in which more than 40 percent of the island's inhabitants became infected with CHIKV. This viral strain—which acquired a mutation that increased its ability to replicate in *Ae. Albopictus*—was eventually introduced to northern Italy, and from there spread through Europe. Upon recognition of this introduction, blood collection was halted in at-risk areas of northern Italy, and in France, donors who had recently traveled to Réunion were deferred and nucleic acid testing for the virus was instituted, she said. The French also used this crisis to test a process known as platelet pathogen inactivation, which employs a broad-spectrum agent to prevent blood-borne transmission, which they found to be both safe and effective (Rasongles et al., 2009). The local collection of platelets, with an intervention, was required because of the short shelf life of platelets. Similar processes have since been shown to inactivate other pathogens, including DENV, in plasma and other blood products (Musso et al., 2014). Subsequent to this report (December 2014), the FDA cleared the process for use in the United States.

CHIKV was detected in blood samples from Caribbean donors within a few months of its emergence there (Gallian et al., 2014). Concerns about the blood supply in Puerto Rico, which has become endemic for CHIKV, and in the United States at large, have been discussed and are summarized below (Katz, 2014):

- Do nothing and watch, as we did before the emergence of WNV in summer 2002, responding if and when transfusion-transmission risk is demonstrated.
- Enhance our ability to identify the approximately 80 percent of donors who would be expected to have symptoms, by effectively eliciting callbacks by donors who get sick after a donation, so that we can recall their products.
- Understand donor travel and temporal donation patterns following travel, allowing us to model the effects of a short-term deferral for travel to affected areas. While operationally challenging, this may mitigate many acute tropical virus "sins." (Stramer added, "We can temporarily stop collections in areas where we see focal outbreaks"; however, this is not sustainable and is costly.)
- Engage our test builders to have "on-the-shelf" nucleic acid assays to detect CHIKV using available test platforms. (Stramer characterized this option as cost prohibitive.)

An option not listed above could use pathogen inactivation systems as used by the French in Réunion to inactivate CHIKV as well as other emerging arboviruses.

In August 2014, under administrative order by the Puerto Rican Ministry of Health, blood donation centers began asking potential donors whether they or anyone in their neighborhood had experienced either symptoms or a diagnosis of CHIKV and/or DENV, and they told donors repeatedly to report postdonation symptoms within 3 days (and in the case of platelet or plasma donors, to confirm their symptom-free status if contacted, or their donation would be discarded). This order was subsequently modified to allow the use of pathogen-reduction systems available as licensed or through treatment-use studies.

Like CHIKV, no transmission of the recently emerged Zika virus has been reported to date, even though it is very closely related to DENV, according to Stramer; however, similar interventions have been taken to prevent its transfusion-associated transmission. In Oceana, she observed, "They have multiple outbreaks ongoing simultaneously: there is Zika, dengue viruses -1, -2, and -3, and chikungunya [virus], so the three can occur quite successfully together." Because of these risks, several research blood donation screening interventions and pathogen reduction have been introduced in remote settings where importing blood components is not feasible, she explained.

Low Threat for Chagas

Few cases of blood-borne Chagas disease have occurred in the United States, Stramer reported, and blood-borne transmissions have only been documented by platelets, due to the fragility of the parasite (platelets are stored at room temperature and agitated to promote oxygen availability, likely enhancing survival of the parasite over their 5-day shelf life). Those transmission-associated cases that have occurred involved long-infected donors who came from endemic areas, she said. An extensive incidence study that followed over 4 million donors and greater than 6 million person-years of observation over the course of 4 years did not find any cases of incident infection, thus supporting a policy of selective testing involving testing each donor only once. The risk of missing a new case of infection was estimated 0.61 per million. Meanwhile, among more than 24 million donations screened between 2007 and 2014, the American Red Cross found about 1 in 36,000 positive donors, she stated.

Documenting and Preventing Transfusion-Associated Babesiosis

Of several tick-borne pathogens of concern to the blood supply, parasites of the genus *Babesia*—which infect red blood cells—are the most important, according to Stramer. General mortality for babesiosis, a malaria-like illness, ranges from 6 to 9 percent, but it is much higher for transfused recipients with underlying comorbidities and for other typically vulnerable patients, she observed.

There are hundreds of apparent transfusion-associated cases of babesiosis primarily confined to the northeastern United States and upper Midwest, she said, but fewer than 170 cases have been well documented. There is no FDA-licensed screening test for *Babesia microti*, the agent responsible for nearly all transfusion transmissions. Current interventions are limited to questions asked of donors, such as "Have you had babesiosis?" Potential improvements should not include questioning patients about their history of tick bites, since most donors do not know if they have been bitten, and if they have, which includes up to 9 percent of donors in endemic areas, such donors likely removed attached ticks during the grace period prior to *B. microti* infection. The only currently realistic intervention is to test all donations in endemic U.S. states for *B. microti* using both antibody and DNA tests. Investigational testing, including a retrospective study of donated blood, supports the use of these tests as an intervention against further blood-borne transmission of *B. microti* (Moritz et al., 2014).

ASSESSING AND ADDRESSING DRIVERS OF VECTOR-BORNE DISEASES

Three workshop speakers described diverse research efforts to investigate a spectrum of factors that potentially influence the transmission dynamics of vector-borne diseases, and to elucidate their mechanisms of action.

Weather, Agriculture, Climate, and Outbreak Patterns

Recent weather extremes have influenced agricultural production and created conditions conducive to outbreaks of certain vector-borne diseases, according to speaker Ken Linthicum of the U.S. Department of Agriculture (Anyamba et al., 2014; see adaptation, Linthicum et al., in Appendix A7). He described how he and coworkers investigated a series of extreme weather events between 2010 and 2012 that strongly affected agricultural production in major growing regions of Australia, East Africa, Russia, Southern Africa, and the continental United States—and where, sometimes simultaneously, outbreaks of vector-borne diseases (including dengue, Rift Valley fever, and West Nile virus disease) occurred. Using satellite data that track both vegetation density and land surface temperature, along with data on rainfall during the growing season, the researchers mapped anomalous conditions in these areas in detail, and compared these locations with places where major vector-borne disease outbreaks occurred during this period.

Linthicum and coworkers observed, for example, that when Texas experienced a 100-year drought in 2012, the overall vegetation index declined by 66 percent, production of cotton—a major crop—was cut in half, and a record-setting outbreak of West Nile virus disease erupted, he said. Drought in East Africa resulted in a loss of sorghum production at the same time as a large dengue

outbreak. Meanwhile, in areas where there was increased rainfall during this period, as occurred in southern Africa and southeast Australia, corn and cotton production increased coincident with outbreaks of Rift Valley fever in southern Africa, and Murray Valley encephalitis[18] outbreaks took place in Australia (Anyamba et al., 2014).

In addition to these acute, short-term impacts of weather anomalies, shifts in climate affect vector-borne disease patterns over the long term, Linthicum stated. There is a close link between the climate fluctuation phenomenon known as the El Niño/Southern Oscillation (ENSO)—as illustrated in Figure WO-12—and global rainfall anomalies.

Global patterns of floods and droughts influence the emergence, propagation, and survival of mosquito vectors and ultimately the transmission of mosquito-borne pathogens associated with diseases that include Rift Valley fever, dengue and dengue hemorrhagic fever (DHF), and chikungunya, he explained (Anyamba et al., 2012; see adaptation, Linthicum et al., in Appendix A7). The result, he observed, is "episodic patterns of disease outbreaks that are in tune with climate variability." For example, he noted:

- Hot and dry periods that occur during El Niño events in Southeast Asia have preceded significant peaks in DHF cases.
- Chikungunya outbreaks occurring between 2004 and 2010 were in some locations associated with extremely hot temperatures and/or drought, but in others with extremely wet conditions (Anyamba et al., 2012).
- In the Horn of Africa, recent outbreak clusters of chikungunya (2004–2006) were associated with severe drought, and Rift Valley fever (2006–2009) with heavy rainfall (Anyamba et al., 2012).
- *Plasmodium vivax* malaria reemerged in the Republic of Korea (post-eradication in the late 1970s) in 1993 during an extremely hot and dry period, and gained in incidence during subsequent periods of similar conditions (Linthicum et al., 2014).
- Global sea surface temperatures and rainfall patterns during the spring, summer, and fall 2014, and winter-spring of 2015, suggested that an El Niño event was imminent (see Figure WO-13). Figure WO-14 illustrates predicted regions of elevated risk for outbreaks of several vector-borne diseases if such an event occurred in 2014–2015.

[18] Murray Valley encephalitis (MVEV) is caused by a mosquito-borne virus that is found across Australia, Papua New Guinea, and Irian Jaya. MVEV is endemic to northern Australia and causes occasional outbreaks across southeastern Australia. 2011 saw a dramatic increase in MVEV activity in endemic regions and the reemergence of MVEV in southeastern Australia. This followed significant regional flooding and increased numbers of the main mosquito vector, *Culex annulirostris*, and was evident from the widespread seroconversion of sentinel chickens, fatalities among horses, and several cases in humans, resulting in at least three deaths. The last major outbreak in Australia was in 1974, during which 58 cases were identified and the mortality rate was about 20 percent (Knox et al., 2012).

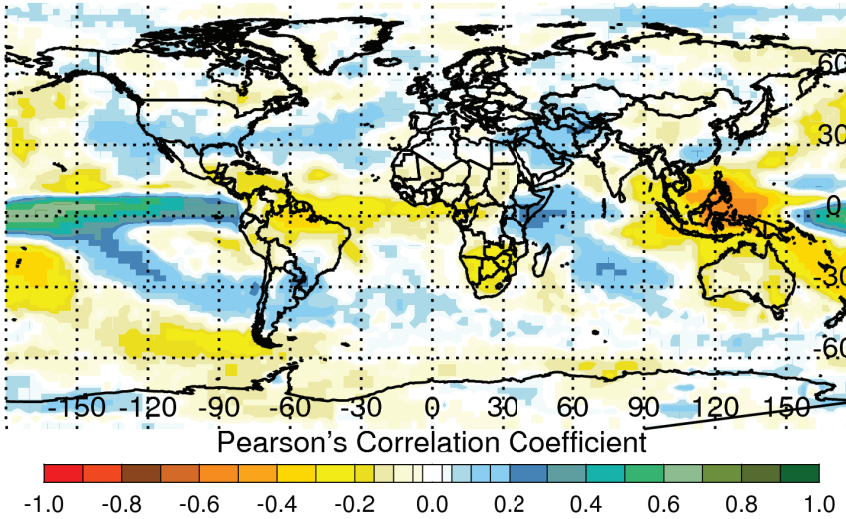

FIGURE WO-12 Summary correlation map between monthly NINO3.4 SST and rainfall anomalies, 1979–2008. Correlation of sea surface temperatures and rainfall anomalies illustrate ENSO teleconnection patterns. There is a tendency for above (below) normal rainfall during El Niño (La Niña) events over East Africa (Southern Africa, Southeast Asia). Similar differential anomaly patterns were observed for other regions, especially within the global tropics. These extremes (above or below) in rainfall influence regional ecology and consequently dynamics of mosquito disease vector populations and patterns of mosquito-borne disease outbreaks.
SOURCE: Anyamba et al., 2012. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

While extremes of temperature and precipitation have significant implications for the emergence and spread of vector-borne diseases, the magnitude of ENSO influence on some of these extremes cannot currently be predicted, Linthicum cautioned. Disease transmission dynamics in different environments and populations may vary widely, he observed, reflecting a broad spectrum of influences on vector species, vector population sizes, and vectorial capacity. Nevertheless, he continued, "There's obviously a need to invest in early ground surveillance during periods of unusual weather conditions"—including rapid field diagnostics for vector identification and virus isolation.

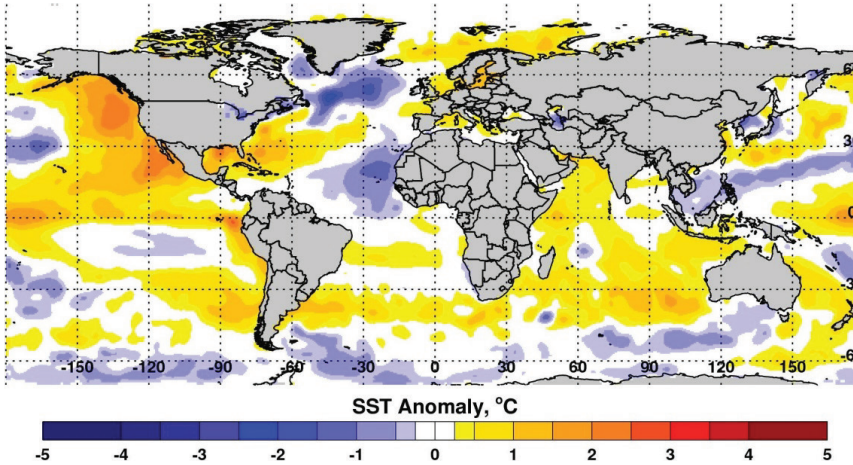

FIGURE WO-13 Global sea surface temperature anomalies for April 2015 expressed in degrees Celsius with respect to the 1982–2014 base mean period. Positive anomalies in the equatorial eastern Pacific Ocean are a manifestation of the late maturing 2014–2015 El Niño event and may portend continued El Niño conditions through the summer and fall of 2015.
SOURCES: NOAA, 2015; Reynolds et al., 2002.

Globalization, Land Use, Global Warming, and the Invasion of West Nile Virus

Continuing the discussion on WNV, A. Marm Kilpatrick of the University of California, Santa Cruz, described its emergence in the United States as a case study in the intersection of multiple disease drivers, including novel pathogen introductions, land use, climate change, and the evolution of pathogen, host, and vector, as illustrated in Figure WO-15 (Kilpatrick, 2011) (see also Appendix A6). While West Nile viral disease patterns are often characterized as complex, he noted, "I think it's actually our job as scientists to take that complexity and distill it down to those factors that matter the most." Hence in his presentation, he posed—and to a large extent, answered—a series of questions intended to accomplish this goal.

How might WNV have arrived in North America? There are five main vehicles for such zoonotic vector-borne pathogens, Kilpatrick stated: infected humans, wind-transported mosquitoes, human-transported mosquitoes (e.g., on planes or boats), human-transported nonhuman hosts (e.g., poultry), and migratory hosts (e.g., birds). Using mathematical models of these scenarios, Kilpatrick and coworkers projected that WNV was most likely to be introduced to Hawaii (Kilpatrick et al., 2004) and the Galapagos (Kilpatrick et al., 2006) by mosquitoes

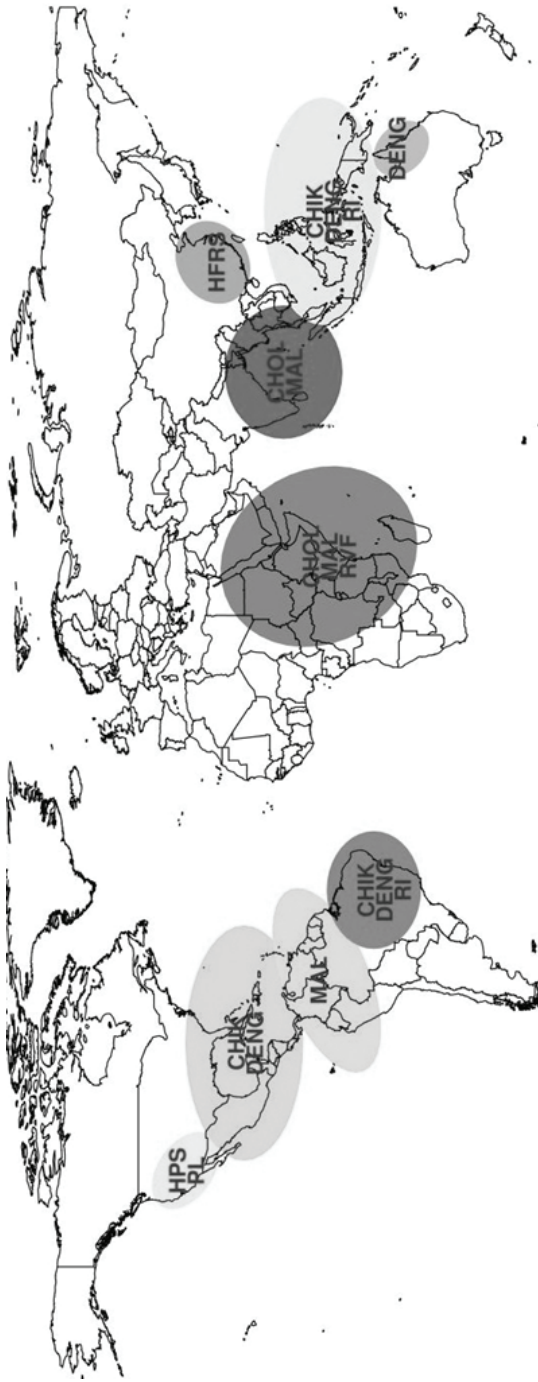

FIGURE WO-14 Potential El Niño regional teleconnections with patterns of vector-borne disease, rodent-borne disease, water-borne disease, and environment-linked respiratory illness patterns.
NOTE: CHIK = chikungunya; CHOL = cholera; DENG = dengue fever; HFRS = hemorrhagic fever with renal syndrome; HPS = hantavirus pulmonary syndrome; MAL = malaria; PL = plague; RI = respiratory illness; RVF = Rift Valley fever.
SOURCE: Chretien et al., 2015. Available from *PLoS Current Outbreaks* under Creative Commons license.

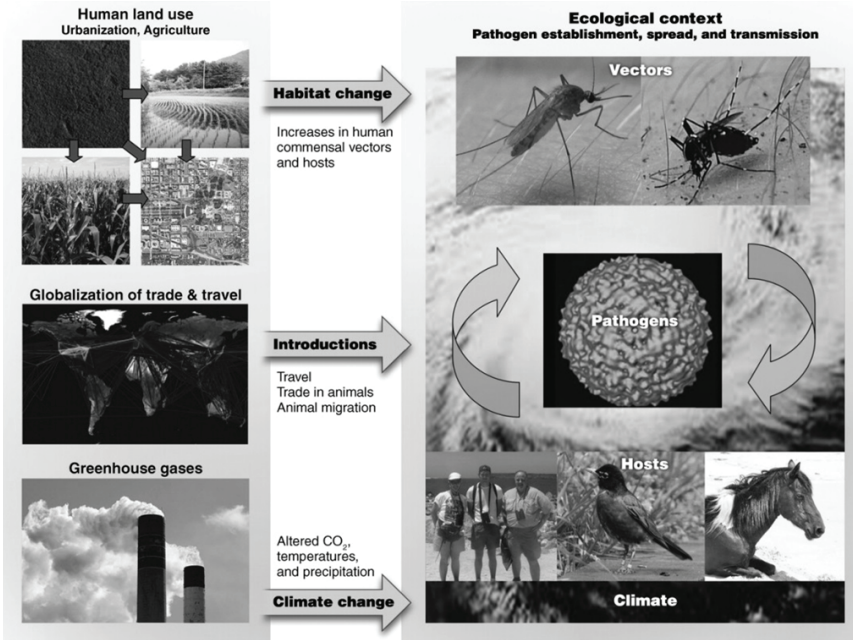

FIGURE WO-15 Anthropogenic processes that facilitate the introduction and establishment of novel pathogens and increase their transmission. Trade, travel, and animal movement introduce new pathogens. Climate, hosts, and the abundance and feeding ecology of vectors determine establishment and transmission intensity. Land use modifies animal communities that serve as hosts and vectors for pathogens, and climate change alters pathogen and vector demographic rates. [Image credits: Google and Tele Atlas (aerial photos); Edward Canda (rice paddy); Photos8.com (cornfield); L. Hufnagel (air traffic map); Dori (dori@merr.info) (smokestacks); Joe Hoyt (left mosquito); Andrew Flemming (right mosquito); Richard Kuhn, Purdue Department of Biological Sciences (virus); NASA (clouds); Marm Kilpatrick (others)].
SOURCE: Kilpatrick, 2011, © AAAS.

that arrived on airplanes, but that migratory birds would provide an easier entry for WNV into the Caribbean (Douglas et al., 2007), he explained.

Key WNV Vectors and Hosts

What are the important vectors and hosts for WNV in the United States? Taking a quantitative approach, Kilpatrick and coworkers determined that in New York—where the epidemic began—only two of the more than 170 species of North American mosquitoes—*Culex pipiens* and *Culex restuans*—dominated WNV transmission among both birds and humans (Kilpatrick et al., 2005). These

two species were similarly dominant vectors of WNV in the Washington, D.C., area (Kilpatrick unpublished data, 2004–2012); in Colorado, *C. pipiens* and *Culex tarsalis* are the primary vectors of WNV (Kilpatrick and Pape, 2013). Such information is crucial to targeted vector control, he observed.

Kilpatrick also noted several reasons for identifying the primary hosts of an introduced vector-borne disease: in order to direct wildlife vaccination or cull host species, should those measures be adopted; to predict hot spots for epidemic disease; and to map temporal-spatial variation in transmission. In the case of WNV, that means identifying which among hundreds of North American bird species are likeliest to transmit the virus. Their quantitative approach led them to the American robin, which proved both highly infectious to WNV, and highly preferred by mosquitoes, he reported (Hamer et al., 2011; Kilpatrick et al., 2006). Thus, he concluded, "It turns out that really there are relatively few mosquito [species] involved in any given place in West Nile Virus transmission, and even relatively few bird [species] involved as well, and I think that's actually quite good news." Kilpatrick quickly noted that he and coworkers found that WNV has evolved since its introduction to be transmitted more efficiently by mosquitoes (Kilpatrick et al., 2008), and also to more efficiently infect birds (Duggal et al., 2014).

Urbanization and WNV Transmission

Briefly summarizing a large body of research on factors influencing spatial variation in West Nile transmission, Kilpatrick noted abundant evidence that transmission intensity is higher in urban areas; however, he added, the mechanisms driving that pattern remain to be determined. His own investigations suggest that one reason is an increase of larval habitat for mosquitoes in urban areas, which increases the density of vectors that transmit WNV. Another reason is that vector species in the forests differ from those in urban settings, and the urban vector, *C. pipiens*, feeds on an especially infective host, the American robin, he stated.

This research also led Kilpatrick to conclude that spatial variation in WNV transmission must be understood on a smaller scale. In the Washington, DC, area, for example, he and coworkers found that mosquito traps placed as close as 100 meters will vary widely both in the number of mosquitoes they capture, and the percentage of those mosquitoes infected with WNV. Based on these observations he concluded, "The proper scale for analyzing transmission is probably in the tens of meters." Castellanos of the PAHO came to a similar conclusion based on PAHO's surveys of malaria transmission in Latin America. "We have made the analysis down to a house unit, and we have houses producing malaria repetitively and houses not producing malaria," he reported. On the other hand, Kilpatrick noted, there is significant variation at higher scales as well—encompassing different types of land uses, such as open fields versus parking lots. It may actually

be possible to predict patterns in WNV transmission at the small scale based on an understanding of both spatial and temporal drivers, he observed.

Environmental Influences on Malaria Transmission

Speaker Matt Thomas of Pennsylvania State University expanded upon the discussion of small-scale variations in vector-borne disease transmission by considering the effects of temperature. Far more immediate than the anticipated effects of climate change, daily temperature ranges and extremes in temperature influence pathogen transmission, he stated, and these effects are tempered by other environmental factors—claims he illustrated with examples from his work on malaria.

Diurnal Temperature Range

Malaria, Thomas observed, is the most important and longest studied of all vector-borne diseases, yet there remain "massive gaps in our knowledge." Taking what he termed a mechanistic approach, he began his remarks by explaining the components of vectorial capacity, a measure of the transmission potential of a vector population (a specific mosquito species, in the case of malaria). The vectorial capacity equation incorporates variables representing the density of vector species in a given area, their rate of biting and of feeding, their longevity as compared with the developmental period of the pathogen (the malarial parasite), and the degree of vector competence: in this case, how effectively the mosquito picks up the parasite, harbors it and supports its development, and transmits it to the host (humans or experimental animals).

Temperature has long been considered a key driver of malaria transmission because the ecology, physiology, and behavior of the mosquito vector—an ectoderm—are strongly influenced by variations in temperature, Thomas said. All of the previously noted contributors to malarial vector capacity are strongly and differently affected by temperature in nonlinear ways, he reported (Mordecai et al., 2013). Despite this fact, experiments examining the effects of temperature on various aspects of vector capacity have been conducted over a range of temperatures, but at constant temperature within each experimental cohort. For example, he said, researchers measuring the effect of temperature on mosquito development rate might measure that trait in separate mosquito populations in incubators set constantly at 20°C, 25°C, 30°C, and 35°C, to represent the possible range of mean monthly temperatures in the mosquito's natural environment. However, recent studies comparing malaria transmission within traditional mud huts and modern brick and tin homes in Tanzania found significant differences that were ascribable not to the mean temperature (which was largely equivalent between the two dwelling types), but to the diurnal temperature range, which could be much broader in the tin-roofed huts (von Seidlein et al., 2012).

As a result of this finding, Thomas and coworkers have been studying the effects of diurnal variation on vector competence in the Asian malaria vector *Anopheles stephensi*. Using incubators programmed to run at a constant temperature, or at that same temperature as its mean, but with variable diurnal highs and lows around it, the researchers monitored the development rate and survivorship of mosquito larvae (Paaijmans et al., 2013). The results, shown in Figure WO-16, reveal that while diurnal temperature variation did not have a significant effect on survivorship under optimum mean temperature conditions, similar variation under high average temperature conditions slows development, he reported—and "the bigger the daily temperature variation the worse things get," he said. "This is rather an important result," he added, because these effects would not be discernable in an experiment that did not feature variations in temperature. Moreover, he added, "You get the reverse effect at the cold end: temperature variation matters there too, but actually it makes things better." For example, he noted, at a constant temperature of 18°C, few larvae survive, but many more do if the temperature varies diurnally around that mean. Thus, he concluded, "You can't define the upper or lower limit for this mosquito's survivorship simply based on

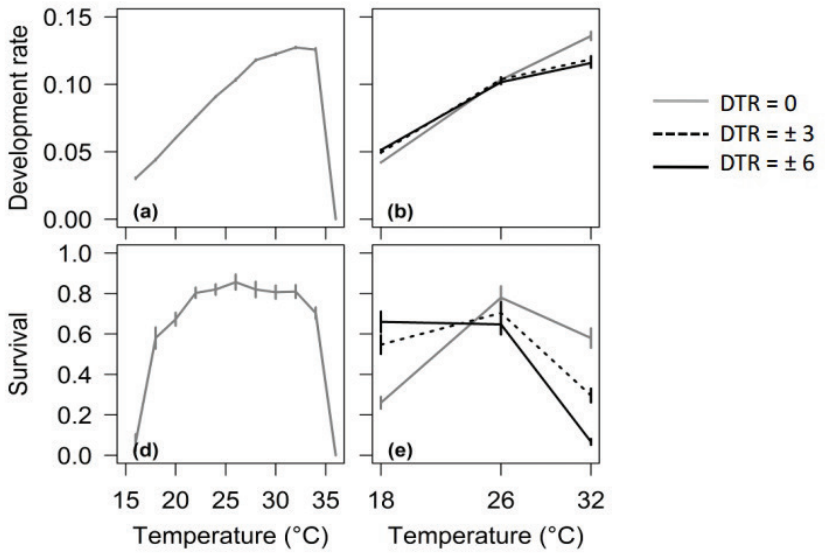

FIGURE WO-16 Influence of temperature fluctuation on larval development and survival of *Anopheles stephensi*.
NOTE: DTR = daily temperature range.
SOURCE: © Paaijmans et al., 2013. Published in *Global Change Biology*, John Wiley & Sons Ltd.

mean temperatures. [Yet] all the models, all the studies that we do, nearly all of them use mean temperatures." Those include recent, apparently promising attempts to stably infect mosquitoes with the endosymbiotic bacterium, *Wolbachia*, and thereby interrupt transmission of the malarial parasite to humans (Murdock et al., 2014).

Thomas also shared results of recent experiments examining additional effects of temperature on vector competence in *A. stephensi*, as well as in *A. gambiae*, the most important African mosquito vector for *P. falciparum*, the most important human malarial parasite. Given a meal of infected blood, they asked, how did temperature affect the proportion of mosquitos that became infectious? At the standard temperature for raising mosquito vectors of malaria, 27°C, about half of the mosquitoes of both species became infected, he reported; if the mean temperature increases, fewer mosquitoes become infected. Diurnal temperature range exaggerates this effect, and at high temperatures and broad range, inhibits infection altogether, they found. Thomas and coworkers found a similar pattern of temperature effects on the proportion of *A. stephensi* that actually harbored sporozoites in their salivary glands following an infected blood meal—the mosquitoes that, Thomas noted, "are the ones that can actually transmit the disease . . . the ones that are going to kill you." However, in this case, *A. gambiae* responded far less strongly to both mean temperature and to diurnal temperature variation.

It would be very informative to collect microenvironmental data in many different locations actually inhabited by mosquitoes, rather than in insectaries or other artificial settings, Thomas suggested—for example, within houses. "What's the temperature in the house? We don't actually know," he acknowledged. "We need to go out and do the leg work."

Larval Nutrition

The quality of habitat available to mosquito larvae can vary greatly within a short time (e.g., whether it is a wet or dry year) and also within a small geographic space, as the result of land use changes that may affect larval nutrition. Little work has been conducted to measure the effects of such variations in larval habitat quality on the ability of adult mosquitoes to transmit malaria, according to Thomas—a question he and colleagues are now attempting to address.

In their recently completed study of *A. stephensi* infected with *P. falciparum* malaria, larvae were raised in either "high-food" or "low-food" environments that differed threefold in the quantity of available food. Adult mosquitoes emerging from the "high-food" environment were significantly more likely than the "low-food" adults to be infected with the parasite, particularly at the sporozoite stage, Thomas reported. "That's just the vector competence component of the vectorial capacity equation," he noted. The investigators also measured the impact of food availability on additional variables including vector density, biting rate, survival, and how quickly the malaria parasite developed within them. When compared on

the basis of complete vectorial capacity, they found a 45-fold difference in transmission potential between the two groups, favoring the high-food mosquitoes.

Could these findings be applied to vector control? Perhaps, Thomas remarked, in the sense that habitat manipulation strategies—already a keystone of integrated vector management—should target larval habitats. However, he added, the main purpose of these nutritional studies is to better understand how changes in the quality of larval habitats influence patterns of seasonality in malaria transmission.

Small Changes with Large Effects?

In contrast to climate change, with its event horizon measured in decades, the environmental changes Thomas described can happen overnight, he observed—and their impacts on the risk for vector-borne disease transmission can be dramatic. Very few of the many modeling and empirical studies that have been conducted in an attempt to predict future patterns of disease, or to explain current variations in transmission risk, take such short-term effects into account, he observed.

Reflecting on earlier speculation by Kilpatrick that many small-scale effects on transmission either cancel each other out or amount to little more than noise as compared with a very few important influences in a given disease system, Thomas observed that without measuring such effects, we cannot be certain of their magnitude. Moreover, he later noted, "If we're going to understand local transmission and understand better the consequences of change in a local context, then we really need to think about understanding the local ecology and the sympatric pairings between those vectors and the parasites."

Perspectives on Disease Drivers

Global Change and Transmission Risk

In the discussion that followed these presentations, and also in comments raised in earlier sessions, several participants expressed concern that disproportionate attention was being paid to the potential influence of climate change on transmission risk for vector-borne diseases, and particularly for West Nile viral disease, where evidence for climate and temperature effects appears particularly thin. "Are we really focusing on the right type of global change by doing so much work on issues around climate change, when really the impact of WNV in the United States may vary with temperature, or it may not?" asked forum member Peter Daszak, of EcoHealth Alliance. Instead, he suggested, it seems that trade routes, travel, globalization, and land use change issues may be the most potent drivers of vector-borne disease transmission over the next two to three decades.

Kilpatrick agreed, asserting that the total number of West Nile viral disease cases driven by changes in either temperature or precipitation is orders of magnitude lower than those driven by changes in land use—and that the same could be said about many vector-borne diseases, because land use changes increase humans' exposure to biting vectors and, thereby, to disease. Climate does have an impact on transmission, he explained, and climate change may increase transmission in certain circumstances, such as at the geographical limits of vector distribution (Siraj et al., 2014). Mercedes Pascal's group has been looking for climate links to malaria and other pathogens for a while, and the message that comes relatively clearly from both that work as well as the larger body of work is that climate at the distributional edges of a pathogen or a vector can have a huge role in changing the geographic distribution of a disease, but in the middle of a pathogen's range it appears that other variables are much more important (Gething et al., 2010; Rogers and Randolph, 2000; Siraj et al., 2014).

So, for example, Pascal's article was suggesting that at the upper altitudinal limits of malaria, climate could drive it up and down for Lyme disease. There has been some nice work by Nick Ogden and candidates showing that in fact the vector is kind of moving north more in warmer years than other years, and there are a number of cases like that (Ogden et al., 2008).

Many environmental parameters are changing faster than the climate, Linthicum acknowledged, "But I think we have to also keep in mind the long-term impacts of climate change," he advised. Thomas described his work with climate modelers to attempt to anticipate how transmission pattern effects of diurnal temperature range might change with predicted shifts in mean temperature. While there seems to be a narrow range of responses to a mean change in temperature, it is unclear whether that change will occur equally across the temperature spectrum, he observed. "Perhaps we've done a pretty good job with the climate models," he suggested. Far less is understood about how environmental changes predicted by climate models would affect pathogens, vectors, hosts, and their interactions, Thomas said. Solid empirical data are needed to characterize these relationships.

From Models to Mitigation

Impressed with the variety and depth of models of vector-borne disease transmission risk, and with Linthicum's Rift Valley fever model in particular, forum member Julie Pavlin of the Armed Forces Health Surveillance Center asked to what extent these models were being translated into actions that benefited public health. The responses she received were mixed. While Linthicum expressed frustration at initially having tried and failed in part to initiate timely responses from public health and agriculture officials to mitigate predicted Rift Valley fever activity that then occurred, they later were able to achieve better communication and ultimately better response. Kilpatrick said that some of his

group's work had been used by many local health departments to guide vector control activities.

Thomas noted that implementing studies of malaria transmission risk is very challenging. "Early warning systems might be useful in terms of allowing for some level of preparedness," he explained, but studies of transmission drivers might more productively be used to analyze trends in malaria cases in order to determine the effects of specific preventive measures, such as bed net use on transmission rates, separate from environmental variables, including seasonal precipitation (Aregawi et al., 2014).

What does one do when there is disagreement among multiple models of transmission risk for a vector-borne disease? Both Kilpatrick and Thomas recommended that all models be tested with rigorous local studies. "I personally won't be confident in the mechanisms we think are driving . . . [variations in vector-borne disease transmission] until we have both broad scale correlational patterns and local-scale studies that support the actual mechanisms," Kilpatrick asserted. For example, he noted, links between remotely sensed climate and human cases of malaria are well established, but the mechanisms that connect those phenomena have not been defined. "If you can actually open up that black box and show that . . . when we have a higher temperature that does lead to an earlier transmission season, higher mosquito abundance, higher mosquito infection rates, and then more human cases, then I'll start to believe," he said. "Short of that I think we're just waving our hands, and we can get it completely wrong."

On the other hand, Linthicum noted, such high-resolution information may not be necessary to have a significant impact on public health. In Africa, for example, where Rift Valley fever occurs over very large geographic areas affecting many hundreds of thousands of animals, "There's no point in becoming very specific; what you need to do is to warn people when those risks are going to be elevated," he argued, "and then there are a number of things that could be done on a large scale to really mitigate that [threat]."

"One could spend forever doing elegant research with exquisite temperature fluctuations in the lab," Thomas imagined, "but ultimately we need to get that out in the real world, we need to have it . . . inform practice." In the real world, there will be broad patterns and significant variation and context dependence, he observed. Only by examining what actually happens, by analyzing case histories, can we discern the most important drivers of transmission risk in a given situation. He therefore advocated in favor of focal studies of disease transmission at sentinel sites, with the goal of trying to identify and understand the drivers involved and their interactions, and to gauge the effectiveness of possible interventions. "The best way of progressing is to learn by doing," Thomas insisted.

NOVEL APPROACHES AND INTERVENTION STRATEGIES

In the final workshop session, speakers reviewed past efforts to address vector-borne diseases and described a range of strategies and methods to tackle key obstacles in their prevention, diagnosis, and treatment.

History and Current Challenges of Dengue Vector Control (Or, Why Did Gorgas Succeed? And Why Have We Failed?)

Vector control has an important role in addressing vector-borne diseases, observed speaker Paul Reiter of the Institut Pasteur. In recounting the history of this approach, he began with Carlos Finlay's hypothesis that yellow fever is transmitted by *Aedes aegypti*. This was experimentally confirmed on the arrival of Walter Reed. With this knowledge, William Gorgas[19] had spectacular success in eliminating yellow fever from Havana, Cuba, and later during the construction of the Panama Canal. Fred Soper, director of the Pan American Sanitary Bureau (later PAHO) between 1947 and 1959, implemented Gorgas's approach throughout Latin America (Johns Hopkins Bloomberg School of Public Health, 1991)—a contribution to public health that Reiter deemed "quite exceptional."

Fast-forward to the mid-1970s in Singapore. From the late 1960s until the mid-1980s, dengue—also transmitted by *Ae. aegypti* and once a major cause of illness in that country—had been drastically reduced by vector source reduction but began to rise thereafter until it became a major public health problem once again. As consultant to the Singapore government, Reiter hypothesized that suppression of the disease had been so successful that half the population was now nonimmune; i.e., the herd immunity had been greatly reduced in the host populations so that, even in low numbers, mosquitoes were now more efficient in transmission. "We made certain recommendations, and things looked like they were getting better," he recalled—until a massive epidemic struck in 2005. Again Reiter was consulted, along with Duane Gubler ("sort of the emperor of dengue epidemiology worldwide"), and after their suggestions were implemented, case numbers declined—"until 2013, when, despite major control efforts, dengue suddenly took off again," he reported.

Singapore spends some $60 billion a year on *Ae. aegypti* control yet incidence continues to rise. For decades, government sources state that Havana (about the same size as Singapore) was the only country in the New World that was free of dengue but despite official statistics it is well known that dengue is rampant there. In truth, there is nowhere on this Earth where dengue is under control. . . . If they can't do it in Singapore, no one is going to be able to do it with the weapons that we have at present.

[19] Later Sir William Gorgas. Although an American citizen, he was knighted by King George IV for his achievements and given a funeral in St. Paul's Cathedral upon his death.

Why not? Reiter outlined his response in the form of this poem, entitled "Ode to *Ae. aegypti* Control":

> Those golden days . . . .
> The perfect way . . . .
> Let us-(s)pray
> The mess today!

Source Reduction

The "golden days" began with Gorgas, whose military pursuit of *Ae. aegypti* through "source reduction"—the elimination of breeding sites—purged Havana of yellow fever within 5 months, Reiter stated. Mosquito habitats were eliminated by various means: water storage vessels and wash basins were covered, water in horse troughs was exchanged regularly, gutters were made to drain properly, and so on. The results were certainly impressive but it is important to note this was an entirely different era from today: breeding sites were much less common; there were few motor vehicles, so used tires, a classic mosquito breeding site, were absent; cities were much smaller; and there were no plastics or other disposable items that could serve as water collection breeding sites. Moreover, fear of yellow fever increased acceptance of the intrusive measures used to enforce control, he added.

When Gorgas was subsequently assigned to combat yellow fever and malaria during construction of the Panama Canal (mosquito-borne disease was an important reason why the French canal project had failed), he first attempted to rely on the insecticide pyrethrum to do the job, Reiter said. Three attempts failed before he returned to source reduction, with which he finally achieved success.

Insecticides

Insecticide treatment would not become "the perfect way" to combat mosquito-borne diseases until after World War II, when DDT became available. Soper employed it to eradicate *Ae. aegypti* from 22 countries in less than 10 years, or so it has been claimed, Reiter noted. Certainly, dengue and yellow fever transmission was brought to a halt.

With the banning of DDT, beginning in the 1960s, a new era of insecticide-based vector control began. While DDT had been applied directly to infested containers and their immediate (50 cm) perimeter, post-DDT insecticides were broadcast as aerosols—"let us spray"—by hand-held foggers, road vehicles, or aircraft, Reiter stated. *Ae. Aegypti,* however, is an indoor mosquito; this, among other behavioral traits, may explain why spraying has not effectively reduced the diseases they carry (Reiter, 2007). Nevertheless, he pointed out, "There's a noisy machine with a nasty smell with a big loud noise and the flashing lights.

So that really persuades people that they're being looked after." But, he added, "the bottom line is that many countries are trying to control dengue, and they fail . . . if you look at any of the public health data it's absurd to say that we are actually controlling it."

Now What?

Our cities are huge, human populations are dense and mobile, public health funding is scarce—"It's a perfect paradise for the mosquito," Reiter lamented. It is often difficult to access areas that should be treated to reduce populations of *Ae. aegypti*, insecticide resistance is a problem, and public participation in cleanup campaigns is inadequate to achieve source reduction, he noted. How does one face "the mess today?"

There are a lot of things we don't know about the biology and ecology of *Ae. aegypti*, Reiter observed, and that missing information may provide routes to effective vector control. For example, he said, "We don't know how many [water] containers we have to reduce in order to stop transmission, how many mosquitoes, the comparative economics of the different approaches to control, or their sustainability." According to his group's mathematical models, a 90 percent reduction in the numbers of mosquitos would still produce very little on the overall transmission rate for dengue.

The same model demonstrated that only a few mosquitoes could effectively transmit dengue in a human population that has low herd immunity (see Figure WO-17). This, he concluded, reveals why Gorgas and Soper succeeded where we are now failing: their effective reduction of the vector occurred in human populations with high herd immunity (Reiter, 2014).

If that is indeed the key to successful dengue control, we need to better understand how to build and exploit herd immunity, Reiter insisted. "I don't believe that vector control on its own is going to be the answer, even though I'm a medical entomologist. But I do believe that augmentation of the herd immunity by vaccination, in combination with vector control, may prove more effective than either approach on its own."

Reiter was emphatic that new and novel approaches to vector control are urgently needed. These may include a return to focal insecticide application; the use of *Wolbachia* to reduce mosquito infectivity, as mentioned by Thomas; and the use of juvenile hormone mimic—a compound that disrupts mosquito metamorphosis, and which can be distributed by female mosquitoes among multiple breeding sites. In his opinion, however, the method that shows the most promise is based on transgenics: males of a strain of *Ae. aegypti* that carry a dominant lethal gene are released to mate with "wild" females but the gene ensures that the resulting progeny cannot survive to adulthood. Studies in a number of countries have demonstrated remarkable results.

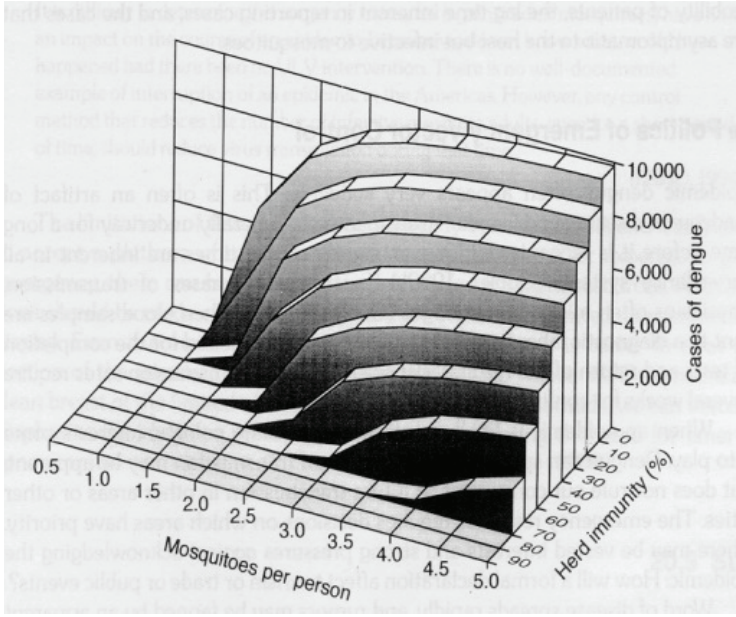

FIGURE WO-17 Vector density, herd immunity, and dengue transmission. Effective dengue control will reduce herd immunity, thereby increasing the transmission efficiency of residual mosquito populations.
SOURCE: Reiter, 1992. Reproduced with permission from CAB International.

The Public Health Imperative for Improved *Ae. aegypti* Control

According to Barry Beaty from Colorado State University, "it has been humankind's great misfortune to share time and space with *Aedes aegypti* (see Figure WO-18). Following domestication of this mosquito in Africa, it traveled with humans throughout the tropical and subtropical world (Powell and Tabachnick, 2013). Beaty describes *Ae. aegypti* as the Norway rat of mosquitoes. Because of its intimate association with humans in homes, schools, and work places and its extreme preference for feeding on humans, it is an excellent vector of yellow fever, dengue, and chikungunya viruses, and this mosquito has caused and continues to cause inestimable morbidity and mortality in humans (García-Rejón et al., 2008, 2011). The same biological and behavioral attributes that allowed domesticated *Ae. aegypti* to colonize the tropical world via sailing ships have served the vector well in the modern era.

Aedes aegypti is uniquely adapted to the modern urban environment, exploiting new breeding sites such as septic systems and storm drains, and it has proven intractable to sustainable control in large urban areas. Classically used control methods such as environmental source reduction and space spraying have not

sustainably stemmed the pandemics of dengue and chikungunya. Indeed, *Ae. aegypti* is essentially now hyperabundant throughout the tropical world, and notably in areas where it had previously been eliminated or greatly reduced in abundance (Gubler, 2011). Large urban areas, with their sizeable *Ae. aegypti* populations, are now very receptive to the introduction, spread, and trafficking of arboviruses (see Beaty et al. in Appendix A3). The introduction of chikungunya virus in 2013, and its explosive spread throughout Latin America (Nasci, 2014) in a very short time, is testimony to the epidemic potential of *Ae. aegypti*–transmitted pathogens. Unfortunately, our ability to intervene in such epidemics is likely to worsen.

The alarming and rapid emergence of pyrethroid resistance in *Ae. aegypti* threatens the efficacy of many of the chemical control efforts for this important vector. The emergence of knockdown resistance (kdr) in *Ae. aegypti* in Mexico (Garcia et al., 2009) has been mirrored in *Ae. aegypti* throughout much of the tropical and subtropical world. Similarly kdr has exploded in *Anopheles gambiae* threatening the efficacy of long-lasting insecticide-treated nets for malaria control. Pyrethroids are also the insecticide of choice for control of vectors of other globally important diseases such as Chagas, lymphatic filariasis, and leishmaniasis, and they are widely used by public health agencies for control of other insect vectors, such as for those that transmit West Nile virus, and other insect vectors of globally important pathogens. The evolution of kdr has been associated with dramatic increases in metabolic resistance in important vector species. Evidence

FIGURE WO-18 *Aedes aegypti* feeding on a human.
SOURCES: James Gathany/CDC, 2006.

is accumulating that pyrethroid resistance is becoming operationally significant, which could lead to the loss of this key class of insecticides in the armamentarium for vector control.

According to Beaty, this would be a public health catastrophe on the order of emerging antibiotic resistance in bacteria and parasites. For the foreseeable future, chemical insecticides will remain critically important for controlling vector populations. Development of new, environmentally sensitive insecticides with new modes of action from existing insecticides is a public health imperative. One novel approach in this regard has been the development of the Innovative Vector Control Consortium (IVCC). The IVCC partners with industry to develop new insecticides with different modes of action than pyrethroids, which will permit rotational or mosaic applications of insecticides to minimize development of resistance and to provide improved stewardship of existing and new insecticides. New insecticides as well as new innovative strategies for vector control are needed, including insecticide resistance blocking strategies (Devine et al., 2009), *Wolbachia* interruption of DENV transmission by *Ae. aegypti* mosquitoes (Moreira et al., 2009), genetic strategies to reduce vector populations (Black et al., 2011), and other innovative approaches to be vigorously pursued to prevent and control *Ae. aegypti* transmitted diseases. The burden of these diseases is too great to bear, and it is clearly time to declare "war" on *Ae. aegypti* and to sustainably control this enemy of humankind.

Strategies for Malaria Eradication

As recently as 1998, it seemed quite unrealistic that malaria could be eradicated, according to speaker Alan Magill of The Bill & Melinda Gates Foundation. That year, malaria killed two million people and infected half a billion. Resistance had developed to key therapeutics, and insecticide-treated bed nets—a preventive measure recently proven in clinical trials—were not yet widely distributed. Epidemic HIV in East Africa led to co-infection, increasing the burden of both diseases.

By 2013, however, the picture had changed drastically (White et al., 2014). As compared with 1998, acute malaria cases had declined by about 60 percent, and mortality by nearly 70 percent, Magill reported. Massive increases in donor funding spurred the implementation of new preventive measures—long-lasting insecticide-treated bed nets and indoor residual insecticide spraying—along with rapid diagnostic tests and targeted interventions for vulnerable populations. This turnaround demonstrates how adequate resources in support of effective tools can produce significant gains in public health, he concluded—and it offers hope that the next step, the eradication of malaria, can be achieved by both applying current interventions continuously and at high coverage, and by applying current interventions with new strategies. "There's really no backing off on this," he insisted.

But Magill also noted daunting challenges to continued progress against malaria, including emerging resistance to drugs and insecticides, and maintaining current levels of support of funding required for malaria eradication. The disease has been controlled many times in many locations, but resurgence predictably has occurred because efforts were not sustained (Cohen et al., 2012). Therefore, he argued, global eradication is the only permanent solution for malaria. Moreover, he pointed to evidence that complete eradication has been stable in 46 out of 50 countries where it has been achieved (Smith et al., 2013). "The trick here is getting to zero," he emphasized. "What often happens is you get to very low levels, you have many residual pockets of transmission, and if you don't actually finish the job then resurgence is pretty much inevitable." Taking that final step from control to eradication is both difficult and expensive, he added, "but if you really do get parasites out of people . . . then maintaining that state is a little easier."

Interrupting Transmission

In 2007, Melinda Gates described the foundation's commitment to eradicating malaria as a quest for equity. "It's about a recognition that those areas of the world that suffer from malaria really can't get ahead," Magill explained. The debate as to whether poverty causes malaria or vice versa isn't useful, he added, since eradicating malaria will surely advance some of the world's poorest people.

Malaria has three possible futures, Magill observed: resurgence, control (sustaining and slowly improving progress against the disease to date), or accelerating toward eradication. To achieve the latter outcome, the Gates Foundation has defined a strategy called Accelerate to Zero, intended to focus current and future tools in an intensive effort to interrupt malaria transmission. The cornerstones of this strategy are the detection of the human parasite reservoir, the elimination of that reservoir, and the effective prevention of transmission, he explained. "If you can't cure people and prevent transmission concurrently, then both of those approaches will ultimately fail," he insisted.

Because the malaria parasite biomass resides almost entirely in humans, it must be diagnosed and treated in infected people in order to be reduced, Magill stated. Thus the Gates Foundation supports current efforts to test, treat, and track malaria infections. Every person who presents with a fever in a malaria-endemic country should have a reliable diagnostic test, and if proven to be infected,[20]

[20] In the discussion that followed this session, forum member Lonnie King of The Ohio State University asked what would be done for those patients who tested negative for malaria. Magill responded that unfortunately, there are not good point-of-care diagnostics for other common febrile diseases, such as Q fever and leptospirosis; however, an initiative is under way to address nonmalarial febrile diseases collectively, with antibiotics for suspected or confirmed bacterial infections, and supportive care in other cases. Moreover, he said, even people who test positive for malaria may actually be suffering from another infection. Forum member Gerald Keusch, of Boston University, noted that a rapid diagnostic test capable of differentiating between malaria and pneumonia in children was shown to reduce the use of antimalarial drugs by about two-thirds, with excellent survival rates.

get treatment with best available therapy, he said—and then, their case should be tracked along with others in a surveillance system. However, not all malarial infections are symptomatic, he noted; "This is the classic iceberg . . . there is a vast reservoir of infected people out there in the community who are happily carrying their parasites and their gametocytes, and they are going on in this uninterrupted circle of transmission with their mosquito vectors, and we're doing absolutely nothing about that today" (Lindblade et al., 2013). To address this problem, the Gates Foundation advocates the complete cure of asymptomatic fathers and mothers as a way to save their children's lives. This will demand new rapid diagnostic tests that can identify asymptomatic people in communities, as well as mass "screen and treat" campaigns, he said.

These efforts will be most effective if they are targeted toward communities that serve as sources for widespread malaria transmission, Magill continued. The Gates Foundation supports efforts to map malaria transmission patterns, analyses to determine the most strategic areas to focus treatment efforts, and the creation of databases for use by ministries of health in affected countries, he reported.

The goal of malaria eradication is the interruption of transmission, Magill emphasized. But today, while many people with malaria get treated and recover, they may continue to carry viable *P. falciparum* gametocytes for the next 4 to 8 weeks, and therefore continue to transmit the disease. "There's never been a single attempt to actually interrupt transmission by targeting the gametocyte," he asserted. "What we need is what we call complete cure, which is complete parasitologic cure. We need a drug regimen that will not just make you better . . . but we also need them to get rid of the parasites that transmit." Thus, he said, the Gates Foundation is working with partners to develop drugs that will kill malaria gametocytes. Two lead candidates "have extremely significant and very promising transmission-blocking and gametocidal effects," he reported; ultimately, it is hoped that they can be delivered as a single pill, along with drugs that cure clinical disease—and that this would be achieved for both *P. falciparum* and *P. vivax* malaria.

Situational Solutions

In 1937, malariologist Lewis Hackett observed,

> Everything about malaria is so molded and altered by local conditions that it becomes a thousand different diseases and epidemiological puzzles. Like chess, it is played with a few pieces, but is capable of an infinite variety of situations.

Recognizing the enduring truth of this depiction, the Gates Foundation supports a variety of means to tackling malaria, and to applying them as targeted, locally adapted solutions, Magill explained. For example, despite the deployment of insecticide-treated bed nets and indoor residual spraying of insecticides, significant residual malaria transmission occurs as mosquito populations adapt both

genetically and behaviorally, he reported (Killeen, 2014). Vector control could, in theory, reduce these problems, but rigorous field trials are rarely conducted on these measures, he noted (Vontas et al., 2014). To meet this need, the Gates Foundation initiated and supports the UK-based IVCC,[21] a group of experts who partner with agrichemical companies worldwide to develop novel insecticides to address a broad range of vector-borne diseases.

The pairing of insecticide-treated bed nets with artemisinin-based combination treatment for malaria has proven extremely effective in reducing cases, as shown in a long-standing epidemiological study in Senegal (Trape et al., 2014). However, resistance to artemisinin has emerged in Southeast Asia (Ashley et al., 2014), while in Haiti, where the primary vector for malaria is the outdoor-dwelling mosquito *Anopheles albimanus*, bed nets are not a useful preventive measure. There, in partnership with PAHO, The Bill & Melinda Gates Foundation is working to eradicate the disease through the identification of transmission hot spots, with a combination of focal indoor residual insecticide spraying and drug treatment to eliminate the human parasite reservoir, Magill said.

The Gates Foundation also supports vaccine development, but with the emphasis on preventing infection to interrupt malaria transmission, rather than targeting disease prevention, Magill stated. An antidisease focus for vaccination could actually enable the continuation of asymptomatic parasitemia, he asserted.

In conclusion, Magill emphasized that eradicating malaria will require new concepts, tools, and strategies, and an end to a "one size fits all" approach to addressing this complex and varied disease. "The next decade will be a period of intense experimentation and learning, leading to a rapidly evolving policy environment for new tools and technologies," he predicted.

Transgenic Insects for Vector Control

Effective control strategies for arboviral diseases can target their insect vectors, observed speaker Luke Alphey of the United Kingdom's Pirbright Institute. He described work under way to create and deploy genetically modified *Ae. aegypti* mosquitoes, designed to reduce dengue transmission by this species, which also transmits the chikungunya and Zika viruses (see Alphey et al. in Appendix A2).

The number of people becoming infected with dengue each year is now approaching that for malaria, Alphey noted, although far fewer people die of dengue. There are no drugs that specifically treat it, nor are bed nets effective deterrents, since the mosquitoes that carry the virus do not bite at night. Thus dengue control has focused on reducing mosquito populations—with varying degrees of success, as previously described by Reiter.

[21] See www.ivcc.com (accessed March 25, 2016).

As an alternative to vector control through source reduction or insecticide treatments, genetic strategies involve changing the mosquito genome so as to spread a repressible developmental defect that would interfere with reproduction or a modification that would interfere with disease transmission, Alphey explained (Alphey et al., 2010). This is not a new idea, he noted: irradiated sterile insects have been used for more than 50 years, on very large scales, to control several agricultural pests. Since then, several additional genetic control strategies have been developed that could be used to combat dengue or other mosquito-borne diseases (see Figure WO-19).

There are two possible goals of genetic control strategies, Alphey explained: to reduce the size of a vector population or to change it in a way that reduces disease transmission—that is, by somehow reducing vector competence throughout the population. The genetic changes introduced to accomplish these goals may either be self-limiting—they will eventually be washed out by lethality or by natural selection—or they may be self-sustaining, becoming established in the target vector population and possibly spreading to other populations, he added. Genetic control strategies share several key features, Alphey stated, including the fact that they protect every person within the area where they are deployed. By way of contrast, human-directed disease control programs often disproportionately favor the wealthy, powerful, and educated. All genetic control strategies exploit insect mate-seeking behavior to disperse the control agent, and also to ensure species-specific effects. Thus, he reassured the audience, "We are not talking about eliminating all mosquitoes."

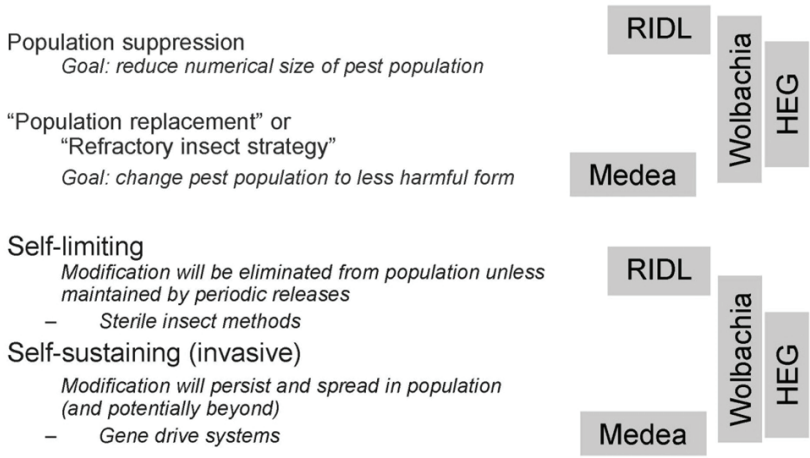

FIGURE WO-19 Classifying genetic control strategies.
NOTE: HEG = homing endonuclease gene; RIDL = release of insects carrying dominant lethal.
SOURCE: As presented by Luke Alphey on September 17, 2014.

RIDL Technology

Alphey's presentation focused primarily on a genetic control strategy known as "release of insects carrying dominant lethal" (RIDL), which features genetically engineered male mosquitoes carrying repressible dominant lethal transgenes that are released to mate with wild females, producing doomed progeny (Alphey, 2014b). For example, he described one transgene with a promoter that is expressed only in female flight muscle tissue and which, when passed to the female offspring of genetically engineered male mosquitoes, compromises the females' ability to fly (Fu et al., 2010). "Of course flightless mosquitoes can't survive in the wild," he observed. "They can't move away from the breeding site, they can't find a host, they can't avoid predators. Actually they can't mate, even in the laboratory." Male offspring, on the other hand, can fly and mate, and thereby spread the lethal gene through the population.

Alphey reported that another type of RIDL system—in which the repressible transgene kills all of the progeny from the engineered father and wild mother (Phuc et al., 2007)—has proven effective in suppressing *Ae. aegypti* populations throughout a series of phased tests, culminating in a successful field release in the Cayman Islands, which achieved an 82 percent reduction in the target mosquito population (Harris et al., 2012). The researchers have also conducted field trials with RIDL mosquitoes in Malaysia, Brazil, and Panama, he added. In Brazil, Alphey observed, target mosquito populations in different settings were reduced by 94 to 99 percent.

To gain public support for these potentially controversial experiments, the researchers used informal presentations in a variety of settings—door-to-door visits, television, radio, and print media—to explain their work and to demonstrate its safety. "Dengue control is widely recognized as desirable, and it's also recognized that current methods aren't adequate and new methods are needed," Alphey said, explaining the high levels of public approval these projects received. At the time of the workshop, a proposal for a field trial in the Florida Keys was under consideration, he added. While there has been some objection to this trial by environmental activists, an independent pollster found that 61 percent of residents questioned supported the use of genetically engineered mosquito technology, as compared with 18 percent who did not (Florida Keys Mosquito Control District, 2013). Moreover, 81 percent of respondents considered genetically engineered mosquito technology safe, as compared with 73 percent who considered the use of chemicals and insecticides to be safe.

Appropriate Application

While not a "magic bullet," RIDL technology is appropriate for certain disease vectors in certain settings, Alphey said. For example the two mosquito species *Ae. aegypti* and *Ae. albopictus* (for which RIDL technology has also been developed)—which spread DENV, CHIKV, and Zika, among other pathogens—could be

specifically targeted with the technology. *Ae. aegypti* eggs are dry and easily stored, he added. "They could just be shipped out in packets for people to rear at the bottom of their yard." Likewise, Kilpatrick's narrowing of the mosquito species most likely to transmit WNV suggests that RIDL could also be brought to bear on that disease.

Because *Ae. aegypti* and *Ae. albopictus* are alien invasive species in the Americas, "If you could eliminate them, then that might be seen as ecologically desirable rather than undesirable thing," Alphey observed. Indeed, he added, when *Ae. aegypti* was eliminated from about 20 South American countries during the DDT era, no adverse ecological effects were reported. On the other hand, a native vector species could serve an important ecological role; in that case, he advocated using methods that make vectors less able to transmit pathogens but without reducing their populations. "There are different options, and you look at this on a case-by-case basis," he concluded.

Exploiting Virus–Vector Interactions

Arthropod-borne plant pathogens—and plant viruses in particular—present a major threat to global food security, according to speaker Anna Whitfield of Kansas State University. The viruses she studies, tomato spotted wilt virus (TSWV) and maize mosaic virus (MMV), are related to viruses that infect animals, and their transmission cycles resemble those of other vector-borne animal and human viruses such as CHIKV, she noted. TSWV and MMV are acquired by their insect vectors when they feed on infected plants. The viruses then infect the insect's gut, and eventually move to its salivary glands, where they replicate, and from which they are transmitted to naïve host plants during feeding (Blanc et al., 2014).

Each step in this transmission cycle can potentially be disrupted, Whitfield said; thus her group and other researchers are exploring many opportunities to control plant disease (see Whitfield and Rotenberg in Appendix A12). "We don't view these strategies as a silver bullet," she explained. Managing plant diseases generally requires an integrated approach; therefore, she characterized the methods she described as "just another tool in the toolbox for plant production."

Viral Acquisition

TSWV, a *Bunyavirus*, is related to RVFV and other members of the genus *Hantavirus*, Whitfield noted. TSWV is globally distributed, has an exceptionally wide host range of more than 1,000 plant species, and annually contributes to more than $1 billion in losses of crops that include tomatoes, peanuts, and peppers. There is no single effective control strategy for TSWV, as it easily overcomes genetic resistance bred into crop plants, and its thrips vector develops resistance to pesticides. By pursuing the interruption of TSWV transmission by molecular means, she and coworkers are trying to provide another tool to add to their current integrated pest management system.

Whitfield described a glycoprotein known as G_N that projects from the membrane surface of TSWV and mediates viral attachment to the thrips vector's midgut tissue. The researchers made G_N in soluble form, fed it to thrips, and found that it not only specifically bound their midgut tissue, but that it inhibited TSWV transmission—presumably by blocking its binding site, she explained. They then made transgenic plants that expressed the G_N protein, infected them with TSWV, and let thrips feed on them; compared with insects fed on equivalent nontransgenic plants, the thrips fed on G_N plants had significantly lower TSWV titers and rates of viral transmission, she reported (Montero-Astúa et al., 2014). The transgenic G_N plants sustain an initial infection with TSWV, she said, but because they are expressing the viral attachment protein, they block subsequent transmission of the virus. "We think that these could be a promising tool for control of TSWV spread from secondary infection," she concluded.

A similar strategy has been demonstrated in another vector-borne plant virus system, Whitfield noted. Bonning and coworkers (2014) produced transgenic plants that expressed a soluble luteoviral coat protein fused to a spider toxin. Aphids fed on these plants internalized the chimeric protein as if it were a virus, delivering the toxin to its body cavity and killing the insect. This approach provides much-needed options for controlling aphids, a major transmitter of plant viruses—and it could be applied to other virus–insect systems, employing a range of potential toxins (Whitfield et al., 2014).

Viral Dissemination and Transmission

Whitfield's laboratory is also exploring vector proteins that interact with TSWV and MMV, enabling these viruses to traverse multiple barriers and reach the vector's salivary glands, where they replicate and from which they are disseminated. The researchers have identified a suite of proteins consistent among members of the *Rhabdovirus* genus like MMV and among members of the *Tospovirus* genus like TSWV that appear to interact with or respond to the insect vectors of several types of plant viruses, she reported. "These are the type of proteins that we would like to follow up on by targeting and disrupting vector acquisition and transmission," she stated.

RNAi for Vector Control

Viral-vector interactions could be further exploited as a means of vector control through the use of RNA interference (RNAi),[22] Whitfield observed (Kupferschmidt, 2013). Research in several different insect species has

[22] The term RNA interference was coined to describe a cellular mechanism that uses the gene's own DNA sequence to turn it off, a process that researchers call silencing. In a wide variety of organisms, including animals, plants, and fungi, RNAi is triggered by double-stranded RNA (dsRNA). http://www.umassmed.edu/rti/biology/how-rnai-works (accessed August 12, 2016).

demonstrated the effectiveness of this method for silencing expression of the crucial enzyme, vacuolar ATPase (v-ATPase) (Yao et al., 2013). When Whitfield and coworkers delivered double-stranded RNA fragments either orally or by microinjection to nymphs of the insect vector of MMV, the corn planthopper *Peregrinus maidis*, it experienced higher rates of mortality, as well as reduced egg production (apparently due to abnormal development of female reproductive organs). Similar decreases in survival and fecundity were achieved with thrip vectors when treated with dsRNA of v-ATPase (Badillo-Vargas et al., 2015). Studies are currently under way to treat plants, including crops threatened by citrus greening disease, with double-stranded RNA to control its insect vector, the Asian citrus psyllid (*Diaphorina citri*).

RNAi techniques have also been developed to control arthropod vectors of animal viruses, Whitfield noted. For example, Kang and coworkers (2014) showed that silencing v-ATPase and another gene (inhibitor of inosine-5′-monophosphate dehydrogenase) in *Ae. aegypti* not only reduced mosquito survival and egg production, but it also suppressed host factors for DENV-2, thereby decreasing infectivity. The successful results with RNAi to silence the v-ATPase genes of plant and animal disease insect vectors highlight not only the similarities in basic vector biology but also that of emerging vector control strategies. These commonalities suggest that increased communication and collaboration between investigators working with plant and animal vectors could be beneficial for the control of vector-borne diseases.

Outlook for West Nile and Chikungunya Vaccines

Vaccines represent the most cost-effective means of controlling many infectious diseases, and they offer significant prevention against zoonotic vector-borne diseases for which humans serve as a dead-end host, observed speaker Thomas Monath of Hookipa BioTech AG and PaxVax, Inc. Repeated vaccination of humans can prevent them from contracting such diseases, which he said cannot be eradicated due to the size or persistent infection of animal reservoirs.

Figure WO-20 provides an overview of the state of vaccine development for vector-borne diseases. “Yellow fever vaccine is probably the closest to a silver bullet that we have,” Monath said. “The Nobel Prize was awarded for it, and it really has driven that disease nearly to extinction, though it still remains a threat.” He highlighted vaccines against WNV and Japanese encephalitis virus as good examples of prevention against diseases that pose an ongoing threat to human hosts. By contrast, he noted, vaccines against vector-borne pathogens that can be transmitted by humans, such as dengue and chikungunya, could potentially contribute to the eradication of those diseases.

Focusing on vaccines to prevent West Nile viral disease and chikungunya, Monath suggested that lessons learned in the process of developing the WNV vaccine could inform the development of vaccines against other emerging

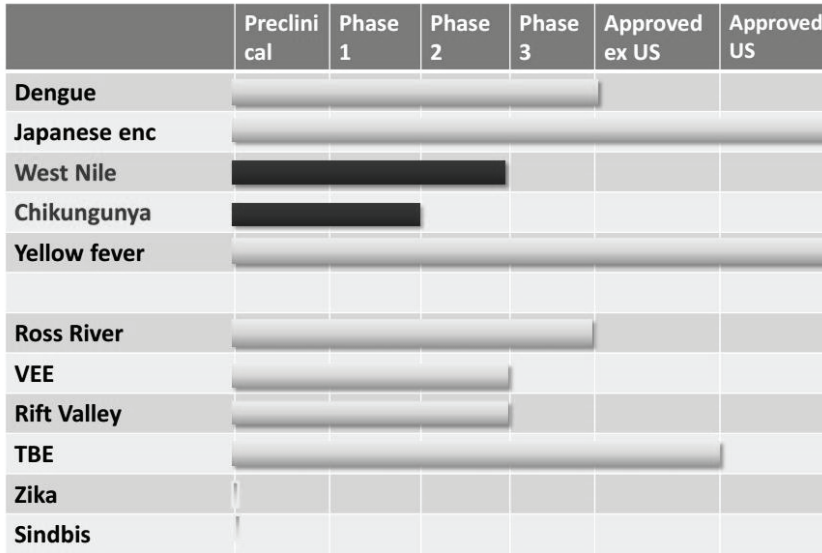

FIGURE WO-20 Vaccines against vector-borne diseases with potential for introduction and spread into the United States.
NOTE: TBE = Tick-borne encephalitis; VEE = Venezuelan equine encephalitis.
SOURCE: As presented by Thomas Monath on September 17, 2014.

pathogens—most notably CHIKV, with which WNV shares several common threads, as shown in Box WO-2.

A Vaccine Against WNV?

The first vaccine initiative against WNV started within months of its emergence in New York in 1999, Monath recalled. "The first vaccine for horses was approved in 2001," he said. "By that time there were at least six companies engaged in vaccine development for humans," he added—all of which have since halted their efforts. Today, only the National Institutes of Health (NIH) continues to pursue a vaccine against WNV—and without industry involvement, he expressed doubt that such a candidate would reach advanced stages of development.

Technical obstacles to developing a WNV or CHIKV vaccine are minimal, Monath stated. "These are fairly straightforward targets." The real problems, he continued, are economic: uncertain market size, high development costs, a challenging regulatory pathway, and the risk that the disease will be controlled by natural immunity before the vaccine is developed (a possibility for any emerging infection, he pointed out, and for chikungunya in particular).

BOX WO-2
West Nile and Chikungunya Common Threads

- Cyclical or intermittent epidemics
- Absent for a long period and then reappear in explosive form
- Changing geographical distribution, rapid spread
- Overwintering, transovarial transmission
- Potentially severe, life threatening
- Sequelae, chronic disease syndrome in 20–30 percent
- Threat to blood supply
- No specific treatment
- Regulatory pathway challenging
- Uncertain recommendations and sustainable market for vaccines
- High cost of vaccine development, limited access to capital

SOURCE: As presented by Thomas Monath on September 17, 2014.

Given these hurdles, who is developing vaccines for emerging diseases? "It's mostly biotech companies," Monath said. "They're cash constrained," he noted, and to pursue a vaccine, they must invest a significant amount [of capital] without guarantee of return—which tends to discourage investors. Due to this reality, small companies with candidate vaccines have partnered with large ones to defray the expenses associated with late-stage development. Large pharmaceutical companies, Monath observed, have limited interest in vaccines. "They were, as you saw, engaged in West Nile at the beginning, but they've put a tiny fraction of their treasure to work on these kinds of targets, and they're basically watching and waiting to see what happens," he noted.

Monath was part of the effort to develop a WNV vaccine at his former employer, Acambis, now owned by Sanofi Pasteur. Their product, ChimeriVax-WN, is a live, attenuated, virus vaccine based on the yellow fever vaccine, which they produced within months of the 1999 outbreak (Dayan et al., 2013). A single inoculation provided long-lasting immunity in nearly everyone who received it, he reported. It was shown to be safe, even in elderly people, and it couldn't be transmitted by mosquitoes (and thus would not stimulate antivector immunity). "It really looked like an ideal vaccine," he observed.

In 2001, Acambis began advanced preclinical studies in nonhuman primates and demonstrated the vaccine's effectiveness in horses, he said. The horse vaccine was licensed in 2002 to a large company, Intervet. With Phase 1 clinical trials completed, ChimeriVax-WN was manufactured at final scale by 2004—but by then, the epidemic had peaked, and soon afterward, market surveys revealed

a public wary of a live viral vaccine for a disease they and their physicians perceived as low risk. Acambis also learned that primary care physicians, who would be primarily responsible for administering the vaccine, would likely not be mandated to provide it. Nevertheless, in 2007, Sanofi licensed ChimeriVax-WN.

The next year, Sanofi bought Acambis. Development of ChimeriVax-WN stalled during the next 2 years of declining disease incidence and unfavorable market and regulatory conditions, Monath recalled. In 2010, Sanofi ceased further development of the vaccine. Other companies made the same decision, he noted. By the time of the next WNV outbreak in 2012, few vaccines were in development. "Arbovirologists understood that this was going to come back and we would have a problem, and it would be nice to have a vaccine as part of the armamentarium against a reemerging disease," he observed, "but it's very hard to rekindle or restart a program that's become dormant in a company."

"I think we can conclude that if past is prologue, we'll have more episodes like 2012," Monath continued. Nevertheless, taking into account a variety of regulatory issues and the cost of a Phase 3 trial, Sanofi concluded that it still did not make financial sense to develop the vaccine. Given that decision, should the U.S. government fund the development of a WNV vaccine stockpile in order to respond to future outbreaks? Even a stockpile restricted to immunizing the elderly, who are most susceptible to neuroinvasive disease, would cost about $156,000 per case averted, Monath warned.

Moreover, he wondered, "How are you going to use a vaccine like this in an emergency? These epidemics tend to evolve quite quickly . . . [and] in the United States we really don't have any experience with mass immunization campaigns, especially those involving adults." On the other hand, a growing burden of long-term symptoms associated with WNV could tip the scales to justify further vaccine development, Monath observed (Garcia et al., 2014; Maxmen, 2012; Murray et al., 2014).

What About Chikungunya?

Fifteen years after the emergence of WNV in the United States, the threat of chikungunya is déjà vu, Monath observed (Morens and Fauci, 2014). As was illustrated in Box WO-2, the two diseases are quite similar in terms of epidemiology, clinical impact, and apparent long-term sequelae (Weaver et al., 2012). However, there are some important differences in the challenges these viruses present for vaccine development, he added. For example, humans are a dead-end host for WNV, but they participate in the transmission of chikungunya. "I think that could actually help us develop a vaccine, speed up the clinical development in part, and be a driver for an intervention," he observed. The rarity of asymptomatic CHIKV infections as compared with WNV could also expedite vaccine development, he added.

Much has occurred with the emergence of WNV in the United States: the emergence of CHIKV in the Americas has spurred several companies and government institutes to pursue development of a vaccine using a broad range of approaches, as shown in Figure WO-21. NIH researchers have produced a CHIKV-like particle that so far has proven safe and immunogenic, Monath reported (Akahata et al., 2010; Chang et al., 2014). "This is a real potential product, and I think there are a number of companies interested in licensing it," he said.

As with WNV, the obstacles to developing a CHIKV vaccine are not technical, but economic, Monath said. Once again, key issues involve target population, regulatory pathway, timing, and funding sources. Chikungunya's dramatic emergence has raised the profile of a potential vaccine, as does the likelihood that CHIKV will persist for decades in a large human population with abundant mosquito vectors, through which many travelers pass, he noted. There is also the potentially significant burden of chronic, long-term disease. Whereas the WNV vaccine was relatively feasible but had low market interest, a CHIKV vaccine, though potentially more difficult to develop, could have a larger potential market both in the United States and worldwide if chikungunya continues to spread, Monath predicted.

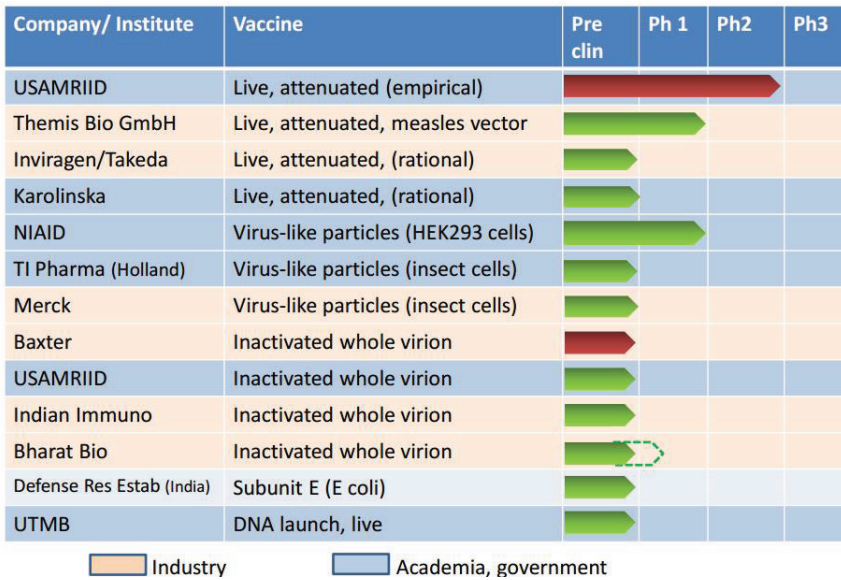

FIGURE WO-21 Chikungunya vaccine competitive landscape, 2014.
SOURCE: As presented by Thomas Monath on September 17, 2014.

Protecting Humans with Animal Vaccines

Monath briefly discussed prospects for immunizing animal species that are reservoirs for emerging human pathogens, a strategy that is being investigated to prevent several vector-borne diseases, including Rift Valley fever (in livestock), Venezuelan equine encephalitis, and leishmaniasis (in dogs). Some innovative approaches to animal vaccines include oral bait vaccines for Lyme disease, as well as "generic" immunization against ticks, according to Monath. Given Kilpatrick's indictment of robins as a key reservoir for West Nile virus, he noted, "An intriguing idea now obviously has to be a recombinant worm that robins eat," he observed.

Monath remarked that the work of Ken Linthicum (see Appendix A7) and others demonstrates that if you can predict Rift Valley fever activity and immunize the hosts that contribute to amplification of the virus, you can prevent human disease and prevent direct animal-to-human transmission, which may provide a model for other vaccine-preventable diseases. He noted that the approval process for animal vaccines is less onerous than for human vaccines, and therefore potentially a more attractive investment for pharmaceutical companies.

Both WNV and CHIKV vaccines present obstacles to industry that could be reduced through push/pull incentives, Monath suggested, as well as direct government funding for advanced development. The accumulation of a government stockpile, which could begin when the vaccine was still under emergency use authorization or not yet licensed, would also boost development. Ultimately, he concluded, "We need a faster, easier, less expensive way to get these kinds of vaccines through the regulatory process, reduce the cost of development, and improve the return on the investment for industry."

REFERENCES

Akahata, W., Z. Y. Yang, H. Andersen, S. Sun, H. A. Holdaway, W. P. Kong, M. G. Lewis, S. Higgs, M. G. Rossmann, S. Rao, and G. J. Nabel. 2010. A virus-like particle vaccine for epidemic chikungunya virus protects nonhuman primates against infection. *Nature Medicine* 16(3):334-338. doi: 10.1038/nm.2105.

Almeida, R. 2007. *Vector-borne plant diseases: Factors driving the emergence and spread of pathogens*. Presentation at the Forum on Microbial Threats workshop entitled "Vector-borne diseases: Understanding the environmental, human health, and ecological connections," June 19–20, Ft. Collins, CO.

Almeida, R. 2014. *The past, present, and future of vector-borne plant diseases*. Presentation at IOM workshop on vector-borne diseases, Washington, DC.

Alphey, L. 2014a. *Development and evaluation of transgenic insects for use in the control of vector-borne disease*. Presentation at IOM workshop on vector-borne disease, Washington, DC.

Alphey, L. 2014b. Genetic control of mosquitoes. *Annual Review of Entomology* 59:205-224. doi: 10.1146/annurev-ento-011613-162002.

Alphey, L., M. Benedict, R. Bellini, G. G. Clark, D. A. Dame, M. W. Service, and S. L. Dobson. 2010. Sterile-insect methods for control of mosquito-borne diseases: An analysis. *Vector Borne Zoonotic Diseases* 10(3):295-311. doi: 10.1089/vbz.2009.0014.

Anderson, P. K., A. A. Cunningham, N. G. Patel, F. J. Morales, P. R. Epstein, and P. Daszak. 2004. Emerging infectious diseases of plants: Pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution* 19(10):535-544. doi: 10.1016/j.tree.2004.07.021.

Angerami, R. N., A. M. da Silva, E. M. Nascimento, S. Colombo, M. Y. Wada, F. C. dos Santos, D. M. Mancini, R. C. de Oliveira, G. Katz, E. C. Martins, and L. J. da Silva. 2009. Brazilian spotted fever: Two faces of a same disease? A comparative study of clinical aspects between an old and a new endemic area in Brazil. *Clinical Microbiology and Infection* 15(Suppl 2):207-208. doi: 10.1111/j.1469-0691.2008.02160.x.

Antinori, S., L. Schifanella, and M. Corbellino. 2012. Leishmaniasis: New insights from an old and neglected disease. *European Journal of Clinical Microbiology and Infectious Disease* 31(2):109-118. doi: 10.1007/s10096-011-1276-0.

Anyamba, A., K. J. Linthicum, J. L. Small, K. M. Collins, C. J. Tucker, E. W. Pak, S. C. Britch, J. R. Eastman, J. E. Pinzon, and K. L. Russell. 2012. Climate teleconnections and recent patterns of human and animal disease outbreaks. *PLoS Neglected Tropical Diseases* 6(1):e1465. doi: 10.1371/journal.pntd.0001465.

Anyamba, A., J. L. Small, S. C. Britch, C. J. Tucker, E. W. Pak, C. A. Reynolds, J. Crutchfield, and K. J. Linthicum. 2014. Recent weather extremes and impacts on agricultural production and vector-borne disease outbreak patterns. *PLoS ONE* 9(3):e92538. doi: 10.1371/journal.pone.0092538.

Aregawi, M., M. Lynch, W. Bekele, H. Kebede, D. Jima, H. S. Taffese, M. A. Yenehun, A. Lilay, R. Williams, M. Thomson, F. Nafo-Traore, K. Admasu, T. A. Gebreyesus, and M. Coosemans. 2014. Time series analysis of trends in malaria cases and deaths at hospitals and the effect of antimalarial interventions, 2001-2011, Ethiopia. *PLoS ONE* 9(11):e106359. doi: 10.1371/journal.pone.0106359.

ARS (Agricultural Research Service, USDA). 2013. *Phytoplasma Resource Center*. http://plantpathology.ba.ars.usda.gov/phytoplasma.html (accessed July 26, 2013).

Ashley, E. A., M. Dhorda, R. M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J. M. Anderson, S. Mao, B. Sam, C. Sopha, C. M. Chuor, C. Nguon, S. Sovannaroth, S. Pukrittayakamee, P. Jittamala, K. Chotivanich, K. Chutasmit, C. Suchatsoonthorn, R. Runcharoen, T. T. Hien, N. T. Thuy-Nhien, N. V. Thanh, N. H. Phu, Y. Htut, K. T. Han, K. H. Aye, O. A. Mokuolu, R. R. Olaosebikan, O. O. Folaranmi, M. Mayxay, M. Khanthavong, B. Hongvanthong, P. N. Newton, M. A. Onyamboko, C. I. Fanello, A. K. Tshefu, N. Mishra, N. Valecha, A. P. Phyo, F. Nosten, P. Yi, R. Tripura, S. Borrmann, M. Bashraheil, J. Peshu, M. A. Faiz, A. Ghose, M. A. Hossain, R. Samad, M. R. Rahman, M. M. Hasan, A. Islam, O. Miotto, R. Amato, B. MacInnis, J. Stalker, D. P. Kwiatkowski, Z. Bozdech, A. Jeeyapant, P. Y. Cheah, T. Sakulthaew, J. Chalk, B. Intharabut, K. Silamut, S. J. Lee, B. Vihokhern, C. Kunasol, M. Imwong, J. Tarning, W. J. Taylor, S. Yeung, C. J. Woodrow, J. A. Flegg, D. Das, J. Smith, M. Venkatesan, C. V. Plowe, K. Stepniewska, P. J. Guerin, A. M. Dondorp, N. P. Day, N. J. White, and Tracking Resistance to Artemisinin Collaboration. 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine* 371(5):411-423. doi: 10.1056/NEJMoa1314981.

AVMA (American Veterinary Medical Association). 2008. *One Health: A new professional imperative*. Schaumburg, IL: American Veterinary Medical Association.

Badillo-Vargas, I., D. Rotenberg, B. A. Schneweis, and A. E. Whitfield. 2015. RNA interference tools for *Frankliniella occidentalis*. *Journal of Insect Physiology* 76:36-46.

Bean, A. G. D., M. L. Baker, C. R. Stewart, C. Cowled, C. Deffrasnes, L.-F. Wang, and J. W. Lowenthal. 2013. Studying immunity to zoonotic diseases in the natural host—Keeping it real. *Nature Reviews Immunology* 13:851-861.

Black, W. C., 4th, L. Alphey, and A. A. James. 2011. Why RIDL is not SIT. *Trends in Parasitology* 27(8):362-370.

Blanc, S., M. Drucker, and M. Uzest. 2014. Localizing viruses in their insect vectors. *Annual Review of Phytopathology* 52:403-425. doi: 10.1146/annurev-phyto-102313-045920.

Bonning, B. C., N. Pal, S. Liu, Z. Wang, S. Sivakumar, P. M. Dixon, G. F. King, and W. A. Miller. 2014. Toxin delivery by the coat protein of an aphid-vectored plant virus provides plant resistance to aphids. *Nature Biotechnology* 32(1):102-105.

Brehm, M. A., N. Jouvet, D. L. Greiner, and L. D. Shultz. 2013. Humanized mice for the study of infectious diseases. *Current Opinion in Immunology* 25(4):428-435. doi: 10.1016/j.coi.2013.05.012.

Carribean 360. 2014. Chikungunya declared an epidemic in the Caribbean. http://www.caribbean360.com/news/chikungunya-declared-an-epidemic-in-the-caribbean (accessed December 12, 2014).

Carrillo, E., J. Moreno, and I. Cruz. 2013. What is responsible for a large and unusual outbreak of leishmaniasis in Madrid? *Trends in Parasitology* 29(12):579-580. doi: 10.1016/j.pt.2013.10.007.

CDC (Centers for Disease Control and Prevention). 2006. Assessing capacity for surveillance, prevention, and control of West Nile virus infection—United States, 1999 and 2004. *Morbidity and Mortality Weekly Report* 55(6):150-153.

CDC. 2013. Three sudden cardiac deaths associated with Lyme carditis—United States, November 2012-July 2013. *Morbidity and Mortality Weekly Report* 62(49):993-996.

CDC. 2014a. Chikungunya nowcast for the Americas. http://www.cdc.gov/chikungunya/modeling (accessed November 24, 2014).

CDC. 2014b. First chikungunya case acquired in the United States reported in Florida. http://www.cdc.gov/media/releases/2014/p0717-chikungunya.html (accessed November 24, 2014).

CDC. 2014c. Geographic distribution: Where has chikungunya virus been found? http://www.cdc.gov/chikungunya/geo/index.html (accessed November 24, 2014).

CDC. 2014d. World Health Day—Vector-borne diseases. http://www.cdc.gov/features/worldhealthday2014 (accessed October 20, 2014).

CDC. 2015. Dengue. http://www.cdc.gov/Dengue (accessed October 5, 2015).

Chang, L. J., K. A. Dowd, F. H. Mendoza, J. G. Saunders, S. Sitar, S. H. Plummer, G. Yamshchikov, U. N. Sarwar, Z. Hu, M. E. Enama, R. T. Bailer, R. A. Koup, R. M. Schwartz, W. Akahata, G. J. Nabel, J. R. Mascola, T. C. Pierson, B. S. Graham, J. E. Ledgerwood, and VRC 311 Study Team. 2014. Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: A phase 1 dose-escalation trial. *Lancet* 384(9959):2046-2052. doi: 10.1016/S0140-6736(14)61185-5.

Chretien, J.-P., A. Anyamba, J. L. Small, S. C. Britch, J. L. Sanchez, A. C. Halbach, C. J. Tucker, and K. J. Linthicum. 2015. Global climate anomalies and potential infectious disease risks: 2014-2015. *PLoS Currents Outbreaks* Jan 26. Edition 1. doi: 10.1371/currents.outbreaks.95fbc4a8fb4695e049baabfc2fc8289f.

Clarke, C. F., K. K. Bradley, J. H. Wright, and J. Glowicz. 2013. Case report: Emergence of autochthonous cutaneous leishmaniasis in northeastern Texas and southeastern Oklahoma. *American Journal of Tropical Medicine and Hygiene* 88(1):157-161. doi: 10.4269/ajtmh.2012.11-0717.

Cohen, J. M., D. L. Smith, C. Cotter, A. Ward, G. Yamey, O. J. Sabot, and B. Moonen. 2012. Malaria resurgence: A systematic review and assessment of its causes. *Malaria Journal* 11:122. doi: 10.1186/1475-2875-11-122.

Cologna, R., P. M. Armstrong, and R. Rico-Hesse. 2005. Selection for virulent dengue viruses occurs in humans and mosquitoes. *Journal of Virology* 79(2):853-859. doi: 10.1128/JVI.79.2.853-859.2005.

COMTF (California Oak Mortality Task Force). 2013. Sudden oak death overview. http://www.suddenoakdeath.org/about-sudden-oak-death/history-background (accessed August 11, 2015).

Cox, J., J. Mota, S. Sukupolvi-Petty, M. S. Diamond, and R. Rico-Hesse. 2012. Mosquito bite delivery of dengue virus enhances immunogenicity and pathogenesis in humanized mice. *Journal of Virology* 86(14):7637-7649. doi: 10.1128/JVI.00534-12.

Cragun, W. C., B. L. Bartlett, M. W. Ellis, A. Z. Hoover, S. K. Tyring, N. Mendoza, T. J. Vento, W. L. Nicholson, M. E. Eremeeva, J. P. Olano, R. P. Rapini, and C. D. Paddock. 2010. The expanding spectrum of eschar-associated rickettsioses in the United States. *Archives of Dermatology* 146(6):641-648. doi: 10.1001/archdermatol.2010.48.

Crowder, D. W., E. A. Dykstra, J. M. Brauner, A. Duffy, C. Reed, E. Martin, W. Peterson, Y. Carriere, P. Dutilleul, and J. P. Owen. 2013. West Nile virus prevalence across landscapes is mediated by local effects of agriculture on vector and host communities. *PLoS ONE* 8(1):e55006. doi: 10.1371/journal.pone.0055006.

Damsteegt, V. D., R. Scorza, A. L. Stone, W. L. Schneider, K. K. Webb, M. A. Demuth, and F. E. Gildow. 2007. *Prunus* host range of plum pox virus (PPV) in the United States by aphid and graft inoculation. *Plant Disease* 91:18-23.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife—Threats to biodiversity and human health. *Science* 287(5452):443-449.

Dayan, G. H., K. Pugachev, J. Bevilacqua, J. Lang, and T. P. Monath. 2013. Preclinical and clinical development of a YFV 17 D-based chimeric vaccine against West Nile virus. *Viruses* 5(12):3048-3070. doi: 10.3390/v5123048.

Devine, G. J., E. Z. Perea, G. F. Killeen, J. D. Stancil, S. J. Clark, and A. C. Morrison. 2009. Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proceedings of the National Academy of Sciences of the United States of America* 106(28):11530-11534.

Dodd, R. Y. 2012. Emerging infections and transfusion safety. In *Practical transfusion medicine*, 4th ed., edited by M. F. Murphy, D. H. Pamphilon, and N. M. Heddle. Chichester, UK: John Wiley & Sons. Pp. 161-167.

Douglas, K. O., A. M. Kilpatrick, P. N. Levett, and M. C. Lavoie. 2007. A quantitative risk assessment of West Nile virus introduction into Barbados. *West Indian Medical Journal* 56(5):394-397.

Duggal, N. K., A. Bosco-Lauth, R. A. Bowen, S. S. Wheeler, W. K. Reisen, T. A. Felix, B. R. Mann, H. Romo, D. M. Swetnam, A. D. T. Barrett, and A. C. Brault. 2014. Evidence for co-evolution of West Nile virus and house sparrows in North America. *PLoS Neglected Tropical Diseases* 8.

Ebi, K. L., E. Lindgren, J. E. Suk, and J. C. Semenza. 2013. Adaptation to the infectious disease impacts of climate change. *Climatic Change* 118:355-365.

Eisen, L., and C. G. Moore. 2013. *Aedes* (*Stegomyia*) *aegypti* in the continental United States: A vector at the cool margin of its geographic range. *Journal of Medical Entomology* 50(3):467-478.

European Food Safety Authority. 2014. Schmallenberg virus: State of art. *EFSA Journal* 12(5).

Fischer, M., J. E. Staples, National Center for Emerging Arboviral Diseases Branch, and CDC Zoonotic Infectious Diseases. 2014. Notes from the field: Chikungunya virus spreads in the Americas–Caribbean and South America, 2013-2014. *Morbidity and Mortality Weekly Report* 63(22):500-501.

Fletcher, J., and A. Wayadande. 2002. Fastidious vascular-colonizing bacteria. http://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/Fastidious.aspx (accessed December 12, 2014).

Florida Keys Mosquito Control District. 2014. http://keysmosquito.org/wp-content/uploads/2013/02/All-Keys-Survey-Results-2013.pdf (accessed June 2, 2015).

Freedman, D. O., L. H. Weld, P. E. Kozarsky, T. Fisk, R. Robins, F. von Sonnenburg, J. S. Keystone, P. Pandey, M. S. Cetron, and Network GeoSentinel Surveillance. 2006. Spectrum of disease and relation to place of exposure among ill returned travelers. *New England Journal of Medicine* 354(2):119-130. doi: 10.1056/NEJMoa051331.

Fu, G., R. S. Lees, D. Nimmo, D. Aw, L. Jin, P. Gray, T. U. Berendonk, H. White-Cooper, S. Scaife, H. Kim Phuc, O. Marinotti, N. Jasinskiene, A.A. James, and L. Alphey. 2010. Female-specific flightless phenotype for mosquito control. *Proceedings of the National Academy of Sciences of the United States of America* 107:4550-4554.

Funk, S., T. L. Bogich, K. E. Jones, A. M. Kilpatrick, and P. Daszak. 2013. Quantifying trends in disease impact to produce a consistent and reproducible definition of an emerging infectious disease. *PLoS ONE* 8:e69951.

Gallian, P., X. de Lamballerie, N. Salez, G. Piorkowski, P. Richard, L. Paturel, R. Djoudi, I. Leparc-Goffart, P. Tiberghien, J. Chiaroni, and R. N. Charrel. 2014. Prospective detection of chikungunya virus in blood donors, Caribbean 2014. *Blood* 123(23):3679-3681. doi: 10.1182/blood-2014-03-564880.

Garcia, G. P, A. E. Flores, I. Fernández-Salas, K. Saavedra-Rodríguez, G. Reyes-Solis, S. Lozano-Fuentes, J. Guillermo Bond, M. Casas-Martínez, J. M. Ramsey, J. García-Rejón, M. Domínguez-Galera, H. Ranson, J. Hemingway, L. Eisen, and W. C. Black 4th. 2009. Recent rapid rise of a permethrin knock down resistance allele in *Aedes aegypti* in México. *PLoS Neglected Tropical Diseases* 3(10):e531.

Garcia, G., N. Gonzalez, A. B. Perez, B. Sierra, E. Aguirre, D. Rizo, A. Izquierdo, L. Sanchez, D. Diaz, M. Lezcay, B. Pacheco, K. Hirayama, and M. G. Guzman. 2011. Long-term persistence of clinical symptoms in dengue-infected persons and its association with immunological disorders. *International Journal of Infectious Diseases* 15(1):e38-e43. doi: 10.1016/j.ijid.2010.09.008.

Garcia, M. N., A. M. Hause, C. M. Walker, J. S. Orange, R. Hasbun, and K. O. Murray. 2014. Evaluation of prolonged fatigue post-West Nile virus infection and association of fatigue with elevated antiviral and proinflammatory cytokines. *Viral Immunology* 27(7):327-333. doi: 10.1089/vim.2014.0035.

García-Rejón, J., M. A. Loroño-Pino, J. A. Farfan-Ale, L. Flores-Flores, E. Del Pilar Rosado-Paredes, N. Rivero-Cardenas, R. Najera-Vazquez, S. Gomez-Carro, V. Lira-Zumbardo, P. Gonzalez-Martinez, S. Lozano-Fuentes, D. Elizondo-Quiroga, B. J. Beaty, and L. Eisen. 2008. Dengue virus-infected *Aedes aegypti* in the home environment. *American Journal of Tropical Medicine and Hygiene* 79(6):940-950.

García-Rejón, J. E., M. A. Loroño-Pino, J. A. Farfán-Ale, L. F. Flores-Flores, M. P. López-Uribe, R. Najera-Vazquez Mdel, G. Nuñez-Ayala, B. J. Beaty, and L. Eisen. 2011. Mosquito infestation and dengue virus infection in *Aedes aegypti* females in schools in Merida, Mexico. *American Journal of Tropical Medicine and Hygiene* 84(3):489-496. doi: 10.4269/ajtmh.2011.10-0654.

Gergerich, R. C., and V. V. Dolja. 2006. Introduction to plant viruses, the invisible foe. http://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/PlantViruses.asp (accessed December 12, 2014).

Gething, P. W., D. L. Smith, A. P. Patil, A. J. Tatem, R. W. Snow, and S. I. Hay. 2010. Climate change and the global malaria recession. *Nature* 465:342-346.

Gray, S. M., and N. Banerjee. 1999. Mechanisms of arthropod transmission of plant and animal viruses. *Microbiol and Molecular Biology Reviews* 63(1):128-148.

Gubler, D. J. 1998. Resurgent vector-borne diseases as a global health problem. *Emerging Infectious Diseases* 4(3):442-450. doi: 10.3201/eid0403.980326.

Gubler, D. J. 2011. Dengue, urbanization and globalization: The unholy trinity of the 21st century. *Tropical Medicine and Health* 39:3-11. doi:10.2149/tmh.2011-S05.

Hackett. L. W. 1937. *Malaria in Europe, an ecological study.* London: Oxford University Press.

Hadler, J. L., D. Patel, K. Bradley, J. M. Hughes, C. Blackmore, P. Etkind, L. Kan, J. Getchell, J. Blumenstock, J. Engel, and Control Centers for Disease and Prevention. 2014. National capacity for surveillance, prevention, and control of West Nile virus and other arbovirus infections–United States, 2004 and 2012. *Morbidity and Mortality Weekly Report* 63(13):281-284.

Hamer, G. L., L. F. Chaves, T. K. Anderson, U. D. Kitron, J. D. Brawn, M. O. Ruiz, S. R. Loss, E. D. Walker, and T. L. Goldberg. 2011. Fine-scale variation in vector host use and force of infection drive localized patterns of West Nile virus transmission. *PLoS ONE* 6:e23767.

Harris, A. F., A. R. McKemey, D. Nimmo, Z. Curtis, I. Black, S. A. Morgan, M. N. Oviedo, R. Lacroix, N. Naish, N. I. Morrison, A. Collado, J. Stevenson, S. Scaife, T. Dafa'alla, G. Fu, C. Phillips, A. Miles, N. Raduan, N. Kelly, C. Beech, C. A. Donnelly, W. D. Petrie, and L. Alphey. 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology* 30(9):828-830. doi: 10.1038/nbt.2350.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296(5576):2158-2162.

Hayes, E. B. 2009. Zika virus outside Africa. *Emerging Infectious Diseases* 15(9):1347-1350. doi: 10.3201/eid1509.090442.

Hogenhout, S. A., E.-D. Ammar, A. E. Whitfield, and M. G. Redinbaugh. 2008. Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology* 46:327-359. doi: 10.1146/annurev.phyto.022508.092135.

IOM (Insitute of Medicine). 1992. *Emerging infections: Microbial threats to health in the United States*. Washington, DC: National Academy Press.

IOM. 2008. *Vector-borne diseases: Understanding the environment, human health, and ecological connections*. Washington, DC: The National Academies Press.

Johns Hopkins Bloomberg School of Public Health. 1991. Fred Soper, DrPH, MPH. http://www.jhsph.edu/about/history/heroes-of-public-health/fred-soper.html (accessed December 5, 2014).

Johnston, S. H., C. A. Glaser, K. Padgett, D. A. Wadford, A. Espinosa, N. Espinosa, M. E. Eremeeva, K. Tait, B. Hobson, S. Shtivelman, C. Hsieh, and S. L. Messenger. 2013. *Rickettsia* spp. 364D causing a cluster of eschar-associated illness, California. *Pediatric Infectious Disease Journal* 32(9):1036-1039. doi: 10.1097/INF.0b013e318296b24b.

Jongejan, F., and G. Uilenberg. 2004. The global importance of ticks. *Parasitology* 129(Suppl):S3-S14.

Kang, S., A. R. Shields, N. Jupatanakul, and G. Dimopoulos. 2014. Suppressing dengue-2 infection by chemical inhibition of *Aedes aegypti* host factors. *PLoS Neglected Tropical Diseases* 8(8): e3084.

Katz, L. 2014. Not much happening in the real world, so i'll update you on mine. America's Blood Centers. http://www.americasblood.org/blog/not-much-happening-in-the-real-world,-so-i'll-update-you-on-mine.aspx (accessed November 30, 2014).

Khan, K., I. Bogoch, J. S. Brownstein, J. Miniota, A. Nicolucci, W. Hu, E. O. Nsoesie, M. Cetron, M. I. Creatore, M. German, and A. Wilder-Smith. 2014. Assessing the origin of and potential for international spread of chikungunya virus from the Caribbean. *PLoS Currents* 6. doi: 10.1371/currents.outbreaks.2134a0a7bf37fd8d388181539fea2da5.

Killeen, G. F. 2014. Characterizing, controlling and eliminating residual malaria transmission. *Malaria Journal* 13:330.

Kilpatrick, A. M. 2011. Globalization, land use, and the invasion of West Nile virus. *Science* 334(6054):323-327. doi: 10.1126/science.1201010.

Kilpatrick, A. M. 2014. *Globalization, land use, global warming, and the invasion of West Nile virus*. Presentation at IOM workshop on vector-borne diseases, Washington, DC.

Kilpatrick, A. M., and W. J. Pape. 2013. Predicting human West Nile virus infections with mosquito surveillance data. *American Journal of Epidemiology* 178(5):829-835. doi: 10.1093/aje/kwt046.

Kilpatrick, A. M., and S. E. Randolph. 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* 380(9857):1946-1955. doi: 10.1016/S0140-6736(12)61151-9.

Kilpatrick, A. M., Y. Gluzberg, J. Burgett, and P. Daszak. 2004. A quantitative risk assessment of the pathways by which West Nile virus could reach Hawaii. *Ecohealth* 1(2):205-209.

Kilpatrick, A. M., L. D. Kramer, S. R. Campbell, E. O. Alleyne, A. P. Dobson, and P. Daszak. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerging Infectious Diseases* 11(3):425-429. doi: 10.3201/eid1103.040364.

Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra, and L. D. Kramer. 2006. Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society B: Biological Sciences* 273:2327-2333.

Kilpatrick, A. M., M. A. Meola, R. M. Moudy, and L. D. Kramer. 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathogens* 4(6):e1000092. doi: 10.1371/journal.ppat.1000092.

Knox, J., R. U. Cowan, J. S. Doyle, M. K. Ligtermoet, J. S. Archer, J. N. Burrow, S. Y. Tong, B. J. Currie, J. S. Mackenzie, D. W. Smith, M. Catton, R. J. Moran, C. A. Aboltins, and J. S. Richards. 2012. Murray Valley encephalitis: A review of clinical features, diagnosis and treatment. *Medical Journal of Australia* 196(5):322-326.

Kupferschmidt, K. 2013. A lethal dose of RNA. *Science* 341(6147):732-733. doi: 10.1126/science.341.6147.732.

Lindblade, K. A., L. Steinhardt, A. Samuels, S. P. Kachur, and L. Slutsker. 2013. The silent threat: Asymptomatic parasitemia and malaria transmission. *Expert Review of Anti-Infective Therapy* 11(6):623-639. doi: 10.1586/eri.13.45.

Lindgren, E., Y. Andersson, J. E. Suk, B. Sudre, and J. C. Semenza. 2012. Public health. Monitoring EU emerging infectious disease risk due to climate change. *Science* 336(6080):418-419. doi: 10.1126/science.1215735.

Lindsey, N. P., J. A. Lehman, J. E. Staples, M. Fischer, National Center for Emerging Division of Vector-Borne Diseases, and CDC Zoonotic Infectious Diseases. 2014. West Nile virus and other arboviral diseases—United States, 2013. *Morbidity and Mortality Weekly Report* 63(24):521-526.

Linthicum, K. J., A. Anyamba, B. Killenbeck, W. J. Lee, H. C. Lee, T. A. Klein, H. C. Kim, J. A. Pavlin, S. C. Britch, J. Small, C. J. Tucker, and J. C. Gaydos. 2014. Association of temperature and historical dynamics of malaria in the Republic of Korea, including reemergence in 1993. *Military Medicine* 179(7):806-814. doi: 10.7205/MILMED-D-13-00545.

Maxmen, A. 2012. The hidden threat of West Nile virus. *Nature* 489(7416):349-350. doi: 10.1038/489349a.

McMullan, L. K., S. M. Folk, A. J. Kelly, A. MacNeil, C. S. Goldsmith, M. G. Metcalfe, B. C. Batten, C. G. Albarino, S. R. Zaki, P. E. Rollin, W. L. Nicholson, and S. T. Nichol. 2012. A new phlebovirus associated with severe febrile illness in Missouri. *New England Journal of Medicine* 367(9):834-841. doi: 10.1056/NEJMoa1203378.

Mielke-Ehret, N., and H. P. Muhlbach. 2012. *Emaravirus*: A novel genus of multipartite, negative strand RNA plant viruses. *Viruses* 4(9):1515-1536. doi: 10.3390/v4091515.

Miller, W. A., and L. Rasochova. 1997. Barley yellow dwarf viruses. *Annual Review of Phytopathology* 35:167-190.

Minjauw, B., and A. McLeod. 2003. *Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal livestock owners in India and eastern and southern Africa*. University of Edinburgh, UK: DFID Animal Health Programme, Centre for Tropical Veterinary Medicine.

Monath, T. P. 2014. *Dengue, Japanese encephalitis, West Nile, chikungunya, and yellow fever: Challenges for the development and use of vaccines*. Presentation at IOM workshop on vector-borne diseases, Washington, DC.

Montero-Astúa, M., D. Rotenberg, A. Leach-Kieffaber, B. A. Schneweis, S. Park, J. K. Park, T. L. German, and A. E. Whitfield. 2014. Disruption of vector transmission by a plant-expressed viral glycoprotein. *Molecular Plant-Microbe Interactions Journal* 27(3):296-304. doi: 10.1094/MPMI-09-13-0287-FI.

Montgomery, S. P., M. C. Starr, P. T. Cantey, M. S. Edwards, and S. K. Meymandi. 2014. Neglected parasitic infections in the United States: Chagas disease. *American Journal of Tropical Medicine and Hygiene* 90(5):814-818. doi: 10.4269/ajtmh.13-0726.

Mordecai, E. A., K. P. Paaijmans, L. R. Johnson, C. Balzer, T. Ben-Horin, E. de Moor, A. McNally, S. Pawar, S. J. Ryan, T. C. Smith, and K. D. Lafferty. 2013. Optimal temperature for malaria transmission is dramatically lower than previously predicted. *Ecology Letters* 16(1):22-30. doi: 10.1111/ele.12015.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, L. E. Hugo, K. N. Johnson, B. H. Kay, E. A. McGraw, A. F. van den Hurk, P. A. Ryan, and S. L. O'Neill. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and plasmodium. *Cell* 139(7):1268-1278. doi: 10.1016/j.cell.2009.11.042.

Morens, D. M., and A. S. Fauci. 2014. Chikungunya at the door—déjà vu all over again? *New England Journal of Medicine* 371(10):885-887. doi: 10.1056/NEJMp1408509.

Moritz, E. D., C. S. Winton, S. T. Johnson, D. E. Krysztof, R. L. Townsend, G. A. Foster, P. Devine, P. Molloy, E. Brissette, V. P. Berardi, and S. L. Stramer. 2014. Investigational screening for *Babesia microti* in a large repository of blood donor samples from nonendemic and endemic areas of the United States. *Transfusion* 54(9):2226-2236. doi: 10.1111/trf.12693.

Murdock, C. C., S. Blanford, G. L. Hughes, J. L. Rasgon, and M. B. Thomas. 2014. Temperature alters *Plasmodium* blocking by *Wolbachia*. *Scientific Reports* 4:3932. doi: 10.1038/srep03932.

Murray, K. O., M. N. Garcia, M. H. Rahbar, D. Martinez, S. A. Khuwaja, R. R. Arafat, and S. Rossmann. 2014. Survival analysis, long-term outcomes, and percentage of recovery up to 8 years post-infection among the Houston West Nile virus cohort. *PLoS ONE* 9 (7):e102953. doi: 10.1371/journal.pone.0102953.

Musso, D., V. Richard, J. Broult, and V. M. Cao-Lormeau. 2014. Inactivation of dengue virus in plasma with amotosalen and ultraviolet A illumination. *Transfusion* 54(11):2924-2930. doi: 10.1111/trf.12713.

Nasci, R. S. 2014. Movement of chikungunya virus into the Western Hemisphere. *Emerging Infectious Diseases* 8:1394-1395. doi: 10.3201/eid2008.140333.

The National Oceanic and Atmospheric Administration's (NOAA) Climate Prediction Center (CPC) 2014, El Niño conditions advisory. http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/enso_advisory/index.shtml (accessed March 25, 2016).

NOAA (National Oceanic and Atmospheric Administration). 2015. Global temperature record. http://ncdc.noaa.gov/cag/timeseries/global/globe/land_ocean/ytd/12/1880-2014 (accessed March 25, 2016).

NRC (National Research Council). 2004. *California agricultural research priorities: Pierce's disease*. Washington, DC: The National Academies Press.

Ogden, N. H., L. St-Onge, I. K. Barker, S. Brazeau, M. Bigras-Poulin, D. F. Charron, C. M. Francis, A. Heagy, L. Robbin Lindsay, A. Maarouf, P. Michel, F. Milord, C. J. O'Callaghan, L. Trudel, and R. A. Thompson. 2008. Risk maps for range expansion of the Lyme disease vector, *Ixodes scapularis*, in Canada now and with climate change. *International Journal of Health Geographics* 7:24.

Paaijmans, K. P., R. L. Heinig, R. A. Seliga, J. I. Blanford, S. Blanford, C. C. Murdock, and M. B. Thomas. 2013. Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology* 19(8):2373-2380. doi: 10.1111/gcb.12240.

Paddock, C. D., and M. J. Yabsley. 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. *Current Topics in Microbiology and Immunology* 315:289-324.

Paddock, C. D., R. W. Finley, C. S. Wright, H. N. Robinson, B. J. Schrodt, C. C. Lane, O. Ekenna, M. A. Blass, C. L. Tamminga, C. A. Ohl, S. L. McLellan, J. Goddard, R. C. Holman, J. J. Openshaw, J. W. Sumner, S. R. Zaki, and M. E. Eremeeva. 2008. *Rickettsia parkeri* rickettsiosis and its clinical distinction from Rocky Mountain spotted fever. *Clinical Infectious Diseases* 47(9):1188-1196. doi: 10.1086/592254.

PAHO (Pan American Health Organization). 2014a. Dengue Laboratory Network of the Americas (RELDA). http://www.paho.org/hq/index.php?option=com_content&view=article&id=4497&Itemid=39306&lang=en (accessed December 13, 2014).

PAHO. 2014b. *Number of reported cases of chikungunya fever in the Americas, by country or territory, epidemiological week 47 (November 21, 2014)*. Washington, DC: Pan American Health Organization.

Paz, S., D. Malkinson, M. S. Green, G. Tsioni, A. Papa, K. Danis, A. Sirbu, C. Ceianu, K. Katalin, E. Ferenczi, H. Zeller, and J. C. Semenza. 2013. Permissive summer temperatures of the 2010 European West Nile fever upsurge. *PLoS ONE* 8(2):e56398. doi: 10.1371/journal.pone.0056398.

Petersen, L.R. 2014. *Emerging vector-borne diseases in the United States: What's next and are we prepared?* Presentation at the IOM workshop on vector-borne diseases, Washington, DC.

Phuc, H. K., M. H. Andreasen, R. S. Burton, C. Vass, M. J. Epton, G. Pape, G, Fu, K. C. Condon, S. Scaife, C. A. Donnelly, P. G. Coleman, H. White-Cooper, and L. Alphey. 2007. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biology* 5:11.

Powell, J. R., and W. J. Tabachnick. 2013. History of domestication and spread of *Aedes aegypti*—a review. *Memórias do Instituto Oswaldo Cruz* 108(Suppl 1):11-17. doi: 10.1590/0074-0276130395.

Purcell, A. H. 1982. Insect vector relationships with procaryotic plant pathogens. *Annual Review of Phytopathology* 20(1):397-417.

Ramos, M. M., H. Mohammed, E. Zielinski-Gutierrez, M. H. Hayden, J. L. Lopez, M. Fournier, A. R. Trujillo, R. Burton, J. M. Brunkard, L. Anaya-Lopez, A. A. Banicki, P. K. Morales, B. Smith, J. L. Munoz, S. H. Waterman, and Group Dengue Serosurvey Working. 2008. Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico border: Results of a household-based seroepidemiologic survey, December 2005. *American Journal of Tropical Medicine and Hygiene* 78(3):364-369.

Rasongles, P., M. F. Angelini-Tibert, P. Simon, C. Currie, H. Isola, D. Kientz, M. Slaedts, M. Jacquet, D. Sundin, L. Lin, L. Corash, and J. P. Cazenave. 2009. Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during a chikungunya virus epidemic in Ile de La Reunion. *Transfusion* 49(6):1083-1091. doi: 10.1111/j.1537-2995.2009.02111.x.

Rassi, A., Jr., A. Rassi, and J. A. Marin-Neto. 2010. Chagas disease. *Lancet* 375(9723):1388-1402. doi: 10.1016/S0140-6736(10)60061-X.

Reisen, W. K. 2010. Landscape epidemiology of vector-borne diseases. *Annual Review of Entomology* 55:461-483. doi: 10.1146/annurev-ento-112408-085419.

Reiter, P. 2007. Oviposition, dispersal, and survival in *Aedes aegypti*: Implications for the efficacy of control strategies. *Vector Borne Zoonotic Diseases* 7(2):261-273. doi: 10.1089/vbz.2006.0630.

Reiter, P. 2014. Surveillance and control of urban dengue vectors. In *Dengue and dengue hemorrhagic fever*, 2nd ed., edited by D. J. Gubler, E. E. Ooi, S. Vasudevan, and J. Farrar. London, United Kingdom: CAB International. Pp. 481-518.

Reynolds, R. W., N. A. Rayner, T. M. Smith, D. C. Stokes, and W. Wanqiu. 2002. An improved in situ and satellite SST analysis for climate. *Journal of Climate* 15(13):1609-1625.

Rico-Hesse, R. 2009. Dengue virus markers of virulence and pathogenicity. *Future Virology* 4(6):581. doi: 10.2217/fvl.09.51.

Rogers, D. J., and S. E. Randolph. 2000. The global spread of malaria in a future, warmer world. *Science* 289:1763-1766.

Sabchareon, A., C. Sirivichayakul, K. Limkittikul, P. Chanthavanich, S. Suvannadabba, V. Jiwariyavej, W. Dulyachai, K. Pengsaa, H. S. Margolis, and G. W. Letson. 2012. Dengue infection in children in Ratchaburi, Thailand: A cohort study. I. Epidemiology of symptomatic acute dengue infection in children, 2006-2009. *PLoS Neglected Tropical Diseases* 6(7):e1732. doi: 10.1371/journal.pntd.0001732.

Schilte, C., F. Staikowsky, T. Couderc, Y. Madec, F. Carpentier, S. Kassab, M. L. Albert, M. Lecuit, and A. Michault. 2013. Chikungunya virus-associated long-term arthralgia: A 36-month prospective longitudinal study. *PLoS Neglected Tropical Diseases* 7(3):e2137. doi: 10.1371/journal.pntd.0002137.

Semenza, J. C., and D. Domanovic. 2013. Blood supply under threat. *Nature Climate Change* 3(5):432-435.

Semenza, J. C., J. E. Suk, V. Estevez, K. L. Ebi, and E. Lindgren. 2012. Mapping climate change vulnerabilities to infectious diseases in Europe. *Environmental Health Perspectives* 120(3):385-392. doi: 10.1289/ehp.1103805.

Semenza, J. C., B. Sudre, T. Oni, J. E. Suk, and J. Giesecke. 2013. Linking environmental drivers to infectious diseases: The European environment and epidemiology network. *PLoS Neglected Tropical Diseases* 7(7):e2323. doi: 10.1371/journal.pntd.0002323.

Semenza, J. C., B. Sudre, J. Miniota, M. Rossi, W. Hu, D. Kossowsky, J. E. Suk, W. Van Bortel, and K. Khan. 2014. International dispersal of dengue through air travel: Importation risk for Europe. *PLoS Neglected Tropical Diseases* 8(12):e3278. doi: 10.1371/journal.pntd.0003278.

Siraj, A. S., M. Santos-Vega, M. J. Bouma, D. Yadeta, D. Ruiz Carrascal, and M. Pascual. 2014. Altitudinal changes in malaria incidence in highlands of Ethiopia and Colombia. *Science* 343(6175):1154-1158.

Smith, D. L., J. M. Cohen, C. Chiyaka, G. Johnston, P. W. Gething, R. Gosling, C. O. Buckee, R. Laxminarayan, S. I. Hay, and A. J. Tatem. 2013. A sticky situation: The unexpected stability of malaria elimination. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 368(1623):20120145. doi: 10.1098/rstb.2012.0145.

Stramer, S. L., J. M. Linnen, J. M. Carrick, G. A. Foster, D. E. Krysztof, S. Zou, R. Y. Dodd, L. M. Tirado-Marrero, E. Hunsperger, G. A. Santiago, J. L. Munoz-Jordan, and K. M. Tomashek. 2012. Dengue viremia in blood donors identified by RNA and detection of dengue transfusion transmission during the 2007 dengue outbreak in Puerto Rico. *Transfusion* 52(8):1657-1666. doi: 10.1111/j.1537-2995.2012.03566.x.

Streit, J. A., M. Yang, J. E. Cavanaugh, and P. M. Polgreen. 2011. Upward trend in dengue incidence among hospitalized patients, United States. *Emerging Infectious Diseases* 17(5):914-916. doi: 10.3201/eid1705.101023.

Sudre, B., M. Rossi, W. Van Bortel, K. Danis, A. Baka, N. Vakalis, and J. C. Semenza. 2013. Mapping environmental suitability for malaria transmission, Greece. *Emerging Infectious Diseases* 19(5):784-786. doi: 10.3201/eid1905.120811.

Suk, J. E., and J. C. Semenza. 2011. Future infectious disease threats to Europe. *America Journal of Public Health* 101(11):2068-2079. doi: 10.2105/AJPH.2011.300181.

Thiberville, S. D., N. Moyen, L. Dupuis-Maguiraga, A. Nougairede, E. A. Gould, P. Roques, and X. de Lamballerie. 2013. Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research* 99(3):345-370. doi: 10.1016/j.antiviral.2013.06.009.

Tran, A., B. Sudre, S. Paz, M. Rossi, A. Desbrosse, V. Chevalier, and J. C. Semenza. 2014. Environmental predictors of West Nile fever risk in Europe. *International Journal of Health Geographics* 13:26. doi: 10.1186/1476-072X-13-26.

Trape, J. F., A. Tall, C. Sokhna, A. B. Ly, N. Diagne, O. Ndiath, C. Mazenot, V. Richard, A. Badiane, F. Dieye-Ba, J. Faye, G. Ndiaye, F. Diene Sarr, C. Roucher, C. Bouganali, H. Bassene, A. Toure-Balde, C. Roussilhon, R. Perraut, A. Spiegel, J. L. Sarthou, L. P. da Silva, O. Mercereau-Puijalon, P. Druilhe, and C. Rogier. 2014. The rise and fall of malaria in a West African rural community, Dielmo, Senegal, from 1990 to 2012: A 22-year longitudinal study. *Lancet Infectious Diseases* 14(6):476-488. doi: 10.1016/S1473-3099(14)70712-1.

University of California Agriculture and Natural Resources. 2013. Asian citrus psyllid and huanglongbing disease. http://www.ipm.ucdavis.edu/PMG/PESTNOTES/pn74155.html (accessed December 12, 2014).

Vega-Rua, A., K. Zouache, R. Girod, A. B. Failloux, and R. Lourenco-de-Oliveira. 2014. High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of chikungunya virus. *Journal of Virology* 88(11):6294-6306. doi: 10.1128/JVI.00370-14.

von Seidlein, L., K. Ikonomidis, R. Bruun, M. Jawara, M. Pinder, B. G. Knols, and J. B. Knudsen. 2012. Airflow attenuation and bed net utilization: Observations from Africa and Asia. *Malaria Journal* 11:200. doi: 10.1186/1475-2875-11-200.

Vontas, J., S. Moore, I. Kleinschmidt, H. Ranson, S. Lindsay, C. Lengeler, N. Hamon, T. McLean, and J. Hemingway. 2014. Framework for rapid assessment and adoption of new vector control tools. *Trends in Parasitology* 30(4):191-204. doi: 10.1016/j.pt.2014.02.005.

Weaver, S. C., and W. K. Reisen. 2010. Present and future arboviral threats. *Antiviral Research* 85(2):328-345. doi: 10.1016/j.antiviral.2009.10.008.

Weaver, S. C., J. E. Osorio, J. A. Livengood, R. Chen, and D. T. Stinchcomb. 2012. Chikungunya virus and prospects for a vaccine. *Expert Review of Vaccines* 11(9):1087-1101. doi: 10.1586/erv.12.84.

Weintraub, P. G., and L. Beanland. 2006. Insect vectors of phytoplasmas. *Annual Review of Entomology* 51:91-111. doi: 10.1146/annurev.ento.51.110104.151039.

White, N. J., S. Pukrittayakamee, T. T. Hien, M. A. Faiz, O. A. Mokuolu, and A. M. Dondorp. 2014. Malaria. *Lancet* 383(9918):723-35. doi: 10.1016/S0140-6736(13)60024-0.

Whitfield, A. E., and D. Rotenberg. 2015. Disruption of insect transmission of plant viruses. *Current Opinion in Insect Science* 8:79-87.

Whitfield, A. E., D. Rotenberg, and T. L. German. 2014. Plant pest destruction goes viral. *Nature Biotechnology* 32(1):65-66. doi: 10.1038/nbt.2787.

WHO (World Health Organization). 2014a. *A global brief on vector-borne diseases*. Geneva, Switzerland: World Health Organization.

WHO. 2014b. Chikungunya Fact Sheet No. 327. http://www.who.int/mediacentre/factsheets/fs327/en/ (accessed December 29, 2014).

Wilcox, B. A., and D. J. Gubler. 2005. Disease ecology and the global emergence of zoonotic pathogens. *Environmental Health and Preventive Medicine* 10(5)263-272.

Wiser, M. F. 2013. Kinetoplastids. http://www.tulane.edu/~wiser/protozoology/notes/kinet.html (accessed November 25, 2014).

Yao, J., D. Rotenberg, A. Afsharifar, K. Barandoc-Alviar, and A. E. Whitfield. 2013. Development of RNAi methods for *Peregrinus maidis*, the corn planthopper. *PLoS ONE* 8(8):e70243. doi: 10.1371/journal.pone.0070243.

Zhou, J., and I. E. Tzanetakis. 2013. Epidemiology of soybean vein necrosis-associated virus. *Phytopathology* 103(9):966-971. doi: 10.1094/PHYTO-12-12-0322-R.

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EMERGING INSECT-TRANSMITTED PLANT DISEASES: THE BACTERIUM *XYLELLA FASTIDIOSA* AS A CASE STUDY[1]

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Introduction

Emerging vector-borne plant diseases may have severe economic, social, environmental, and cultural impacts. Factors driving the emergence of these diseases include vector and/or pathogen introductions into new areas where susceptible plant host species occur, the adaptation of pathogens and their vectors to management strategies such as pesticides or pathogen resistant plant varietal selections, the emergence of novel pathogens, as well as human-mediated environmental changes such deforestation and climate. Unlike animal and human emerging diseases, however, there is no recent large scale analysis of global trends of the types of emerging diseases affecting plants, or what are the main factors driving the emergence of these diseases (the last analysis being Anderson et al., 2004). For this reason, we chose to address the issue of the emergence of plant diseases by choosing one representative pathogen, and exploring some of the factors responsible for its rise from relative obscurity one or two decades ago (Hopkins and Purcell, 2002; Purcell, 2013). Thus, in this Chapter we address in detail the factors affecting the emergence of one important insect-transmitted plant pathogen, the bacterium *Xylella fastidiosa*.

Biology of a Plant and Insect Colonizer

Xylella fastidiosa is a bacterium that colonizes two distinct habitats, the xylem network of host plants and the foregut of xylem-sap feeding insects (Chatterjee et al., 2008). Processes leading to plant colonization are yet to be fully understood. Movement from vessel to vessel occurs through intact and damaged pit membranes, and is a necessary process for successful *X. fastidiosa* movement within plants (Baccari and Lindow, 2011; Chatelet et al., 2006; Newman et al., 2003). The specific mechanisms leading to disease remain poorly understood, but recently studies addressing this question from a host plant perspective suggest

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that symptom development is, initially, a consequence of physiological responses commenced by water deficit responses (Choi et al., 2013; Daugherty et al., 2010b; Sun et al., 2013).

Research on *X. fastidiosa* focuses on its role as a plant pathogen; however, to understand its ecology and evolution we propose that a broader view is necessary, recognizing that disease is the outcome of interactions between specific pathogen genotypes and host species (Casadevall and Pirofski, 2014). Infection dynamics of *X. fastidiosa* will be influenced by the extensive list of host plants species that can be infected (at least temporarily), the plant-host specificity of different genotypes, and the wide range of potential insect vectors. In 1995 Hill and Purcell (1995) compiled published data and concluded that plants in 29 families were hosts of this bacterium. More recently, a report listed 309 plant species in 63 families as hosts of *X. fastidiosa* (EFSA PLH Panel, 2015). This bacterium is capable of persisting at the inoculation site in many plant species under greenhouse and field conditions when either insect or mechanically inoculated (Purcell and Saunders, 1999). It can also be recovered from a wide range of weedy plants in infected agricultural areas (e.g., Lopes et al., 2003). *X. fastidiosa* does not appear to cause disease in most of these species; however the available data suggest that these asymptomatic infections typically declined over time (Purcell and Saunders, 1999). Thus, *X. fastidiosa* colonization of plants does not equal disease development.

Even though *X. fastidiosa* is a plant pathogen of considerable economic importance, mechanisms of host plant-pathogen specificity remain unknown and a major question in the field. The limited genomic structural variability within *X. fastidiosa* suggests phylogenetic groups colonizing different host plants have similar pathogenicity machineries (van Sluys et al., 2003). The only study to address the mechanisms of host plant specificity experimentally showed that an isolate could expand its host range if a cell-cell signaling-based gene regulation system was disrupted, suggesting that alleles or gene regulation, but not loci, were associated with specificity (Killiny and Almeida, 2011). But the approach used did not allow for the identification of candidate loci for future testing, and therefore the question remains largely open.

Disease is the outcome of complex *X. fastidiosa*-plant interactions and is obviously important, but it is not known what proportion of the interactions *X. fastidiosa* engages in within natural ecosystems result in plant disease. This leaves open the possibility that disease may represent a relatively small proportion of these interactions, leading to the suggestion that *X. fastidiosa* may be considered primarily an endophyte rather than a pathogen (Chatterjee et al., 2008). It may be notable that in *X. fastidiosa* cell-cell signaling regulates limited virulence to plants while promoting vector plant-to-plant transmission (Newman et al., 2004). Transmission rates can also be directly affected by vectors responding to disease symptoms. In two *X. fastidiosa* disease systems studied, sharpshooter leafhoppers do not avoid infected yet asymptomatic plants, but discriminate against infected and symptomatic plants, or healthy plants painted to simulate disease

symptoms (Daugherty et al., 2011; Marucci et al., 2005). This behavior may be advantageous for these insects: water stressed- and *X. fastidiosa*-infected plants have some shared physiological characteristics, of which xylem sap under high tension is of paramount relevance. Increased tension in the water column leads to a food source that is energetically expensive, resulting in the ingestion of less xylem sap (Andersen et al., 1992; Miranda et al., 2013) and possibly promoting the movement of vectors to another host. Since symptomatic plants are heavily colonized by *X. fastidiosa* (Newman et al., 2003), vector avoidance may act to reduce transmission rates (Zeilinger and Daugherty, 2014) and select for decreased bacterial virulence. This effect could be important when transmission rates are low; however, if vectors are common and transmission rates high, rapid bacterial growth leading to increased virulence may be favored, a pattern often observed in diseases that are transmitted between hosts (e.g., malaria; de Roode et al., 2005).

Experimentally identified insect vectors of *X. fastidiosa* belong to two insect groups, the sharpshooter leafhoppers (Cicadellidae, Cicadellinae) and spittlebugs (superfamily Cercopoidea, with five species of Aphrophoridae and two species of Clastopteridae identified) (Almeida et al., 2005; EFSA PLH Panel, 2015). In addition, there are two reports of cicadas (Cicadidae) transmitting *X. fastidiosa* (Krell et al., 2007; Paião et al., 1996), which need to be confirmed through larger experiments. Colonization of these insects by *X. fastidiosa* occurs in a non-circulative yet persistent manner (Purcell and Finlay, 1979), with the bacterium colonizing the foregut on insect vectors (Purcell et al., 1979). Consequently, there is no transovarial or transtadial transmission (Almeida and Purcell, 2003; Freitag, 1951; Purcell and Finlay, 1979). Colonization of regions in the foregut called cibarium and precibarium were first shown microscopically (Brlansky et al., 1983; Purcell et al., 1979), and later correlated with insect inoculation of plant hosts during feeding (Almeida and Purcell, 2006). So far, no other plant pathogen is known to be transmitted in a similar manner, with the possible exception of *Ralstonia syzigii*, which is transmitted by spittlebugs in the Machaerotidae (Eden-Green et al., 1992).

Transmission efficiency of *X. fastidiosa* increases with both the time an insect feeds on an infected host plant (acquisition) and the subsequent time it feeds on an uninfected host (inoculation), up to 48-96 hours (Almeida and Purcell, 2003; Purcell and Finlay, 1979). Presumably a longer feeding time increases the likelihood of insect vectors reaching colonized xylem vessels in the case of acquisition, and performing specific probing behaviors in the case of inoculation. The colonization of a vector by the bacteria is a critical part of acquisition and is a complex process, similar to biofilm formation on surfaces, which has been explored in some detail (e.g., Killiny and Almeida, 2009a, b, 2014). Specific probing behaviors involved in inoculation are yet to be determined; however the inoculation of *X. fastidiosa* into dormant grapevines with positive xylem sap pressure (positive root pressure) indicates that vector behaviors are required for the inoculation of bacterial cells into plants (Almeida et al., 2005).

One important aspect of *X. fastidiosa* transmission relevant to the emergence of new diseases is that it lacks vector specificity (Almeida et al., 2005). The insect groups that transmit *X. fastidiosa* are distributed worldwide in tropical and temperate climates, and all insect species belonging to the above-mentioned groups should be considered as potential vectors until proven otherwise. For example, one vector species has been shown to transmit *X. fastidiosa* isolates belonging to four different *X. fastidiosa* subspecies (Almeida and Purcell, 2003; Purcell et al., 1999; Sanderlin and Melanson, 2010; Saponari et al., 2014). And a *X. fastidiosa* subspecies originally from South America has been transmitted by various vectors in South America, one in North America, and another in Europe (Brlansky et al., 2002; Damsteegt et al., 2006; Marucci et al., 2008; Saponari et al., 2014). This lack of specificity increases the likelihood that newly introduced *X. fastidiosa* isolates, when reaching a novel environment, will be transmitted by an endemic vector species. However, while the ability to transmit *X. fastidiosa* is widespread, transmission efficiency is highly variable and dependent on a range of vector-plant-pathogen interactions (Lopes et al., 2009). Transmission efficiency may vary for different vector species on the same host plant (Daugherty and Almeida, 2009; Lopes et al., 2009), or the same vector species feeding on different tissues of the same plant (Daugherty et al., 2010a); however, observations suggest that the general mechanisms of transmission are conserved. The one caveat is that most of the research on *X. fastidiosa* transmission has been conducted with two vector species (*Graphocephala atropunctata* and *Homalodisca vitripennis*) and one *X. fastidiosa* subspecies (subsp. *fastidiosa*), so it is important that a broader range of taxa be studied to confirm these results. Until that is done, the effectiveness of individual sharpshooter leafhopper species in transmitting *X. fastidiosa* should not be extrapolated from epidemic to epidemic without considering the novel ecological context.

A Plant Generalist or Not: Revisiting *Xylella fastidiosa* Systematics

Xylella fastidiosa currently is the sole species in the genus *Xylella*; *Xanthomonas* spp. are sister taxa to *X. fastidiosa* (Retchless et al., 2014). As noted earlier, *X. fastidiosa* has traditionally been referred to as a having a "wide host range" or as a "generalist". This is accurate in the sense that a very large number of plant species have been demonstrated to sustain *X. fastidiosa* infections; however, there is mounting evidence suggesting that while this description is accurate it is misleading. Specifically, very few of these plants sustain long-term infections and become symptomatic. Furthermore, it is now clear that specific symptomatic hosts are only susceptible to isolates in one or a limited number of *X. fastidiosa* phylogenetic clades, with the result that specific clades of *X. fastidiosa* have a small number of symptomatic host plant species (Nunney et al., 2013). Such insights are of great relevance in understanding disease outbreaks.

We revisit *X. fastidiosa* taxonomy in face of new findings and, consequently, novel questions, with two important caveats. First, it is fully expected that new clades of *X. fastidiosa* will be reported in the future (e.g., Nunney et al., 2014a). Second, many of the plant species listed as hosts of *X. fastidiosa* should in fact be considered putative hosts since most associations studied so far are derived from symptomatic plant tissue, but without experimental work to confirm the pathogenicity of isolates. Although associations are relevant, the fulfillment of Koch's postulates is a requirement to demonstrate that individual genotypes are pathogenic to specific host plant species. The importance of experimental work to determine the host range of pathogens remains paramount. It is possible that ecological conditions limit the host range and/or virulence of pathogens, which may be 'released' in new environments where other vector species and host plants are present, or abiotic factors such as climate and precipitation vary. In summary, we emphasize the importance of experimentally determining plant species susceptibility to *X. fastidiosa*, as there are plant species-pathogen genotype associations that do not lead to disease. Moreover, even when symptoms eventually develop, a delay of several months following infection is not uncommon. These issues are especially relevant given the economic and quarantine importance of this bacterial species. In this context, it is important to note that so far no native plant hosts of *X. fastidiosa* in South and Central America have been identified. In contrast, a number of native hosts (primarily trees) of the North American subsp. *multiplex* have been identified, including several oak species (*Quercus* spp.), American elm (*Ulmus Americana*), American sycamore (*Platanus occidentalis*), sweetgum (*Liquidambar styraciflua*), and pecan (*Carya illinoinensis*) (for a more complete list see Table 2 in Nunney et al. [2013]).

Studies of *X. fastidiosa* genetic and phenotypic diversity have historically been confusing and inconclusive, despite the efforts of a small and dedicated group of scientists. Purcell (2013) made a case for the importance of researchers naïve to a new field of science being able to address old questions, and the advent of DNA sequencing has facilitated studies of *X. fastidiosa* diversity and host range that were until recently technically intractable. As a result, we can argue that the main drivers of conflicting results can be summarized under four headings. First, isolates from a small range of host plants and geographical distribution have been used for studies, over-representing a limited and narrow sampling of genetic diversity. Second, procedures for typing have relied on within-study comparisons of the above-mentioned small number of available isolates with methods that provided inadequate phylogenetic resolution. Third, methodological differences in the typing of isolates limited comparisons among studies, a problem that is disappearing now that sequencing technology is easily available worldwide. Lastly, *X. fastidiosa* is naturally competent (Kung and Almeida, 2011); gene flow deeply impacts the systematics and evolution of bacteria (Polz et al., 2013).

The current view of *X. fastidiosa* genetic diversity has overcome most of these limitations, largely through the use of a portable multi-locus sequence

typing (MLST) approach (Maiden et al., 1998). MLST for *X. fastidiosa* was first introduced by Scally in 2005 (Scally et al., 2005) and refined by five years later into the form currently employed (Yuan et al., 2010). MLST has been successfully used to study *X. fastidiosa* diversity at the species/subspecies level, and to infer the phylogenetic placement of newly identified isolates. These data have resulted in a robust taxonomy for the species. Furthermore, the MLST classification of isolates into sequence types (STs) (unique genotypes based on the 7 loci used in MLST) has provided insights about *X. fastidiosa* evolution and host specificity. For example, comparing subsp. *pauca* STs found on coffee and citrus, it has been shown that in general they are reciprocally host specific (Almeida et al., 2008; Nunney et al., 2012).

Based on current knowledge, *X. fastidiosa* is primarily a species of the Americas. A distant relative is found in Taiwan (Su et al., 2014), but should probably be classified as a separate species. Two other exceptions that must yet be confirmed and for which no genetic information is available, are reports from Iran (Amanifar et al., 2014) and Turkey (Guldur et al., 2005). Lastly, the recent introduction of *X. fastidiosa* into Italy is an important change to its geographical distribution (Saponari et al., 2013). The American representatives were initially divided into three subspecies subsp. *fastidiosa*, *multiplex* and *pauca* based on DNA-DNA hybridization data (Schaad et al., 2004). MLST sequence data confirmed the status of these subspecies, and suggested a fourth, subsp. *sandyi*, which was not present among the earlier strains that were tested (Scally et al., 2005). Subsequent sampling and analysis based on MLST has indicated that these subspecies evolved in geographical isolation with subsp. *pauca* native to South America (Nunney et al., 2012), subsp. *multiplex* native to temperate and subtropical North America (Nunney et al., 2012, 2014b), subsp. *fastidiosa* is found in Costa Rica and is presumed to be native to southern Central America (Nunney et al., 2010), and subsp. *sandyi* has only been detected in southern regions of the USA (Yuan et al., 2010). Subspecies *morus* represents a new proposal and is discussed below. Historical geographical isolation of the original four subspecies is consistent with the known biology of *X. fastidiosa*: this bacterium can only invade a new region by long-distance dispersal of infected insects or infected plants. In the absence of human intervention, the former is very unlikely and the latter is close to impossible. However, it has become apparent that in the recent past human-mediated invasion is the primary driver of economically costly *X. fastidiosa* introductions. We discuss three main pathways leading to the emergence of *X. fastidiosa* diseases, following examples available in the literature.

Introduction of Exotic Genotypes

The most common pathway leading to *X. fastidiosa* epidemics is the introduction of exotic genotypes into environments that are ecologically prone to the maintenance of the bacterium in the plant community. Although the introduction

of insect vectors carrying *X. fastidiosa* represents a potential pathway, only one vector species is considered invasive (*Homalodisca vitripennis*, Cicadellidae, a sharpshooter leafhopper), and another is distributed beyond its region of origin (*Philaneus spumarius*, Aphrophoridae, a spittlebug). The expansion in the geographical range of these species has not been associated with the spread of *X. fastidiosa*, therefore we consider this an unlikely route. The main dispersal pathway would then be the movement of infected, and potentially asymptomatic, plant material from areas where the pathogen occurs. A recent report by the European Food Safety Authority evaluation on the risk of *X. fastidiosa* introductions into the European Union reached similar conclusions (EFSA Panel on Plant Health, 2015) with a much more detailed and systematic analysis of potential pathways. Here we discuss examples with conclusive evidence from the available literature.

The most recent case of an introduction is the outbreak of rapid olive decline in the Apuglia region in southern Italy, first reported in October 2013 (Saponari et al., 2013). While the distribution and consequences of this introduction are yet to be determined, it is known that this outbreak is associated with a strain of *X. fastidiosa* subsp. *pauca* classified as ST53 (Elbeaino et al., 2014). Subspecies *pauca* is of South American origin but this sequence type so far has not been found in South America; however, it has been detected in Costa Rica infecting primarily oleander (Nunney et al., 2014a). Thus, this particular ST of subsp. *pauca* has now been introduced into two new regions, and infecting novel hosts. While olive is currently considered the primary host in the Italian outbreak, infection of oleander has also been observed, illustrating a common feature of *X. fastidiosa*: oleander and olive are hosts of the same strain, and yet they are in different Orders (Gentianales vs. Lamiales). As a result, given our current knowledge, it is not possible to predict potential hosts following an invasion.

Yet another introduction involved the best studied *X. fastidiosa* disease, Pierce's disease of grapevines. It had been first proposed that the Gulf Coast Plain area of the USA was the center of origin of the etiological agent of the disease based on the fact that species of grapevines (*Vitis* spp.) native to the USA were tolerant to infection, while the exotic European grapevine (*Vitis vinifera*) was susceptible (Hewitt, 1958). With the recent availability of larger datasets on the genetic diversity of *X. fastidiosa*, we now know that the genotype causing disease in grapevines in the USA originated from Central America (Nunney et al., 2010). The lack of genetic diversity among isolates belonging to this clade in the USA is evidence of a relatively recent introduction (Yuan et al., 2010), and it has been proposed that the introduction into the USA of a single genotype was via an infected coffee plant, a known host of *X. fastidiosa* in Central America (Nunney et al., 2010). Isolates derived from this single genotype are now widely distributed through grape-growing regions of the USA, from Florida to California. Interestingly, an isolate from this same almost monomorphic clade found in the USA has now been reported causing Pierce's disease of grapevines in Taiwan (Su et al., 2013), suggesting that *X. fastidiosa* infected-plant material originating from

the USA was inadvertently introduced into the country, eventually leading to an epidemic.

A similar scenario appears to have occurred with the emergence of plum leaf scald in Argentina, Paraguay, and Brazil (French and Kitajima, 1978; Kitajima et al., 1975). The disease in plum and other *Prunus* species were known in the southeast USA, but the origin of the *X. fastidiosa* genotype(s) causing plum leaf scald in South America remained unidentified until Nunes et al. (2003) studied the gene content of several isolates. They determined that the tested plum isolate from Brazil grouped with North instead of South American isolates (i.e., belonging to subsp. *multiplex*), demonstrating yet another introduction, this time from the USA to South America. These examples illustrate the challenges of limiting the inadvertent transportation of *X. fastidiosa*-infected plant material from one country, or continent, to another. For a detailed risk assessment analysis of *X. fastidiosa* introductions we direct readers to a recent review by the European Food Safety Authority (EFSA Panel on Plant Health, 2015).

Introduction of an Invasive Vector

To our knowledge there is only one example of a *X. fastidiosa* vector being considered invasive, spreading over vast geographical distances and reaching large populations at various environmental conditions (Grandgirard et al., 2006; Petit et al., 2008). *Homalodisca vitripennis* (Cicadellidae, Cicadellinae) is native to the southeastern USA (Turner and Pollard 1959; Young, 1958); in 1989 it was first detected in California (Sorensen and Gill, 1996), but only in the late 1990s it became a problem as the vector of *X. fastidiosa* driving the oleander leaf scorch epidemic in Southern California (Purcell et al., 1999), and at the same time a Pierce's disease epidemic in the grape-growing region of Temecula, also in Southern California (Hopkins and Purcell, 2002). Estimates suggested 1–2 million insects per hectare in the region (Coviella et al., 2006), populations which are thought to have allowed for its transportation to French Polynesia (Grandgirard et al., 2006), where biological control successfully controlled very large populations that developed in those tropical regions (Grandgirard et al., 2008). It is notable that these invasions were not associated with the introduction of *X. fastidiosa*, supporting our view that the primary mechanism of *X. fastidiosa* invasions is the movement of infected live plants. In the case of California, the introduction of *H. vitripennis* had several important consequences; we focus here on the emergence of *X. fastidiosa* diseases alone. Newton Pierce, after whom Pierce's disease was later named, studied the first known outbreak of the disease that was in Southern California (Pierce, 1892). Since that time, *X. fastidiosa* has been regularly reported in grapevines, almonds, and alfalfa, indicating it has been continuously present. However, disease outbreaks were primarily limited to small areas, apparently due to habitat specific of endemic vectors and the broader ecological context.

There were two main consequences associated with the extremely large populations of *H. vitripennis* in southern California in the two decades subsequent to its introduction (see Almeida, 2008, for further discussion). The first was the development of a Pierce's disease epidemic, where very large populations of a relatively inefficient vector (*H. vitripennis* is not an efficient vector on grapevines when compared to other species), (Daugherty and Almeida, 2009) led to the effective spread of the pathogen to a focal crop under new ecological conditions, decimating the vineyards of the Temecula region (Hopkins and Purcell, 2002). Chemical control of *H. vitripennis* populations in the region has led to the restoration of the local wine industry to economically profitable levels (M. Daugherty personal communication). The second consequence is based on associations rather than conclusive epidemiological data; yet, the contention is well supported by field observations. We contend that the introduction of the highly polyphagous *H. vitripennis*, led to the establishment of various *X. fastidiosa* diseases in Southern California, notably oleander leaf scorch (Purcell et al., 1999) and scorch diseases of a range of ornamental trees (Hernandez-Martinez et al., 2007, 2009). A large list of diseases associated with *X. fastidiosa* has been generated, albeit Koch's postulates have only been fulfilled for a few of them (e.g., Hernandez-Martinez et al., 2009; Purcell et al., 1999). We suggest that *X. fastidiosa* genotypes had been widely established in Southern California ahead of the *H. vitripennis* invasion, albeit restricted to disease cycles with endemic vectors and asymptomatic hosts or associated with species where it caused disease rarely enough to be overlooked. The presence of *H. vitripennis* resulted in increments of such rare events due to its large populations, or in the displacement of genotypes from endemic cycles to disease cycles that incorporated hosts of this invasive vector. The lack of vector-pathogen specificity is the trait most responsible for this outcome. In fact, *H. vitripennis* is the only vector species shown to transmit *X. fastidiosa* belonging to all currently accepted subspecies (*fastidiosa*, *multiplex*, *sandyi*, and *pauca*), although this should be expected from all known and potential *X. fastidiosa* vector species.

Recombination and Adaptation to New Plant Hosts

The anthropogenic introduction of *X. fastidiosa* subspecies into new regions can have two effects: the emergence of a known disease in a new area, and/or the emergence of a new disease involving a new plant host. In this section we focus on the second of these possibilities. One example of *X. fastidiosa* invading a new host is the case of mulberry leaf scorch. It was first noted in the early 1980s in Washington DC, and subsequent sampling revealed infected trees (the native *Morus rubra*) along the eastern seaboard as far north as southern New York (Kostka et al., 1986). Within a few years the disease was found on the west coast with infected trees (the introduced *Morus alba*) observed in California (Hernandez-Martinez et al., 2007). Initial genetic typing showed that the mulberry

isolates always grouped together, but their relationship to the other subspecies was marker dependent. The reason for this ambiguity was revealed using MLST: the genome is a roughly equal mix of genetic material from subsp. *fastidiosa* and *multiplex*, such that an examination of the 7 MLST loci revealed 3 alleles from subsp. *fastidiosa*, 3 from subsp. *multiplex*, and one chimeric allele containing sequence from both subspecies and consequently a recombination breakpoint (Nunney et al., 2014c). All other forms of *X. fastidiosa* are genetically very distinct from the mulberry type, which themselves show almost no genetic variability. Since they do not group with any pre-existing subspecies, and since they appear to be unique in naturally infecting mulberry, it's been proposed that they define a new subspecies (subsp. *morus*), that was created by one or more massive genetic exchanges between subsp. *fastidiosa* and *multiplex* that created a chimeric genome via inter-subspecific homologous recombination (IHR) (Nunney et al., in preparation).

The genetic exchange that created subsp. *morus* has also resulted in a group of genotypes (recombinant multiplex) that cluster with subsp. *multiplex*, presumably due to repeated backcross exchanges with the native subspecies (*multiplex*) (Nunney et al., 2014b). Of interest is that the isolates from diseased blueberry plants (from Georgia and Florida) were all of only two sequence types, both of which were recombinant subsp. *multiplex*. No non-recombinant subsp. *multiplex* have yet been isolated from blueberry strongly suggesting that we have a second example of genetic mixing between an introduced and native subspecies resulting in the infection of a new host. The involvement of IHR in the genesis of subsp. *morus*, and the subsequent formation of the group of recombinant subsp. *multiplex*, might seem like a special event unlikely to be repeated; however, we now have evidence that a similar genetic exchange occurred in South America. Studies of citrus and coffee *X. fastidiosa* isolates from Brazil have provided evidence of IHR from subsp. *multiplex* to subsp. *pauca* (Almeida et al., 2008; Nunney et al., 2012). Based on MLST data, Nunney et al. (2012) estimated that about half of the genome was polymorphic for subsp. *multiplex* sequence, suggesting that, as in the case of subsp. *morus*, one or more major genetic exchanges had occurred. However, non-recombinant subsp. *pauca* has not been found, although it seems probable that it will eventually be isolated by more thorough sampling away from agricultural areas. These examples highlight the important question of the consequences of gene flow on the emergence of *X. fastidiosa* diseases. We propose that the introduction of novel allelic diversity into countries/regions where *X. fastidiosa* is already present poses a significant risk and should be a major concern to regulatory bodies around the world.

In addition to host species switches induced by IHR, genetic exchange within subspecies occurs (Almeida et al., 2008; Nunney et al., 2013). This, together with IHR, may be highly relevant in determining the ability of *X. fastidiosa* to adapt to resistant plant genotypes. Specifically, the breeding programs that are developing resistant plant material for various *X. fastidiosa* hosts (notably wine grapes) should take into account the potential for *X. fastidiosa* to adapt. The groups of bacterial

genes that are frequently exchanged and maintained in a population and those that are quickly purged have not been identified. Similarly, general patterns of short- and long-term genome evolution have so far not been analyzed. These are essential components for the robust deployment of resistant plant material, transgenic or not, as the strong selective pressure on *X. fastidiosa* populations due to the usage of new technologies will eventually lead to the selection of novel pathogen variants that are capable of breaking down resistance. This process is equivalent to antibiotic resistance strains of human pathogens, such as tuberculosis, or loss of *Bacillus thuringiensis* derived plant resistance to pests. Our argument is not that new technologies will not be successful; our argument is that the evolution of *X. fastidiosa* needs to be considered and incorporated into management practices aimed at prolonging the utilization of such plant lines. That, however, cannot be done with the very superficial and limited knowledge currently available.

Last Thoughts

Xylella fastidiosa is no longer a plant pathogen limited to a few countries in the Americas, where its geographical distribution ranges from Canada to Argentina. The long-term presence of *X. fastidiosa* in Taiwan raises questions about its potential distribution in Asia, and its introduction into Europe and recent report from Iran will dramatically and permanently change its geographic range. Is this bacterium present elsewhere, or where is it not present? And, as shown recently in Central America (Nunney et al., 2014a), how much of the genetic diversity of *X. fastidiosa* remains to be described? Old and unaddressed questions are now more relevant than ever, especially for Europe and the Mediterranean basin, where the plant community has, as far as we know, not been exposed to *X. fastidiosa*. Among those is what drives host specificity in this pathogen, in other words, why do genotypes cause disease in one plant species and not another, while still being able to colonize various plant species with different degrees of success without inducing symptom expression. Finally, we still know very little about *X. fastidiosa* outside of its crop hosts. We are strong believers that much would be gained from studies of *X. fastidiosa* in natural environments, no only in regards to its biology, ecology, and evolution, but also on how to better manage diseases it causes in crops of economic importance.

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References

Almeida, R. P. P. 2008. Ecology of emerging vector-borne plant diseases. In *Institute of Medicine Forum on Vector-Borne Diseases: Understanding the environmental, human health, and ecological connections*. Washington, DC: The National Academies Press.

Almeida, R. P. P., and A. H. Purcell. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera:Cicadellidae). *Journal of Economic Entomology* 96:264–271.

Almeida, R. P. P., and A. H. Purcell. 2006. Patterns of *Xylella fastidiosa* colonization on the precibarium of sharpshooter vectors relative to transmission to plants. *Annals of the Entomological Society of America* 99:884–890.

Almeida, R. P. P., M. J. Blua, J. R. S. Lopes, and A. H. Purcell. 2005a. Vector transmission of *Xylella fastidiosa*: Applying fundamental knowledge to generate disease management strategies. *Annals of the Entomological Society of America* 98:775–786.

Almeida, R. P. P., C. Wistrom, B. L. Hill, J. Hashim, and A. H. Purcell. 2005b. Vector transmission of *Xylella fastidiosa* to dormant grape. *Plant Disease* 89:419–424.

Almeida, R. P. P., F. E. Nascimento, J. Chau, S. S. Prado, C.-W. Tsai, S. A. Lopes, and J. R. S. Lopes. 2008. Genetic structure and biology of *Xylella fastidiosa* strains causing disease in citrus and coffee in Brazil. *Applied and Environmental Microbiology* 74:3690–3701.

Amanifar, N., M. Taghavi, K. Izadpanah, and G. Babaei. 2014. Isolation and pathogenicity of *Xylella fastidiosa* from grapevine and almond in Iran. *Phytopathologia Mediterranea* 53:318–327.

Andersen, P. C., B. V. Brodbeck, and R. F. Mizell III. 1992. Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. *Journal of Insect Physiology* 38:611–622.

Anderson, P. K., A. A. Cunningham, N. G. Patel, F. J. Morales, P. R. Epstein, amd P. Daszak. 2004. Emerging infectious diseases of plants: Pathogen pollution, climate change, and agrotechnology drivers. *Trends in Ecology and Evolution* 19:535–544.

Baccari, C., and S. E. Lindow. 2011. Assessment of the process of movement of *Xylella fastidiosa* within susceptible and resistant grape cultivars. *Phytopathology* 101:77–84.

Brlansky, R. H., L. W. Timmer, W. J. French, and R. E. McCoy. 1983. Colonization of the sharpshooter vectors, *Oncometopia nigricans* and *Homalodisca coagulata*, by xylem-limited bacteria. *Phytopathology* 73:530–535.

Brlansky, R. H., V. D. Damsteegt, and J. S. Hartung. 2002. Transmission of the citrus variegated chlorosis bacterium *Xylella fastidiosa* with the sharpshooter *Oncometopia nigricans*. *Plant Disease* 86:1237–1239.

Casadevall, A., and L.-A. Pirofski. 2014. Microbiology: Ditch the term pathogen. *Nature* 516:165–166.

Chatelet, D. S., M. A. Matthews, and T. L. Rost. 2006. Xylem structure and connectivity in grapevine (*Vitis vinifera*) shoots provides a passive mechanism for the spread of bacteria in grape plants. *Annals of Botany* 98:483–494.

Chatterjee, S., R. P. P. Almeida, and S. E. Lindow. 2008. Living in two worlds: The plant and insect lifestyles of *Xylella fastidiosa*. *Annual Review of Phytopathology* 46:243–271.

Choi, H.-K., A. Iandolino, F. G. Silva, and D. R. Cook. 2013. Water deficit modulates the response of *Vitis vinifera* to the Pierce's disease pathogen *Xylella fastidiosa*. *Molecular Plant-Microbe Interactions* 26:643–657.

Coviella, C. E., J. F. Garcia, D. R. Jeske, R. A. Redak, and R. F. Luck. 2006. Feasibility of tracking within-field movements of *Homalodisca coagulata* (Hemiptera: Cicadellidae) and estimating its densities using fluorescent dusts in mark-release–recapture experiments. *Journal of Economic Entomology* 99:1051–1057.

Damsteegt, V. D., R. H. Brlansky, P. A. Phillips, and A. Roy. 2006. Transmission of *Xylella fastidiosa*, causal agent of citrus variegated chlorosis, by the glassy-winged sharpshooter, *Homalodisca coagulata*. *Plant Disease* 90:567–570.

Daugherty, M. P., and R. P. P. Almeida. 2009. Estimating *Xylella fastidiosa* transmission parameters: Decoupling sharpshooter number and feeding period. *Entomologia Experimentalis et Applicata* 132:84–92.

Daugherty, M. P., J. R. S. Lopes, and R. P. P. Almeida. 2010a. Vector within-host feeding preference mediates transmission of a heterogeneously distributed pathogen. *Ecological Entomology* 35:360–366.

Daugherty, M. P., J. R. S. Lopes, and R. P. P. Almeida. 2010b. Strain-specific alfalfa water stress-induced by *Xylella fastidiosa*. *European Journal of Plant Pathology* 127:333–340.

Daugherty, M. P., A. Rashed, R. P. P. Almeida, and T. M. Perring. 2011. Vector preference for hosts differing in infection status: Sharpshooter movement and *Xylella fastidiosa* transmission. *Ecological Entomology* 36:654–662.

de Roode, J. C., R. Pansini, S. J. Cheesman, M. E. H. Helinski, S. Huijben, A. R. Wargo, A. S. Bell, B. H. K. Chan, D. Walliker, and A. F. Read. 2005. Virulence and competitive ability in genetically diverse malaria infections. *Proceedings of the National Academy of Sciences of the United States of America* 102:7624–7628.

Eden-Green, S. J., R. Balfas, and T. Sutarjo. 1992. Characteristics of the transmission of Sumatra disease of cloves by tube-building cercopoids, *Hindola* spp. *Plant Pathology* 41:702–712.

EFSA PLH Panel (EFSA Panel on Plant Health). 2015. Scientific opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. *EFSA Journal* 13:2989.

Elbeaino, T., F. Valentini, A. K. Kubaa, P. Moubarak, T. Yaseen, and M. Digiaro. 2014. Multilocus sequence typing of *Xylella fastidiosa* isolated from olive affected by 'Olive Quick Decline Syndrome' in Italy. *Phytopathologia Mediterranea* 53:533–542.

Freitag, J. H. 1951. Host range of the Pierce's disease virus of grapes as determined by insect transmission. *Phytopathology* 41:920–633.

French, W. J., and E. W. Kitajima. 1978. Occurrence of plum leaf scald in Brazil and Paraguay. *Plant Disease Reporter* 62:1035–1038.

Grandgirard, J., M. S. Hoddle, G. K. Roderick, J. N. Petit, D. Percy, R. Putoa, C. L. Garnier, and N. Davies. 2006. Invasion of French Polynesia by the glassy-winged sharpshooter, *Homalodisca coagulata* (Hemiptera: Cicadellidae): A new threat to the South Pacific. *Pacific Science* 60:429–438.

Grandgirard, J., M. S. Hoddle, J. N. Petit, G. K. Roderick, and N. Davies. 2008. Engineering an invasion: Classical biological control of the glassy-winged sharpshooter, *Homalodisca vitripennis*, by the egg parasitoid *Gonatocerus ashmeadi* in Tahiti and Moorea, French Polynesia. *Biological Invasions* 10:135–148.

Guldur, M. E., B. K. Caglar, M. A. Castellano, L. Ulnu, S. Guran, M. A. Yilmaz, and G. P. Martelli. 2005. First report of almond leaf scorch in Turkey. *Journal of Plant Pathology* 87:246.

Hernandez-Martinez, R., K. A. de la Cerda, H. S. Costa, D. A. Cooksey, and F. P. Wong. 2007. Phylogenetic relationships of *Xylella fastidiosa* strains isolated from landscape ornamentals in Southern California. *Phytopathology* 97:857–864.

Hernandez-Martinez, R., D. A. Cooksey, and F. P. Wong. 2009. Leaf scorch of purple-leafed plum and sweetgum dieback: Two new diseases in Southern California caused by *Xylella fastidiosa* strains with different host ranges. *Plant Disease* 93:1131–1138.

Hewitt, W. B. 1958. The probable home of Pierce's disease virus. *Plant Disease Reporter* 42:211–215.

Hill, B. L., and A. H. Purcell. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. *Phytopathology* 85:1368–1372.

Hopkins, D. L., and A. H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. *Plant Disease* 86:1056–1066.

Killiny, N., and R. P. P. Almeida. 2009a. *Xylella fastidiosa* afimbrial adhesins mediate cell transmission to plants by leafhopper vectors. *Applied and Environmental Microbiology* 75:521–528.

Killiny, N., and R. P. P. Almeida. 2009b. Host structural carbohydrate induces vector transmission of a bacterial plant pathogen. *Proceedings of the National Academy of Sciences of the United States of America* 106:22416–22420.

Killiny, N., and R. P. P. Almeida. 2011. Gene regulation mediates host specificity of a bacterial pathogen. *Environmental Microbiology Reports* 3:791–797.

Killiny, N., and R. P. P. Almeida. 2014. Factors affecting the initial adhesion and retention of the plant pathogen *Xylella fastidiosa* in the foregut of an insect vector. *Applied and Environmental Microbiology* 80:420–426.

Kitajima, E. W., M. Bakarcic, and M. V. Fernandez-Valiela. 1975. Association of rickettsialike bacteria with plum leaf scald disease. *Phytopathology* 65:476–479.

Kostka, S. J., T. A. Tattar, J. L. Sherald, and S. S. Hurtt. 1986. Mulberry leaf scorch, new disease caused by a fastidious, xylem-inhabiting bacterium. *Plant Disease* 70:690–693.

Krell, R. K., E. A. Boyd, J. E. Nay, Y.-L. Park, and T. M. Perring. 2007. Mechanical and insect transmission of *Xylella fastidiosa* to *Vitis vinifera*. *American Journal of Enology and Viticulture* 58:211–216.

Kung, S. H., and R. P. P. Almeida. 2011. Natural competence and recombination in the plant pathogen *Xylella fastidiosa*. *Applied and Environmental Microbiology* 77:5278–5284.

Lopes, J. R. S., M. P. Daugherty, and R. P. P. Almeida. 2009. Context-dependent transmission of a generalist plant pathogen: Host species and pathogen strain mediate insect vector competence. *Entomologia Experimentalis et Applicata* 131:216–224.

Lopes, S. A., S. Marcussi, S. C. Z. Torres, V. Souza, C. Fagan, S. C. França, N. G. Fernandes, and J. R. S. Lopes. 2003. Weeds as alternative hosts of the citrus, coffee, and plum strains of *Xylella fastidiosa* in Brazil. *Plant Disease* 87:544–549.

Maiden, M. C. J., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, et al., 1998. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America* 95:3140–3145.

Marucci, R. C., J. R. S. Lopes, J. D. Vendramim, and J. E. Corrente. 2005. Influence of *Xylella fastidiosa* infection of citrus on host selection by leafhopper vectors. *Entomologia Experimentalis et Applicata* 117:95–103.

Marucci, R. C., J. R. S. Lopes, and R. R. Cavichioli. 2008. Transmission efficiency of *Xylella fastidiosa* by sharpshooters (Hemiptera: Cicadellidae) in coffee and citrus. *Journal of Economic Entomology* 101:1114–1121.

Miranda, M. P., E. S. Villada, S. A. Lopes, A. Fereres, and J. R. S. Lopes. 2013. Influence of citrus plants infected with *Xylella fastidiosa* on stylet penetration activities of *Bucephalogonia xanthophis* (Hemiptera: Cicadellidae). *Annals of the Entomological Society of America* 106:610–618.

Newman, K. L., R. P. P. Almeida, A. H. Purcell, and S. E. Lindow. 2003. Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. *Applied and Environmental Microbiology* 69:7319–7327.

Newman, K. L., R. P. P. Almeida, A. H. Purcell, and S. E. Lindow. 2004. Cell-cell signaling controls *Xylella fastidiosa* interactions with both insects and plants. *Proceedings of the National Academy of Sciences of the United States of America* 101:1737–1742.

Nunes, L. R. 2003. Microarray analyses of *Xylella fastidiosa* provide evidence of coordinated transcription control of laterally transferred elements. *Genome Research* 13:570–578.

Nunney, L., X. Yuan, R. Bromley, J. Hartung, M. Montero-Astúa, L. Moreira, B. Ortiz, and R. Stouthamer. 2010. Population genomic analysis of a bacterial plant pathogen: Novel insight into the origin of Pierce's disease of grapevine in the U.S. *PLoS ONE* 5:e15488.

Nunney, L., X. Yuan, R. E. Bromley, and R. Stouthamer. 2012. Detecting genetic introgression: High levels of intersubspecific recombination found in *Xylella fastidiosa* in Brazil. *Applied and Environmental Microbiology* 78:4702–4714.

Nunney, L., D. B. Vickerman, R. E. Bromley, S. A. Russell, J. R. Hartman, L. D. Morano, and R. Stouthamer. 2013. Recent evolutionary radiation and host plant specialization in the *Xylella fastidiosa* subspecies native to the United States. *Applied and Environmental Microbiology* 79:2189–2200.

Nunney, L., B. Ortiz, S. A. Russell, R. R. Sánchez, and R. Stouthamer. 2014a. The complex biogeography of the plant pathogen *Xylella fastidiosa*: Genetic evidence of introductions and subspecific introgression in Central America. *PLoS ONE* 9:e112463.

Nunney, L., D. L. Hopkins, L. D. Morano, S. E. Russell, and R. Stouthamer. 2014b. Intersubspecific recombination in *Xylella fastidiosa* strains native to the United States: Infection of novel hosts associated with an unsuccessful invasion. *Applied and Environmental Microbiology* 80:1159–1169.

Nunney, L., E. L. Schuenzel, M. Scally, R. E. Bromley, and R. Stouthamer. 2014c. Large-scale intersubspecific recombination in the plant-pathogenic bacterium *Xylella fastidiosa* is associated with the host shift to mulberry. *Applied and Environmental Microbiology* 80:3025–3033.

Paião, F. G., A. M. Meneguim, E. C. Casagrande, and R. P. Leite, Jr. 1996. Envolvimento de cigarras (Homoptera, Cicadidae) na transmissão de *Xylella fastidiosa* em cafeeiro. *Fitopatologia Brasileira* 27:S67.

Petit, J. N., M. S. Hoddle, J. Grandgirard, G. K. Roderick, and N. Davies. 2008. Invasion dynamics of the glassy-winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in French Polynesia. *Biological Invasions* 10:955–967.

Pierce, N. B. 1892. The California vine disease. *U.S. Department of Agriculture, Division of Vegetable Pathology Bulletin* 2.

Polz, M. F., E. J. Alm, and W. P. Hanage. 2013. Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends in Genetics* 29:170–175.

Purcell, A. H., and A. H. Finlay. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathology 69:393–395.

Purcell, A. H., A. H. Finlay, and D. L. McLean. 1979. Pierce's disease bacterium: Mechanism of transmission by leafhopper vectors. *Science* 206:839–841.

Purcell, A. H., and S. R. Saunders. 1999. Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. *Plant Disease* 83:825–830.

Purcell, A. H., S. R. Saunders, M. Hendson, M. E. Grebus, and M. J. Henry. 1999. Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. *Phytopathology* 89:53–58.

Purcell, A. H. 2013. Paradigms: Examples from the bacterium *Xylella fastidiosa*. *Annual Review of Phytopathology* 51:339–356.

Retchless, A. C., F. Labroussaa, L. Shapiro, D. C. Stenger, S. E. Lindow, and R. P. P. Almeida. 2014. Genomic insights into *Xylella fastidiosa* interactions with plant and insect hosts. In *Genomics of Plant-Associated Bacteria*, edited by D. C. Gross, A. Lichens-Park, and C. Kole. Berlin, Germany: Springer Berlin Heidelberg. Pp. 177–202.

Sanderlin, R. S., and R. A. Melanson. 2010. Insect transmission of *Xylella fastidiosa* to pecan. *Plant Disease* 94:465–470.

Saponari, M., D. Boscia, F. Nigro, and G. P. Martelli. 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond, and olive trees exhibiting leaf scorch symptoms in Apulia (southern Italy). *Journal of Plant Pathology* 95:668.

Saponari, M., G. Loconsole, D. Cornara, R. K. Yokomi, A. De Stradis, D. Boscia, D. Bosco, G. P. Martelli, R. Krugner, and F. Porcelli. 2014. Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. *Journal of Economic Entomology* 107(4):1316–1319.

Scally, M., E. L. Schuenzel, R. Stouthamer, and L. Nunney. 2005. Multilocus sequence type system for the plant pathogen *Xylella fastidiosa* and relative contributions of recombination and point mutation to clonal diversity. *Applied and Environmental Microbiology* 71:8491–8499.

Schaad, N. W., E. Postnikova, G. Lacy, M. Fatmi, and C.-J. Chang. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Systematic and Applied Microbiology* 27:290–300.

Sorensen, J. T., and R. G. Gill. 1996. A range extension of *Homalodisca vitripennis* (Say) (Hemiptera: Clypeorrhyncha: Cicadellidae) to Southern California. *Pan Pacific Entomologist* 72:160–161.

Su, C.-C., C.-J. Chang, C.-M. Chang, H.-T. Shih, K.-C. Tzeng, F.-J. Jan, C.-W. Kao, and W.-L. Deng. 2013. Pierce's disease of grapevines in Taiwan: Isolation, cultivation and pathogenicity of *Xylella fastidiosa*. *Journal of Phytopathology* 161:389–396.

Su, C.-C., W.-L. Deng, F.-J. Jan, C.-J. Chang, H. Huang, and J. Chen. 2014. Draft genome sequence of *Xylella fastidiosa* pear leaf scorch strain in Taiwan. *Genome Announcements* 2:e00166–14.

Sun, Q., Y. Sun, M. A. Walker, and J. M. Labavitch. 2013. Vascular occlusions in grapevines with Pierce's disease make disease symptom development worse. *Plant Physiology* 161:1529–1541.

Turner, W. F., and H. N. Pollard. 1959. Life histories and behavior of five insect vectors of phony peach disease. *U.S. Department of Agriculture Technical Bulletin* 1188.

Van Sluys, M. A., M. C. de Oliveira, C. B. Monteiro-Vitorello, C. Y. Miyaki, L. R. Furlan, L. E. A. Camargo, A. C. R. da Silva, et al., 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. *Journal of Bacteriology* 185:1018–1026.

Young, D. A. 1958. A synopsis of the species of *Homalodisca* in the United States (Homoptera: Cicadellidae). *Bulletin of the Brooklyn Entomology Society* 53:7–13.

Yuan, X., L. Morano, R. Bromley, S. Spring-Pearson, R. Stouthamer, and L. Nunney. 2010. Multilocus sequence typing of *Xylella fastidiosa* causing Pierce's disease and oleander leaf scorch in the United States. *Phytopathology* 100:601–611.

Zeilinger, A. R., and M. P. Daugherty. 2014. Vector preference and host defense against infection interact to determine disease dynamics. *Oikos* 123:613–622.

A2

GENETIC CONTROL OF *AEDES* MOSQUITOES[1]

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Abstract

***Aedes* mosquitoes include important vector species such as *Aedes aegypti*, the major vector of dengue. Genetic control methods are being developed for several of these species, stimulated by an urgent need owing to the poor**

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effectiveness of current methods combined with an increase in chemical pesticide resistance. In this review we discuss the various genetic strategies that have been proposed, their present status, and future prospects. We focus particularly on those methods that are already being tested in the field, including RIDL and *Wolbachia*-based approaches.

Introduction

Aedes mosquitoes transmit a range of pathogens that cause substantial human morbidity, mortality, and suffering. Dengue, the most important mosquito-borne viral disease with 50–400 million infections per year worldwide (Bhatt et al., 2013; WHO, 2012), is transmitted primarily by *Ae. aegypti*. Several other *Aedes* species are competent vectors for dengue in the laboratory and *Ae. albopictus* in particular has been responsible for some transmission in the field, though it appears much less epidemiologically significant than *Ae. aegypti* (Lambrechts et al., 2010). The common name of *Ae. aegypti* is the yellow fever mosquito, indicating another major arbovirus transmitted by mosquitoes of this genus, and there are many more; chikungunya has come to prominence more recently with a major outbreak in the Indian Ocean in 2005–6 (Gérardin et al., 2008; Delatte et al., 2008) and some transmission in Italy in 2006 (Bonilauri et al., 2008). Pathogen transmission is not confined to viruses—lymphatic filariasis in the South Pacific is vectored by *Ae. polynesiensis*; specific characteristics of this vector may have contributed to the failure of drug-based control programmes in the region (Chambers et al., 2011; O'Connor et al., 2012).

A vaccine has long been available for yellow fever, but remains some way off for dengue, following disappointing results from a recent large trial of the leading candidate (Halstead, 2012; Sabchareon et al., 2012). With no licensed vaccine or specific drug (whether prophylactic or therapeutic), dengue control focuses on the major mosquito vector, *Ae. aegypti*—and vector control is expected to remain essential even when drugs or vaccines eventually become available. However, current mosquito control methods have limited effectiveness against some key species which breed in small dispersed bodies of water. For *Ae. aegypti*, these might be water storage containers or rain-water filled artificial containers such as buckets, vases, general refuse, or blocked rainwater gutters. Both private properties and public spaces will have large numbers of such potential breeding sites. Each one may be treated easily by tipping out the water or treating with a chemical or biological toxin, however finding and treating a high enough proportion for effective control is extremely difficult and impractical in most settings. Adulticides are also of limited effectiveness, compounded by increased resistance and the relative ineffectiveness of bednets against day-biting mosquitoes. The inadequacy of current technology is clear: for example, the efficient and well-resourced programme in Singapore, working with a cooperative citizenry, has not been able to prevent epidemic dengue (Egger et al., 2008; Ooi et al., 2006;

National Environment Agency, 2012). This, combined with recent enabling technical advances in mosquito genetics, provides the underlying motivation for the development of new genetics-based approaches.

Genetics-based approaches have several features in common.[5] Since they depend on vertical (mating-based) transmission of heritable elements,[6] they are extremely species-specific. Populations can only be affected by the genetic system if they can interbreed with carriers of that genetic system; other populations will not be directly affected. This species-specific aspect is very attractive from an environmental perspective, as it means that these approaches are exquisitely targeted to the pest or vector species of interest. On the other hand, this feature may be a limitation where multiple pest species are transmitting the same pathogen, in which case a more broad-spectrum approach may be preferred. An additional advantage of genetic control methods is that the control agent, modified insects, will actively disperse and seek mates, so the methods are "homing" or actively target-seeking, as well as specific.

Though some genetic strategies have been developed using classical genetics, such as the Sterile Insect Technique (SIT) (see section below: Population Suppression Strategies—Sterile Males Section), recombinant DNA methods provide a step change in our ability to design and build highly specified, versatile, powerful genetic systems. Several key *Aedes* species have now been transformed, either by recombinant DNA methods using transposon vectors (Labbé et al., 2010; Coates et al., 1998; Jasinskiene et al., 1998; Rodrigues et al., 2006; Fraser, 2012), or by artificial infection with various *Wolbachia*, a diverse group of intracellular bacteria (Chambers et al., 2011; Xi et al., 2005, 2006). This opens the door to the development of powerful new genetics-based tools with which to control major vector-borne diseases.

Classifying Genetic Control Strategies

A bewildering variety of genetic control strategies have been proposed; these can be categorised according to the intended outcome, or according to the expected dynamics of the genetic element in the target population. Regarding intended outcome, this may be to reduce the number of individual vector mosquitoes—population suppression—or to reduce the ability of individual mosquitoes within the population to transmit the pathogen. This latter approach is

[5] Genetic control may be defined as "Dissemination, by mating or inheritance, of factors that reduce pest damage" and area-wide control as "Reducing pest damage using measures whose effectiveness depends on application over large expanses" (Mark Benedict, pers. comm.). All proposed genetic strategies are intended for area-wide use, though the minimum useful area varies by species and strategy.

[6] One exception might be "paratransgenesis," the use of modified microbes to change the phenotype of insects with which the microbes associate. Depending on the microbe, horizontal transfer of the modified microbe between insects might be possible. Paratransgenesis is not discussed further in this review.

known as "population replacement" or, because the mosquitoes are made refractory to transmission of the pathogen, "refractory insect strategy" (Braig and Yan, 2001; James, 2000; Alphey et al., 2002; Alphey, 2009). However, the target population is not really replaced; rather a genetic element is introduced into it through breeding of released modified mosquitoes with wild individuals, thereby changing the phenotype of some or all individuals in that population—those that carry the new genetic element.

Regarding the expected dynamics of the genetic element in the target population, the element may be intended to persist indefinitely in the target population, potentially also increasing in frequency within the target population and spreading to invade additional populations. These are termed "self-sustaining" genetic systems. The alternative is systems which will not spread or persist, rather they will decrease in prevalence over time and can be maintained in the target population only by periodic release of additional carriers. These are known as "self-limiting" genetic systems.

Population Suppression Strategies—Sterile Males

The most familiar genetics-based population suppression strategy is SIT. This relies on the release of large numbers of sterile males to seek, court, and mate wild females, thereby reducing the reproductive potential of the target wild population. If enough of the wild females mate sterile males then the target population will decline and collapse. SIT has been used successfully for more than 50 years against several major agricultural pests, using radiation-sterilised insects (Dyck et al., 2005; Knipling, 1955). However, the use of radiation imposes several undesirable limitations, including logistical issues, and the somatic damage unavoidably caused by the sterilising dose of radiation used (Andreasen and Curtis, 2005; Helinski et al., 2006, 2009; Helinski and Knols, 2008). Several field trials using radiation- or chemo-sterilised mosquitoes have been conducted, with some success, but there are also problems including poor performance of irradiated mosquitoes (Dame et al., 2009). Though classical methods have recently been revisited for *Ae. albopictus* (Bellini et al., 2007; Boyer et al., 2011) and *An. arabiensis* (Helsinki et al., 2008), several alternatives have been explored to avoid the need for irradiation, and to provide additional enhancements, while retaining the many attractive aspects of classical SIT (Alphey et al., 2010). Though "sterile" may strictly indicate agametic sterility, meaning that no gametes are produced, for SIT agametic sterility is not intended or used as it is important that spermatozoa are in fact produced. If aspermic males were used, sperm competition in remating females would likely lead to fertile sperm winning over (non-existent) sperm from sterile males. This would lead to most or all of the eggs from females that mate more than once being fertilised by unmodified sperm and therefore being viable, unless all of their mates are sterile. Increased remating might therefore represent a form of selectable behavioural resistance. However, the barriers to remating

vary; where physical barriers such as mating plugs occur selection for increased remating may be less likely. Instead of "agametic," in the context of SIT and this review "sterile" simply means that some or all of the offspring die. For instance, *Wolbachia* can induce a form of sterility known as Cytoplasmic Incompatibility (CI), in which embryos from uninfected females fertilised by sperm from infected males fail to develop. Infected males are therefore sterile when mated with uninfected females, though fertile when mating with infected females. This can potentially be used as a sterilising principle for SIT, this variant being called the Incompatible Insect Technique, IIT (Brelsfoard et al., 2008). In classical SIT, the radiation doses used induce dominant lethal mutations in the irradiated sperm such that most eggs die after being fertilised by such sperm. About 95–99% sterility is typical for Mediterranean fruit fly SIT programmes (Bakri et al., 2005; Mumford, 2012); higher sterility can be achieved with more radiation, but at the cost of further damaging the insects. *Wolbachia* achieve a similar effect—death of offspring of incompatible crosses—in IIT, though the biochemical and genetic mechanism is unknown.

Sterility—death of most or all offspring—can also be achieved by using dominant lethal alleles introduced into the genome by recombinant DNA methods, rather than by irradiation. In the most direct analogous system, so far described only in *Anopheles*, a nuclease is expressed in the male germline (Windbichler et al., 2008). This gives a sterilising effect much like radiation—and presumably by a similar mechanism, induction of double-stranded breaks in the insect's chromosomes. Interestingly, the system was designed to cut the *X* chromosome exclusively, and thereby selectively kill female offspring, though this was not achieved and would in any case be difficult in *Aedes* mosquitoes that lack a *Y* chromosome. The underlying molecular system, using sequence-specific nucleases called homing endonucleases (HEGs), is remarkably flexible depending on the precise design. In theory, both self-limiting systems like this SIT example and invasive, self-sustaining genetic systems can be developed with these tools (Burt, 2003; Deredec et al., 2008). Furthermore, although the SIT-like systems described here are clearly self-limiting, self-sustaining population suppression strategies using HEGs have been described, in which reduced-fitness traits are driven into the target population using the super-Mendelian inheritance property of HEGs; in principle this could drive a population or even a species to extinction (Burt, 2003; Deredec et al., 2008).

We have developed a SIT-like system called RIDL (Release of Insects carrying a Dominant Lethal) (Thomas et al., 2000). Here, rather than inducing dominant lethals when required, as with radiation, a dominant lethal transgene is inserted, but its expression is artificially repressed to allow the insects to be reared. One advantage of this approach over the use of DNA damage or CI is the ability to select the time of death of the offspring. Radiation and CI kill affected individuals as embryos, but where there is significant larval density-dependence, a later lethal period can be considerably preferable (Phuc et al., 2007; Atkinson

et al., 2007; Yakob and Alphey, 2008; White et al., 2010; Alphey et al., 2011; Barclay, 2005; Bax and Thresher, 2009).

All control interventions place pressures on the target population that may select for various forms of resistance, and genetic control methods are no exception. As mating-based systems, one obvious potential mode of resistance is assortative mating, whereby females are selected to avoid the engineered males. In practice, in decades of use of radiation-based SIT there have been few examples of this, a melon fly control programme in Okinawa being perhaps the only well-documented example (Koyama et al., 2004). Even then, control was successfully achieved simply by releasing more sterile males. Other genetic strategies may have additional potential resistance modes. The use of zygotically active lethal genes in RIDL provides flexibility in terms of engineering the time—and/or sex, see in the following section—of death. In principle, it also allows the possibility of resistance to the zygotic killing mechanism (Alphey et al., 2011b) though this has not yet been observed. Given the large number of effector molecules available, one might expect that new strains could be developed faster than such resistance would emerge; other approaches such as stacking traits may also be useful should this type of resistance prove an issue in practice.

Large-Scale Separation of Males and Females—Genetic Sexing Strains

A further issue is that of sex separation. This is not essential for efficacy—the New World screw-worm was eliminated from a continent by a classical SIT programme releasing both males and females—but it is highly desirable (Dyck et al., 2005). Female mosquitoes will bite and potentially transmit disease even if sterilised. The lifespan of released mosquitoes will likely be reduced by laboratory rearing and handling, significantly reducing their capacity to transmit disease in addition to any effect of the modification itself, nonetheless the possibility of deliberate or accidental release of females may adversely affect public acceptance. Sterile-male methods (e.g., SIT, IIT, RIDL) do not require the release of females, however self-sustaining releases of *Wolbachia* do require the release of some females because *Wolbachia* is maternally inherited. Therefore it has been proposed that special strategies using male-biased release should be used to minimise the number of females released (Hancock et al., 2011), though in fact no sex separation was used for the first such release trial (Hoffman et al., 2011).

For some strategies there are additional reasons to remove females beyond their potential to bite. For SIT, large-scale field experiments with Mediterranean fruit flies showed that male-only releases were 3–5 times more effective per male than mixed-sex releases; the sterile females are thought to "distract" the sterile males from seeking out wild females (Rendón et al., 2004). For the *Wolbachia*-based IIT specifically there is an additional requirement for sex separation—the infected females are fully fertile with both infected and uninfected males, furthermore all their progeny inherit the infection. This means that release of even a

single infected female could potentially lead to the alien *Wolbachia* spreading in the target population. Where the target species is naturally infected with a different, incompatible, strain of *Wolbachia*, the resulting bidirectional incompatibility will likely limit the spread of the new infection beyond the target area, at least for small target areas. However, if the target species is naturally uninfected, this could lead to the spread of the infection throughout the species. The natural history of *Wolbachia*, which indicates many independent invasion events, shows this is possible, but not the likelihood, which may be very low per female. This is likely to be seen as an undesirable outcome and therefore a significant risk, unless species-wide invasion is the intent of the release.

Sex separation can be efficiently achieved for some species of mosquitoes, including *Ae. aegypti*, using physical methods based on the size difference between male and female pupae (Ansari et al., 1977; Focks, 1980; Harris et al., 2011, 2012). Strains that allow genetics-based automated separation of males and females are known as "genetic sexing strains." Several have been developed using classical genetics, notably the "MACHO" strain which contributed greatly to the success of an SIT programme against *An. arabiensis* in El Salvador (Dame et al., 2009; Kaiser et al., 1978). However, modern genetics provides more options and also allows such systems to be transferred more readily from one species to another. Several have been developed (Papathanos et al., 2009; Catteruccia et al., 2005; Fu et al., 2007, 2010; Ant et al., 2012; Jin et al., 2013). In principle, any selectable induced sexual dimorphism could be used, but in practice two approaches have been followed, either sex-specific expression of a fluorescent marker allowing automated sorting (Catteruccia et al., 2005; Marois et al., 2012), or sex-specific conditional lethality allowing facile elimination of one sex from a cohort during rearing (Fu et al., 2007). It is possible to use a repressible female-killing system both for sex separation and also for field control (Thomas et al., 2000; Alphey, 2002; Alphey and Andreasen, 2002; Alphey et al., 2008). Insects are reared with the lethal system repressed to provide a colony. Cohorts for release are then reared without the repressor, so that females are eliminated. The resulting males, homozygous for a dominant female-specific lethal gene are released to mate with wild females. All offspring from such a mating inherit one copy of the female-lethal transgene, so daughters die. These are both the vectors and the reproductive potential of the population. Heterozygous sons will pass the transgene on to half of their offspring, resulting in some additional control, though the high fitness cost of a female-lethal trait means that the transgene will be rapidly eliminated from the target population unless maintained by periodic release of additional homozygous males. This is female-specific RIDL, fsRIDL, which has some similarities to the classical field female-killing (FK) systems developed in *Lucilia cuprina* (Black and Alphey, 2011) and is in principle more efficient than SIT (Schliekelman and Gould, 2000). Furthermore, the use of female-lethal systems may provide additional benefits in terms of resistance management for other approaches used in an integrated vector management

programme (Alphey et al., 2007, 2009). fsRIDL strains have been developed for *Ae. aegypti* (Fu et al., 2010; Wise de Valdez et al., 2011), and *Ae. albopictus* (Labbé et al., 2012), using flightlessness as a lethal trait.

Refractory Insects

Several approaches have been described for making mosquitoes refractory to malaria, including the expression of specific antibodies (Isaacs et al., 2012), peptides (Ito et al., 2002), or manipulating cell signaling (Corby-Harris et al., 2010). For the arboviruses transmitted by *Aedes* mosquitoes, RNAi seems an attractive mechanism for suppressing virus replication. Transgene-based expression of a hairpin RNA corresponding to part of the DEN2 virus in either the midgut (Franz et al., 2006) or salivary glands (Mathur et al., 2010) has been shown to provide a strong block to virus transmission. However, for the midgut-expressing line, expression of the anti-DEN2 hairpin and the associated refractory phenotype were lost after about 13 generations (Franz et al., 2009), suggesting that expression may impose a significant fitness cost, and also perhaps that the unusual inverted repeat structure involved may be subject to some form of epigenetic silencing.

Gene Drive Systems

A refractory gene will only have an epidemiologically useful effect if it is present in a significant fraction of the target population. It will probably also have to keep both prevalence and effectiveness high for many vector generations. How can this be achieved? Getting to a high prevalence by simple introgression is difficult in a numerically large population, though not necessarily impossible (Rasgon, 2009). However, since the refractory gene is likely to impose a fitness cost on the mosquitoes, it is likely that both be selected against in terms of prevalence, and also perhaps in terms of loss of function (Marrelli et al., 2006). A system is therefore required which will increase the prevalence within the population over time, despite a selective disadvantage. Such systems are termed "gene drive systems." Selfish DNA systems (Burt and Trivers, 2006), which have this property of spreading despite not providing an individual fitness benefit, are the main source of inspiration for the design of gene drive systems. Several systems have been proposed (Sinkins and Gould, 2006), but none developed even to proof-of-principle stage in a mosquito. However, a *Medea*-like system has been demonstrated in *Drosophila melanogaster* (Chen et al., 2007), using a design which should in principle be transferable to mosquitoes (Hay et al., 2010).

One interesting proposal is the "killer–rescue" system (Gould et al., 2008). By using a lethal transgene and an unlinked repressor, this provides an initial increase in allele frequency of the repressor, but over time both the lethal transgene and the repressor decline in frequency. Though having some gene drive properties, this is therefore still a self-limiting system, which helps to illustrate that there

is a spectrum of invasiveness or persistence in genetic systems. At one extreme we have high-penetrance dominant lethal systems killing both males and females, where the transgene is not expected to persist beyond the immediate progeny of the released individuals. Then there are female-lethal systems, where the sons survive but the transgene will still disappear rapidly due to its high fitness cost. Refractory genes that are designed to be neutral will also decline in frequency, but much more slowly due to their much lower fitness cost (some fitness penalty seems inevitable). A transient gene drive system like killer–rescue can provide some boost beyond the initial allele frequency, but still eventually declines. Then on the other side of the self-sustaining/self-limiting divide—which is a very real and significant divide, notwithstanding the shades of persistence and invasiveness on either side of it—we have frequency-dependent systems like underdominance (Davis et al., 2001; Magori and Gould, 2006; Curtis, 1968). This has a high invasion threshold making it relatively unlikely to invade non-target populations well isolated from any target populations. *Medea*-like systems have a much lower invasion threshold and so are much more likely to spread aggressively into distant populations (Chen et al., 2007; Hay et al., 2010; Marshall et al., 2011), though modifications can in principle be made to reduce this (Marshall et al., 2011). Transposons, long proposed as the basis for gene drive systems though not yet demonstrated, are also potentially highly invasive (James, 2000).

While the relationship of IIT and RIDL with the well-known SIT is clear, there are not such obvious analogies with current methods to guide the testing, deployment, and use of gene drive systems. Some affinity may be found with classical biological control, where the intention is to introduce a parasitoid or predator to control a pest population, expecting that the biocontrol agent will establish and provide long-lasting control, albeit usually incomplete, for the indefinite future. As with classical biological control, there are concerns regarding the lack of control over the gene drive system once released, its unknown evolutionary trajectory post-release, and the essentially irreversible nature of a release, at least in the case of large-scale releases. For these reasons, self-sustaining systems are seen as higher-risk (FAO/IAEA, 2002; Beech et al., 2012; Alphey and Beech, 2012; Benedict et al., 2008, 2010). On the other hand, while sterile-male control looks economically attractive (Atkinson et al., 2007; Alphey et al., 2011a) self-sustaining systems in principle have an even lower cost to deploy as fewer mosquitoes are required, at least after the initial introduction. This theoretical cost advantage depends on being able to use the gene drive system as a "fire-and-forget" weapon; the more expensive the post-release monitoring required, for example to assure the ongoing prevalence, stability, and effectiveness of the modification, the lower the cost differential is likely to be.

A further issue is the possibility that success may lead to decreased vigilance or the loss of capacity to implement previously effective measures if such existed. While this applies to all control methods, whether genetic or not, it may be a significant concern in respect of the use of long-term self-sustaining systems. The

"forget" part of "fire-and-forget" should therefore not be taken literally—such methods would still require careful ongoing monitoring for field efficacy, and the development of replacement strains prior to breakdown. This is likely to require significant ongoing resource expenditure.

Can *Wolbachia* Provide Both Refractoriness and a Gene Drive System?

One striking exception to the slow progress with refractoriness and gene drive systems has come from work on *Wolbachia* in *Ae. aegypti*. Though originally developed for IIT and life-shortening strategies, it was observed that infection with certain strains of *Wolbachia* dramatically reduced susceptibility to a range of pathogens (Hedges et al., 2008; Kambris et al., 2009; Moreira et al., 2009), though potentially increasing susceptibility to others (Hughes et al., 2012). *Wolbachia* are capable of spreading through insect populations as a heritable modification by manipulating the host's reproductive biology (Burt and Trivers, 2006; Hancock et al., 2011)—in other words, *Wolbachia* has the properties of a gene drive system. This raised the possibility that certain strains of *Wolbachia* might provide a complete gene-drive-plus-refractory-gene package. Attention has focused on wMel, a strain of *Wolbachia* from *Drosophila melanogaster* and a laboratory-isolated pathogenic derivative wMelPop. Interestingly—and highlighting the diversity of *Wolbachia*—wMel infection has a similar dengue-blocking effect in *Ae. albopictus*, even though *Ae. albopictus* is naturally infected with two further strains of *Wolbachia* that do not have this effect (Blagrove et al., 2012). As with cytoplasmic incompatibility, the molecular basis of this pathogen-blocking phenotype is not known, though various studies have implicated upregulation of immune genes or production of reactive oxygen species, or competition for a limited resource such as cholesterol (Kambris et al., 2009; Moreira et al., 2009; Pan et al., 2012; Brennan et al., 2008).

In principle, therefore, a suitable strain of *Wolbachia* could provide an invasive refractoriness phenotype. Though such invasive genetic systems are seen as relatively risky for reasons outlined above, *Wolbachia* is not especially invasive, particularly for a strain that has a significant fitness cost, as appears to be the case for wMelPop (Hancock et al., 2011). Introduction of a single infected female can still lead to *Wolbachia* invading that population, especially if the effective population size is low (Jansen et al., 2008).

Since *Wolbachia* are naturally occurring, albeit not in *Ae. aegypti* and the relevant strains are from rather distantly related insects, this use of *Wolbachia* escapes the regulatory structures and oversight put in place for recombinant DNA technology (De Barro et al., 2011). This may seem rather odd if one considers that addition of any single gene, or less, of DNA from *Wolbachia* would trigger such an oversight, but the addition of the whole genome does not. However, it is clear that here, as for conventional genetic engineering of mosquitoes, the relevant research groups have worked hard to clarify and then to comply with all

applicable regulations (O'Connor et al., 2012; Harris et al., 2012; De Barro et al., 2011; Subramaniam et al., 2012; Beech et al., 2009; Mumford et al., 2009).

For any self-sustaining genetic system, key questions relate to the initial ability to spread and confer the desired phenotype, and the possibility that evolutionary responses will compromise this, or have some other undesirable effect. Though in principle the large-scale use of such systems may be reversible by further genetic intervention, restoring the *status quo ante* is at best uncertain; this irreversibility has been a major discussion point in respect of gene drive systems. In the case of *Wolbachia*, one may predict that the introduced strain will co-adapt with *Ae. aegypti*, reducing the fitness cost of infection but perhaps correspondingly reducing the extent of refractoriness, as both may have the same underlying cause of overproliferation in somatic cells (Lu et al., 2012). However, while the direction seems clear, the rate of decay is very hard to predict, and many generations of protection may be provided. Lack of permanent effect is hardly a reason not to act, but might this tapering protection have some negative aspect? Consequences might include selection for resistant strains of virus. Though initial experiments suggested that wMel infection gave strong refractoriness (Walker et al., 2011), subsequent data using blood from human patients indicated titre-dependent breakthrough (O'Neill, 2011). This suggests that a *Wolbachia* strain with refractoriness that is incomplete—either as its initial phenotype or arising through co-adaptation with the mosquito—could select for virus strains with higher titre in humans, an undesirable trait. It is also striking that, unlike normal uninfected mosquitoes, *Ae. aegypti* infected with wMelPop require human blood to produce viable eggs (McMeniman et al., 2011). This would appear to provide strong selection for increased human biting preference, a trait which is central to the transmission of human-specific pathogens, as well as to biting nuisance. Unlike the more catholic *Ae. albopictus*, *Ae. aegypti* has a strong preference for anthropophagy, but this is far from absolute and could presumably be increased by such selection (Scott et al., 1993; Siriyasatien et al., 2010; Valerio et al., 2010; Barrera et al., 2012).

These issues illustrate the difficulty of predicting the consequences of releasing a self-sustaining genetic system relating to future evolutionary responses. The use of a "black box" system such as *Wolbachia* has advantages and disadvantages relative to genetic engineering using well-characterised components. On the one hand *Wolbachia* is arguably natural—though this may also be true of the elements of an engineered system; in both cases the association with *Aedes aegypti* is artificially induced, a product of modern biotechnology. To further blur the lines, gene transfer from *Wolbachia* to insect nuclear genomes is well known, and this can lead to stable transfer of expressed genes (Klasson et al., 2009). Nonetheless, this "natural" aspect is somewhat reassuring, in that *Wolbachia* strains are already widespread in the environment without known negative effects—though that many strains are harmless does not imply that all are; one could not sustain such an argument for *E. coli*, for example. On the other hand, a complex

uncharacterised system is by definition less well understood and correspondingly more likely to throw up surprises. wMel has an estimated 1,270 protein-coding genes in 1.3 Mb of DNA121—vastly more complex than the 1–4 genes in about 10–20 kb typical for current transgenic insertions. The refractoriness phenotype was a major, beneficial surprise; the human blood requirement was also entirely unexpected, and less welcome. The future evolutionary trajectory of such a complex system may reveal additional surprises—positive or negative.

However, it is a fallacy, sometimes called the nirvana fallacy, to compare actual things with idealised alternatives, for example the risks of future action with a hypothetical risk-free world. Both inaction and alternative actions have risks of their own. Nonetheless, it may be difficult both for regulatory authorities and the general public to compare the relatively well-known risks and hazards of inaction with the unknown aspects of a new technology, even when—as for genetic control—the technology seems likely to offer potentially large net benefits.

Not a "Magic Bullet"

The above discussion has focused on genetic control methods alone. However, current control methods have some strengths as well as weaknesses; an optimal programme is therefore likely to integrate the best of current methods with new technology to achieve the goal of improved control. For example, short-term suppression by conventional methods is likely to be a desirable prelude to either sterile-male or refractory-insect methods as it will reduce the number of modified insects required to achieve a given effect. As further tools become available, such as drugs and vaccines, this integrated vector management approach will naturally expand to integrated disease management—again using an optimal mix of available tools. While there may be a certain inclination simply to "wait for the vaccine," in practice both vaccine and vector control experts anticipate an ongoing requirement for vector control even when a cheap, effective vaccine is generally available (WHO, 2012)—a hoped-for but perhaps rather distant prospect.

Progress to the Field

In fact, after due consideration, national regulators in several countries have approved small-scale field trials as the next step in an incremental testing and scale-up process. Several self-limiting and one self-sustaining genetic system have been tested in the field to date (Hoffman et al., 2011; Harris et al., 2011, 2012; Lacroix et al., 2012; Brelsfoard and Dobson, 2011). Public perception has generally been positive, though these are early days. The use of *Wolbachia*, presented as "natural,"[7] has largely avoided public concerns relating to the use of recombinant DNA methods. Public response to genetic control, either in general

[7] http://www.eliminatedengue.com/, accessed October 18, 2012 and April 17, 2013.

or relating to specific applications, may vary considerably depending on a wide range of social, political, epidemiological, presentational, and cultural factors, of which the genetic element is only one; furthermore, this response may vary over time. Even for a well-established approach such as vaccination, participation rates are rarely as high as programme managers would wish, and scare stories such as that regarding MMR vaccine in the UK can still shake public confidence. However, regulatory and social factors, while crucial to the adoption of any new technology, are not the main focus of this review.

Field trials of genetic control methods known to the authors are:

1. 2009–2010 Cayman Islands: males of a RIDL strain of *Ae. aegypti*, OX513A,43 were shown to be able to compete successfully for mates with wild mosquitoes; 58 sustained release of these "sterile" males led to strong suppression of the target wild population (Harris et al., 2012).
2. 2010 Malaysia: OX513A males were shown to have similar longevity and maximum dispersal to an unmodified comparator (Lacroix et al., 2012).
3. 2010 French Polynesia: sustained release of *Ae. polynesiensis* males infected with a *Wolbachia* strain from *Ae. riversi* for IIT trial (O'Connor et al., 2012; Brelsfoard and Dobson, 2011).
4. 2011–present: Brazil: sustained release of OX513A males led to strong suppression of a target wild population.[8]
5. 2011–present Australia: release of wMel-infected male and female *Ae. aegypti* led to the invasion and establishment of wMel *Wolbachia* in two target wild populations (Hoffman et al., 2011); releases underway in three further areas.
6. Australia: release of wMelPop-infected male and female *Ae. aegypti* undertaken in two target areas; present status unknown.[9]
7. 2013–present Vietnam: release of wMelPop-infected male and female *Ae. aegypti* on an island (Delatte et al., 2008).

To our knowledge, each of these trials has been successful in accomplishing its experimental objectives, and in no case have any negative consequences to human health or the environment been identified.

Prospects for the Future

One may anticipate that each of the programmes described above will develop further over the coming years, though there will doubtless be numerous technical, legal, and social challenges. In addition, one may anticipate that some

[8] http://www.moscamed.org.br/2012/index.php, http://www.oxitec.com, accessed October 18, 2012.
[9] http://www.eliminatedengue.com, accessed April 17, 2013.

of the many approaches at earlier stages of development will progress towards field trials and use. In this regard one may particularly look to synthetic biology approaches to engineered refractoriness and gene drive systems—an approach that has been long heralded and where the daunting technical obstacles are slowly being overcome.

A specific technical question relating to both genetic and conventional vector control is "how low do you have to go?" What is the relationship between the number and competence of vectors and disease transmission? Current dengue control methods rely on population suppression. Genetics-based population suppression has the same aim, so can reasonably be evaluated on the same terms, looking for mosquito suppression, i.e., entomological endpoints. But what about refractory-insect methods, or indeed novel non-genetic methods such as spatial repellents? One would need to show an ability to reduce dengue—an epidemiological endpoint. However, this is extremely difficult for an area-wide intervention, as dengue is highly variable in time and space. Consequently, a trial to show disease suppression would likely need to have many separate treatment and control sites, each of a significant size and with many inhabitants. This is problematic in terms of scale but also in terms of funding—despite the potential, and outstanding early results, funding for genetic control has been extremely low relative to the resources devoted to drugs, vaccines, and insecticides.

Given adequate resources, the future for genetic control looks bright. Numerous research groups are developing exciting approaches; the first of these have successfully completed their first field trials. Genetic control may soon be deployed on a large scale, delivering clean, affordable, sustainable, scalable solutions to major human vector-borne diseases.

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References

Alphey, L. 2002. Re-engineering the sterile insect technique. *Insect Biochemistry and Molecular Biology* 32:1243–7.

Alphey, L. 2009. Natural and engineered mosquito immunity. *Journal of Biology* 8:40.

Alphey, L., and M. Andreasen. 2002. Dominant lethality and insect population control. *Molecular Biochemistry and Parasitology* 121:173–8.

Alphey, L. and C. Beech. 2012. Regulation of agricultural biotechnology: The United States and Canada. In: Chris A Wozniak, Alan McHughen (eds.). The Netherlands: Springer. Ch. 13. p. 281–99.

Alphey L., C.B. Beard, P. Billingsley, M. Coetzee, A. Crisanti, C. Curtis, et al. 2002. Malaria control with genetically manipulated insect vectors. *Science* 298:119–21.

Alphey, N., P.G. Coleman, C.A. Donnelly, and L. Alphey. 2007. Managing insecticide resistance by mass release of engineered insects. *Journal of Economic Entomology* 100:1642–9.

Alphey L., D. Nimmo, S. O'Connell, N. Alphey. 2008. In: Aksoy S, (ed.), Transgenesis and the management of vector-borne disease. Austin, TX: Landes Bioscience. 627: 93–103.

Alphey, N., L. Alphey, and M. Bonsall. 2009. Combining pest control and resistance management: synergy of engineered insects with Bt crops. *Journal of Economic Entomology* 102:717–32.

Alphey, L., M. Benedict, R. Bellini, G.G. Clark, D.A. Dame, M.W. Service et al. 2010. Sterile-insect methods for control of mosquito-borne diseases: an analysis. *Vector Borne Zoonotic Disease* 10:295–311.

Alphey, N., L. Alphey, and M.B. Bonsall. 2011a. A model framework to estimate impact and cost of genetics-based sterile insect methods for dengue vector control. *PLoS One* 6:e25384.

Alphey, N., L. Alphey, and M.B. Bonsall. 2011b. Modeling resistance to genetic control of insects. *Journal of Theoretical Biology* 27042–55.55

Andreasen, M.H. and C.F. Curtis. 2005. Optimal life stage for radiation sterilization of *Anopheles* males and their fitness for release. *Medical Veterinary Entomology* 19:238–44.

Ansari, M.A., K.R. Singh, G.D. Brooks, P.R. Malhotra, and V. Vaidyanathan. 1977. The development of procedures and techniques for mass rearing of *Aedes aegypti*. *Indian Journal of Medical Research* 65(Suppl):91–9.

Ant, T., M. Koukidou, P. Rempoulakis, H.F. Gong, A. Economopoulos, J. Vontas, et al. 2012. Control of the olive fruit fly using genetics-enhanced sterile insect technique. *BMC Biology* 10:51.

Atkinson, M.P., Z. Su, N. Alphey, L.S. Alphey, P.G. Coleman, and L.M. Wein. 2007. Analyzing the control of mosquito-borne diseases by a dominant lethal genetic system. *PNAS* 104:22.

Bakri, A., K. Mehta, and D.R. Lance. 2005. Sterilizing insects with ionizing radiation. In *Sterile insect technique. Principles and practice in area-wide integrated pest management*, edited by V.A. Dyck, J. Hendrichs, and A.S. Robinson. The Netherlands: Springer. Pp. 233–68.

Barclay, H.J. 2005. Mathematical models for the use of sterile insects. In *Sterile insect technique. Principles and practice in area-wide integrated pest management*, edited by V.A. Dyck, J. Hendrichs, and A.S. Robinson. The Netherlands: Springer. Pp. 147–74.

Barrera, R., A.M. Bingham, H.K. Hassan, M. Amador, A.J. Mackay, and T.R. Unnasch. 2012. Vertebrate hosts of *Aedes aegypti* and *Aedes mediovittatus* (Diptera: Culicidae) in rural Puerto Rico. *Journal of Medical Entomology* 49:917–21.

Bax, N.J. and R.E. Thresher. 2009. Ecological, behavioral, and genetic factors influencing the recombinant control of invasive pests. *Ecological Applications* 19:873–88.

Beech, C., J. Nagaraju, S. Vasan, R. Rose, R. Othman, V. Pillai, and T. Saraswasthy. 2009. Risk analysis of a hypothetical open field release of a self-limiting transgenic *Aedes aegypti* mosquito strain to combat dengue. *Asia Pacific Journal of Molecular Biology and Biotechnology* 17:97–108.

Beech, C., M. Koukidou, N.I. Morrison, and L. Alphey. 2012. Genetically modified insects: Science, use, status and regulation. *ICGEB Collection of Biosafety Reviews* 6:66–124.

Bellini, R., M. Calvitti, A. Medici, M. Carrieri, G. Celli, and S. Maini. 2007. In *Area-wide control of insect pests*, edited by M.B. Vreysen, A.S. Robinson, and J. Hendrichs. The Netherlands: Springer. Pp. 505–15.

Benedict, M., P. D'Abbs, S. Dobson, M. Gottlieb, L. Harrington, S. Higgs, et al. 2008. Guidance for contained field trials of vector mosquitoes engineered to contain a gene drive system: Recommendations of a scientific working group. *Vector Borne and Zoonotic Diseases* 8:127–66.

Benedict, M., M. Eckerstorfer, G. Franz, H. Gaugitsch, A. Greiter, A. Heissenberger, B. Knols, S. Kumschick, W. Nentwig, and W. Rabitsch. 2010. Defining environmental risk assessment criteria for genetically modified insects to be placed on the EU market. External report for European Food Safety Authority. http://www.efsa.europa.eu/en/scdocs/scdoc/71e.htm

Bhatt, S., P.W. Gething, O.J. Brady, J.P. Messina, A.W. Farlow, C.L. Moyes, et al. 2013. The global distribution and burden of dengue. *Nature* 496(7446): 504–7.

Black, W.C., 4th, L. Alphey, and A.A. James. 2011. Why RIDL is not SIT. *Trends in Parasitology* 27:362–70.

Blagrove, M.S., C. Arias-Goeta, A.B. Failloux, and S.P. Sinkins. 2012. *Wolbachia* strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. *Proceedings of the National Academy of Sciences* 109:255–60.

Bonilauri, P., R. Bellini, M. Calzolari, R. Angelini, L. Venturi, F. Fallacara et al. 2008. Chikungunya virus in *Aedes albopictus*, Italy. *Emerging Infectious Diseases* 14:852–4.

Boyer, S., J. Gilles, D. Merancienne, G. Lemperiere, and D. Fontenille. 2011. Sexual performance of male mosquito *Aedes albopictus*. *Medical Veterinary Entomology* 25:454–9.

Braig, H. and G. Yan. 2001. The spread of genetic constructs in natural insect populations. In *Genetically engineered organisms: Assessing environmental and human health effects*, edited by D. K. Letournaeu, B. E. Burrows. Boca Raton (Florida): CRC Press. Pp. 251–314.

Brelsfoard, C.L. and Dobson, S.L. 2011. Short note: An update on the utility of *Wolbachia* for controlling insect vectors and disease transmission. *Asia Pacific Journal of Molecular Biology and Biotechnology* 19:85–92.

Brelsfoard, C.L., Y. Sechan, and S.L. Dobson. 2008. Interspecific hybridization yields strategy for South Pacific filariasis vector elimination. *PLoS Neglected Tropical Diseases* 2:e129.

Brennan, L.J., B.A. Keddie, H.R. Braig, and H.L. Harris. 2008. The endosymbiont *Wolbachia pipientis* induces the expression of host antioxidant proteins in an *Aedes albopictus* cell line. *PLoS One* 3:e2083.

Burt, A. 2003. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proceedings of the Royal Society: Biological Sciences* 270:921–8.

Burt, A. and R. Trivers. 2006. *Genes in conflict: The biology of selfish genetic elements*. Cambridge, MA: Belknap Press, Harvard University Press.

Catteruccia, F., J.P. Benton, and A. Crisanti. 2005. An *Anopheles* transgenic sexing strain for vector control. *Nature Biotechnology* 23:1414–7.

Chambers, E.W., L. Hapairi, B.A. Peel, H. Bossin, and S.L. Dobson. 2011. Male mating competitiveness of a *Wolbachia*-introgressed *Aedes polynesiensis* strain under semi-field conditions. *PLoS Neglected Tropical Disease* 5(8):e1271.

Chen, C.H., H. Huang, C.M. Ward, J.T. Su, L.V. Schaeffer, M. Guo, et al. 2007. A synthetic maternal-effect selfish genetic element drives population replacement in *Drosophila*. *Science* 316:597–600.

Coates, C.J., N. Jasinskiene, L. Miyashiro, and A.A. James. 1998. Mariner transposition and transformation of the yellow fever mosquito, *Aedes aegypti*. *Proceedings of the National Academy of Sciences* 95:3748–51.

Corby-Harris, V., A. Drexler, L. Watkins de Jong, Y. Antonova, N. Pakpour, R. Ziegler, et al. 2010. Activation of Akt signaling reduces the prevalence and intensity of malaria parasite infection and lifespan in *Anopheles stephensi* mosquitoes. *PLoS Pathogens* 6:e1001003.

Curtis C.F. 1968. Possible use of translocations to fix desirable genes in insect pest populations. *Nature* 218:368–9.

Dame, D.A., C.F. Curtis, M.Q. Benedict, A.S. Robinson, and B.G. Knols. 2009. Historical applications of induced sterilisation in field populations of mosquitoes. *Malaria Journal* 8:S2.

Davis, S., N. Bax, and P. Grewe. 2001. Engineered underdominance allows efficient and economic introgression of traits into pest populations. *Journal of Theoretical Biology* 212:83–98.

De Barro, P., B. Murphy, C. Jansen, and J. Murray. 2011. The proposed release of the yellow fever mosquito, *Aedes aegypti*, containing a naturally occurring strain of *Wolbachia pipientis*, a question of regulatory responsibility. *Journal für Verbraucherschutz und Lebensmittelsicherheit* 6(Suppl 1):S33–40.

Delatte, H., C. Paupy, J.S. Dehecq, J. Thiria, A.B. Failloux, and D. Fontenille. 2008. *Aedes albopictus*, vector of chikungunya and dengue viruses in Reunion Island: Biology and control. *Parasite* 15:3–13.

Deredec, A., A. Burt, and H.C. Godfray. 2008. Population genetics of using homing endonuclease genes in vector and pest management. *Genetics* 179:2013–26.

Dyck, V.A., J. Hendrichs, and A.S. Robinson. 2005. *Sterile insect technique: Principles and practice in area-wide integrated pest management*. The Netherlands: Springer.

Egger, J.R., E.E. Ooi, D.W. Kelly, M.E. Woolhouse, C.R. Davies, and P.G. Coleman. 2008. Reconstructing historical changes in the force of infection of dengue fever in Singapore: Implications for surveillance and control. *Bulletin of the World Health Organization* 86:187–96.

FAO/IAEA. 2002. *Status and risk assessment of the use of transgenic arthropods in plant protection*. 48 Vienna: FAO/IAEA.

Focks, D.A. 1980. An improved separator for separating the developmental stages, sexes and species of mosquitoes. *Mosquito News* 19:144–47.

Franz, A.W., I. Sanchez-Vargas, Z.N. Adelman, C.D. Blair, B.J. Beaty, A.A. James, et al. 2006. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proceedings of the National Academy of Sciences* 103:4198–203.

Franz, A.W., I. Sanchez-Vargas, J. Piper, M.R. Smith, C.C. Khoo, A.A. James et al. 2009. Stability and loss of a virus resistance phenotype over time in transgenic mosquitoes harbouring an antiviral effector gene. *Insect Molecular Biology* 18:661–72.

Fraser, M.J., Jr. 2012. Insect transgenesis: Current applications and future prospects. *Annual Review of Entomology* 57:267–89.

Fu, G., K.C. Condon, M.J. Epton, P. Gong, L. Jin, G.C. Condon, et al. 2007. Female-specific insect lethality engineered using alternative splicing. *Nature Biotechnology* 25:353–7.

Fu, G., R.S. Lees, D. Nimmo, D. Aw, L. Jin. P. Gray, et al. 2010. Female-specific flightless phenotype for mosquito control. *Proceedings of the National Academy of Sciences* 107:4550–4.

Gérardin, P., V. Guernier, J. Perrau, A. Fianu, K. Le Roux, P. Grivard, et al. 2008. Estimating Chikungunya prevalence in La Reunion Island outbreak by serosurveys: Two methods for two critical times of the epidemic. *BMC Infectious Diseases* 8:99.

Gould, F., Y. Huang, M. Legros, and A.L. Lloyd. 2008. A killer–rescue system for self-limiting gene drive of anti-pathogen constructs. *Proceedings of the Royal Society: Biological Sciences* 275:2823–9.

Halstead, S.B. 2012. Dengue vaccine development: A 75% solution? *Lancet* 380(9853):1535–6.

Hancock, P.A., S.P. Sinkins, and H.C. Godfray. 2011a. Population dynamic models of the spread of *Wolbachia*. *American Naturalist* 177:323–33.

Hancock, P.A., S.P. Sinkins, and H.C. Godfray. 2011b. Strategies for introducing *Wolbachia* to reduce transmission of mosquito-borne diseases. *PLoS Neglected Tropical Diseases* 5:e1024.

Harris, A.F., D. Nimmo, A.R. McKemey, N. Kelly, S. Scaife, C.A. Donnelly, et al. 2011. Field performance of engineered male mosquitoes. *Nature Biotechnology* 29:1034–7.

Harris, A.F., A.R. McKemey, D. Nimmo, Z. Curtis, I. Black, S.A. Morgan, et al. 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology* 30:828–30.

Hay, B.A., C.H. Chen, C.M. Ward, H. Huang, J.T. Su, and M. Guo. 2010. Engineering the genomes of wild insect populations: Challenges, and opportunities provided by synthetic *Medea* selfish genetic elements. *Journal of Insect Physiology* 56:1402–13.

Hedges, L.M., J.C. Brownlie, S.L. O'Neill, and K.N. Johnson. 2008. *Wolbachia* and virus protection in insects. *Science* 322:702.

Helinski, M.E. and B.G. Knols. 2008. Mating competitiveness of male *Anopheles arabiensis* mosquitoes irradiated with a partially or fully sterilizing dose in small and large laboratory cages. *Journal of Medical Entomology* 45:698–705.

Helinski, M.E., A.G. Parker, and B.G. Knols. 2006. Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis*. *Malaria Journal* 5:41.

Helinski, M.E., M.M. Hassan, W.M. El-Motasim, C.A. Malcolm, B.G. Knols, and B. El-Sayed. 2008. Towards a sterile insect technique field release of *Anopheles arabiensis* mosquitoes in Sudan: Irradiation, transportation, and field cage experimentation. *Malaria Journal* 7:65.

Helinski, M.E., A.G. Parker, and B.G. Knols. 2009. Radiation biology of mosquitoes. *Malaria Journal* 8(Suppl 2):S6.

Hoffman, A.A., B.L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P.H. Johnson, F. Muzzi, et al. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476:454–6.

Hughes, G.L., J. Vega-Rodriguez, P. Xue, and J.L. Rasgon. 2012. *Wolbachia* strain wAlbB enhances infection by the rodent malaria parasite *Plasmodium berghei* in *Anopheles gambiae* mosquitoes. *Applied Environmental Microbiology* 78:1491–95.

Isaacs, A.T., N. Jasinskiene, M. Tretiakov, I. Thiery, A. Zettor, C. Bourgouin, et al. 2012. Transgenic *Anopheles stephensi* coexpressing single-chain antibodies resist *Plasmodium falciparum* development. *Proceedings of the National Academy of Sciences* 109:E1922–30.

Ito, J., A. Ghosh, L.A. Moreira, E.A. Wimmer, and M. Jacobs-Lorena. 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* 417:452–5.

James, A. 2000. In: Handler AM, James A.A., (eds.) *Insect transgenesis*. CRC Press.

Jansen, V.A., M. Turelli, and H.C. Godfray. 2008. Stochastic spread of *Wolbachia*. *Proceedings of the Royal Society: Biological Sciences* 275:2769–76.

Jasinskiene, N., C.J. Coates, M.Q. Benedict, A.J. Cornel, C.S. Rafferty, A.A. James et al. 1998. Stable transformation of the yellow fever mosquito, *Aedes aegypti*, with the Hermes element from the housefly. *Proceedings of the National Academy of Sciences* 95:3743–7.

Jin, L., A.S. Walker, G. Fu, T. Harvey-Samuel, T. Dafa'alla, A. Miles, T. Marubbi, D. Granville, N. Humphrey-Jones, S. O'Connell, N.I. Morrison, and L. Alphey. 2013. Engineered female-specific lethality for control of pest Lepidoptera. *ACS Synthetic Biology* 2(3):160-6

Kaiser, P.E., J.A. Seawright, D.A. Dame, and D.J. Joslyn. 1978. Development of a genetic sexing for *Anopheles albimanus*. *Journal of Economic Entomology* 71:766–71.

Kambris, Z., P.E. Cook, H.K. Phuc, and S.P. Sinkins. 2009. Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. *Science* 326:134–6.

Klasson, L., Z. Kambris, P.E. Cook, T. Walker, and S.P. Sinkins. 2009. Horizontal gene transfer between *Wolbachia* and the mosquito *Aedes aegypti*. *BMC Genomics* 10:33.

Knipling E. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology* 48:459–69.

Koyama, J., H. Kakinohana, and T. Miyatake. 2004. Eradication of the melon fly *Bactrocera cucurbitae*, in Japan: Importance of behavior, ecology, genetics, and evolution. *Annual Review of Entomology* 49:331–49.

Labbé, G.M., D.D. Nimmo, and L. Alphey. 2010. piggyBac- and PhiC31-mediated genetic transformation of the Asian tiger mosquito, *Aedes albopictus* (Skuse). *PLoS Neglected Tropical Disease* 4:e788.

Labbé, G.M., S. Scaife, S.A. Morgan, Z.H. Curtis, and L. Alphey. 2012. Female-specific flightless (fsRIDL) phenotype for control of *Aedes albopictus*. *PLoS Neglected Tropical Diseases* 6:e1724.

Lacroix, R., A.R. McKemey, N. Raduan, L. Kwee Wee, W. Hong Ming, T. Guat Ney, et al. 2012. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. *PLoS One* 7:e42771.

Lambrechts, L., T.W. Scott, and D.J Gubler. 2010. Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. *PLoS Neglected Tropical Diseases* 4:e646.

Lu, P., G. Bian, X. Pan, and Z. Xi. 2012. *Wolbachia* induces density-dependent inhibition to dengue virus in mosquito cells. *PLoS Neglected Tropical Diseases* 6:e1754.

Magori, K., and F. Gould. 2006. Genetically engineered underdominance for manipulation of pest populations: A deterministic model. *Genetics* 172:2613–20.

Marois, E., C. Scali, J. Soichot, C. Kappler, E.A. Levashina, and F. Catteruccia. 2012. High-throughput sorting of mosquito larvae for laboratory studies and for future vector control interventions. *Malaria Journal* 11:302.

Marrelli, M.T., C.K. Moreira, D. Kelly, L. Alphey, and M. Jacobs-Lorena. 2006. Mosquito transgenesis: What is the fitness cost? *Trends in Parasitology* 22:197–202.

Marshall, J.M., G.W. Pittman, A.B. Buchman, and B.A. Hay. 2011. Semele: A killer-male, rescue-female system for suppression and replacement of insect disease vector populations. *Genetics* 187:535–51.

Mathur, G., I. Sanchez-Vargas, D. Alvarez, K.E. Olson, O. Marinotti, and A.A. James. 2010. Transgene-mediated suppression of dengue viruses in the salivary glands of the yellow fever mosquito, *Aedes aegypti*. *Insect Molecular Biology* 19:753–63.

McMeniman, C.J., G.L. Hughes, and S.L. O'Neill. 2011. A *Wolbachia* symbiont in *Aedes aegypti* disrupts mosquito egg development to a greater extent when mosquitoes feed on nonhuman versus human blood. *Journal of Medical Entomology* 48:76–84.

Moreira, L.A., I. Iturbe-Ormaetxe, J.A. Jeffery, G. Lu, A.T. Pyke, L.M. Hedges, et al. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* 139:1268–78.

Mumford, J.D. 2012. Science, regulation, and precedent for genetically modified insects. *PLoS Neglected Tropical Diseases* 6:e1504.

Mumford, J., M.M. Quinlan, C.J. Beech, L. Alphey, V. Bayard, M.L. Capurro, P. Kittayapong, J.D. Knight, M.T. Marrelli, K. Ombongi, J.M. Ramsey, and R. Reuben. 2009. MosqGuide: A project to develop best practice guidance for the deployment of innovative genetic vector control strategies for malaria and dengue. *Asia Pacific Journal of Molecular Biology and Biotechnology* 17:91–3.

National Environment Agency. 2012. Campaign against dengue. Available at http://www.dengue.gov.sg/

O'Connor, L., C. Plichart, A.C. Sang, C.L. Brelsfoard, H.C. Bossin, and S.L. Dobson. 2012. Open release of male mosquitoes infected with a *Wolbachia* biopesticide: Field performance and infection containment. *PLoS Neglected Tropical Diseases* 6:e1797.

O'Neill, S.L. 2011. *Wolbachia* infections of *Aedes aegypti* and their potential to control dengue transmission. *American Journal of Tropical Medicine and Hygiene* 85:169.

Ooi, E.E., K.T. Goh, and D.J. Gubler. 2006. Dengue prevention and 35 years of vector control in Singapore. *Emerging Infectious Diseases* 12:887–93.

Pan, X., G. Zhou, J. Wu, G. Bian, P. Lu, A.S. Raikhel, et al. 2012. *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. *Proceedings of the National Academy of Sciences* 109:E23–31.

Papathanos, P.A., H.C. Bossin, M.Q. Benedict, F. Catteruccia, C.A. Malcolm, L. Alphey, et al. 2009. Sex separation strategies: Past experience and new approaches. *Malaria Journal* 8(Suppl 2):S5.

Phuc, H.K., M.H. Andreasen, R.S. Burton, C. Vass, M.J. Epton, G. Pape, et al. 2007. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biology* 5:11.

Rasgon, J.L. 2009. Multi-locus assortment (MLA) for transgene dispersal and elimination in mosquito populations. *PLoS One* 4:e5833.

Rendón, P., D. McInnis, D. Lance, and J. Stewart. 2004. Medfly (Diptera:Tephritidae) genetic sexing: Large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. *Journal of Economic Entomology* 97:1547–53.

Rodrigues, F.G., S.B. Oliveira, B.C. Rocha, and L.A. Moreira. 2006. Germline transformation of *Aedes fluviatilis* (Diptera:Culicidae) with the piggyBac transposable element. *Memórias do Instituto Oswaldo Cruz* 101:755–7.

Sabchareon, A., D. Wallace, C. Sirivichayakul, K. Limkittikul, P. Chanthavanich, S. Suvannadabba, et al. 2012. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: A randomised, controlled phase 2b trial. *Lancet* 380(9853):1559–67.

Schliekelman, P., and F. Gould. 2000. Pest control by the release of insects carrying a female-killing allele on multiple loci. *Journal of Economic Entomology* 93:1566–79.

Scott, T.W., E. Chow, D. Strickman, P. Kittayapong, R.A. Wirtz, L.H. Lorenz, et al. 1993. Blood-feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village. *Journal of Medical Entomology* 30:922–7.

Sinkins, S.P. and F. Gould. 2006. Gene drive systems for insect disease vectors. *National Review of Genetics* 7:427–35.

Siriyasatien, P., T. Pengsakul, V. Kittichai, A. Phumee, S. Kaewsaitiam, U. Thavara, et al. 2010. Identification of blood meal of field caught *Aedes aegypti* (L.) by multiplex PCR. *Southeast Asian Journal of Tropical Medicine and Public Health* 41:43–7.

Subramaniam, T.S., H.L. Lee, W.A. Nazni, and S. Murad. 2012. Genetically modified mosquito: The Malaysian public engagement experience. *Biotechnology Journal* 7:1323–7.

Thomas, D.D., C.A. Donnelly, R.J. Wood, and L.S. Alphey. 2000. Insect population control using a dominant, repressible, lethal genetic system. *Science* 287:2474–6.

Valerio, L., F. Marini, G. Bongiorno, L. Facchinelli, M. Pombi, B. Caputo, et al. 2010. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in urban and rural contexts within Rome province, Italy. *Vector Borne and Zoonotic Diseases* 10:291–4.

Walker, T., P.H. Johnson, L.A. Moreira, I. Iturbe-Ormaetxe, F.D. Frentiu, C.J. McMeniman, et al. 2011. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 476:450–3.

White, S.M., P. Rohani, and S.M. Sait. 2010. Modelling pulsed releases for sterile insect techniques: Fitness costs of sterile and transgenic males and the effects on mosquito dynamics. *Journal Applied Ecology* 47:1329–39.

WHO. 2012. *Global strategy for dengue prevention and control 2012–2020*. Geneva: WHO.

Windbichler, N., P.A. Papathanos, and A. Crisanti. 2008. Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles gambiae*. *PLoS Genetics* 4:e1000291.

Wise de Valdez, M.R, D. Nimmo, J. Betz, H.F. Gong, A.A. James, L. Alphey, et al. 2011. Genetic elimination of dengue vector mosquitoes. *Proceedings of the National Academy of Sciences* 108:4772–5.

Wu, M., L.V. Sun, J. Vamathevan, M. Riegler, R. Deboy, J.C. Brownlie, et al. 2004. Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: A streamlined genome overrun by mobile genetic elements. *PLoS Biology* 2:e69.

Xi, Z., C.C. Khoo, and S.L. Dobson. 2005. *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science*. 310:326–8.

Xi, Z., C.C. Khoo, and S.L. Dobson. 2006. Interspecific transfer of *Wolbachia* into the mosquito disease vector *Aedes albopictus*. *Proceedings of Biological Sciences* 273:1317–22.

Yakob, L., L. Alphey, and M. Bonsall. 2008. *Aedes aegypti* control: The concomitant role of competition, space and transgenic technologies. *Journal of Applied Ecology* 45:1258–65.

A3

THE INTENSIFYING STORM: DOMESTICATION OF *AEDES AEGYPTI*, URBANIZATION OF ARBOVIRUSES, AND EMERGING INSECTICIDE RESISTANCE

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Summary

It has been and continues to be the great misfortune of humankind to share time and space with the *Aedes aegypti* mosquito. Domestication of *Ae. aegypti*, urbanization of arboviruses, and globalization have created a super nidus for *Ae. aegypti*-transmitted diseases that spans the pantropical world. Ongoing pandemics of dengue and chikungunya are testimony to the threat posed by the super nidus. The burdens and threats of *Ae. aegypti*-transmitted diseases are too great to tolerate and are likely to worsen due to emerging insecticide resistance. The situation is grim; it is time to initiate a "war" on *Ae. aegypti* and to exploit new knowledge, tools, and approaches to control this enemy of humankind.

Introduction

As an epidemiological group, vector-borne diseases (VBDs), e.g., malaria, leishmaniasis, filariasis, onchocerciasis, trypanosomiasis, and dengue, continue to cause inestimable misery, morbidity, and mortality in humans. VBDs are major impediments to social and economic development in areas of the world that can least afford them. For the most part, there are no vaccines or therapeutics for these diseases. Thus, vector control is the principal tool to prevent and control these threats. The vectors have proven to be intractable to sustainable control, and emerging resistance to insecticides is of great concern. Notable recent successes in reducing the burden of some of these diseases, such as malaria, are now threatened by the emergence of resistance, most notably to pyrethroid insecticides, in their vectors (Strode et al., 2014; Hemingway et al., 2013). The situation is further complicated by the concomitant reduction in medical entomologists, vector biologists, and vector control personnel available to address VBD emergencies. This situation was addressed in a previous IOM publication (IOM, 2003a, 2008). Unfortunately little progress has been made in this area (IOM, 2008).

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Arthropod-borne viruses (arboviruses) continue to emerge and spread throughout the world. The introduction of West Nile virus into New York and its rapid spread throughout the Western Hemisphere is a textbook example of arbovirus epidemic potential (Petersen et al., 2013). Another great threat to humankind is the urbanization and spread of arboviruses, such as dengue virus (DENV) and chikungunya virus (CHIKV). Historically these viruses and yellow fever virus (YFV) emerged from sylvatic foci in Africa or Asia, were transported with the *Aedes aegypti* mosquito through much of the tropical world in sailing ships, and caused epidemics principally in port cities (Weaver, 2013; Weaver and Reisen, 2010; Nasci, 2014; Halstead, 2015). However, the ability of these viruses to emerge and to become established in tropical urban areas has increased dramatically in the past 50 years. The emergence and spread of epidemic dengue and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in the Americas in the 1980s and the emergence of CHIKV in the Caribbean in 2013 and its rapid spread throughout tropical America are examples of the extraordinary potential of urbanized, *Ae. aegypti*-transmitted arboviruses to traffic and to emerge as public health threats in pantropical urban areas.

Many reviews have addressed factors that have contributed to the emergence and resurgence and public health importance of VBDs. Clearly, lack of vaccines and therapeutics, erosion of public health infrastructure, poverty and social inequalities, population growth, unplanned urbanization, and globalization are major factors for emergence and continued importance of most VBDs. These have been discussed in detail elsewhere (Gratz, 1999; Gubler, 2005, 2011; IOM, 2003a; Weaver and Reisen, 2010). In a recent review, Gubler (2011) proposes three principal drivers that have conditioned the emergence and expansion of dengue and DHF/DSS as major threats to public health in the tropics: (1) urbanization, (2) globalization, and (3) lack of effective mosquito control. Human population growth and urbanization provide unprecedented availability of susceptible amplifying human hosts and an environment conducive to propagation of *Ae. aegypti*, which live in intimate association with humans. These factors in conjunction with nonsustainable mosquito control have conspired to create ideal conditions for transmission and maintenance of urbanized viruses. Globalization further fuels the flames of the epidemic potential of urbanized viruses, resulting in unprecedented trafficking of virus-infected humans and mosquitoes through the pantropical world. The combination of hyperabundant *Ae. aegypti*, cocirculation of multiple DENV serotypes, and increased evolution and trafficking of new virulent genotypes have led to dengue *hyperendemicity* and the global dengue pandemic (Gubler, 2011).

VBD specialists think in terms of the nidus of infection, in which a pathogen, susceptible hosts, and vectors intersect temporally and spatially in an environment conducive to pathogen transmission and maintenance (Weaver and Reisen, 2010). This concept has great utility, for example, in understanding the factors that maintain zoonotic virus endemic sylvatic cycles and the potential approaches

for preventing human infections. Pantropical urban areas now constitute a "super nidus" for *Ae. aegypti* transmitted pathogens. The large human and mosquito populations in tropical urban areas provide the ideal conditions or nidus for *Ae. aegypti* transmitted arbovirus maintenance, transmission, evolution, and trafficking. The large urban areas are interconnected by travel and commerce, which promote movement of viruses to new areas both within and between urban areas. Jet travel is especially efficient in moving people, viruses, and vectors around the world (Gubler, 2011).

The super nidus is certainly a key factor in the dramatic increase in importance of dengue and chikungunya in the Americas. Both DENV and CHIKV can be transmitted by other mosquito vectors, most notably *Ae. albopictus*, which can cause significant outbreaks of disease (Nasci, 2014). However, transmission of these viruses by *Ae. aegypti* in the tropical urban super nidus is an even greater threat for these diseases. Reduced efficacy of insecticides to control *Ae. aegypti* will likely exacerbate this situation.

In the following, we will principally focus upon entomological factors in the super nidus that conditioned emergence of epidemic dengue and DHF/DSS and chikungunya in Latin America. We will also address the implications of emerging insecticide resistance in *Ae. aegypti* for continued emergence, resurgence, and control of arboviruses in the super nidus. *This is not a review of the literature*. Rather the emphasis will be on selected examples from our long term studies of the epidemic potential of dengue and the control of *Ae. aegypti* in Mexico to illustrate the problems and complexity of controlling *Ae. aegypti* and urbanized arbovirus diseases. However, the lessons learned can certainly be extrapolated to most urban areas in the tropical world.

The Origins of the Super Nidus: Domestication of *Aedes aegypti* and Urbanization of Arboviruses

Domestication of Aedes aegypti

One of the great misfortunes of humankind has been the domestication and subsequent urbanization of *Ae. aegypti*. The ancestral form of *Ae. aegypti* is found in Africa; it is a sylvatic mosquito that feeds on nonhuman primates and other forest mammals and oviposits in tree holes and other natural water-containing sites (Powell and Tabachnick, 2013, Tabachnick, 2013). The mosquito is dark in color and is designated as a subspecies—*Ae. aegypti formosus*. A lighter colored subspecies, *Ae. aegypti aegypti* (hereafter called *Ae. aegypti* for simplicity) has adapted to feed on humans and to live and breed in and around human habitation. Domestication has likely occurred multiple times (Brown et al., 2013; Moore et al., 2013, Powell and Tabachnick, 2013). *Ae. aegypti* has expanded from Africa and colonized most of the pantropical world with disastrous public health consequences. Historically, the introduction of *Ae. aegypti* and of YFV, DENV,

and CHIKV into the New World resulted in large epidemics of the respective diseases typically in port cities (Powell and Tabachnick, 2013; Weaver, 2014). Similarly, introduction of *Ae. aegypti* into Asia in the mid-20th century led to spillover of DENV from its sylvatic cycles resulting in large urban epidemics of dengue (Smith, 1956).

Behavioral and genetic changes associated with domestication *of Ae. aegypti* dramatically impacted the vectorial capacity of the species for arbovirus transmission (Table A3-1). *Ae. aegypti* is anthropophilic (feeds on humans) and endophilic (lives in homes). Thus the mosquito is intimately associated with humans, thereby dramatically increasing its potential to transmit pathogens to humans. *Ae. aegypti formosus* is zoophilic (feeds on nonhuman hosts), exophilic (lives outdoors), and sylvatic, thereby limiting its potential to transmit pathogens to humans. However, domestication of *Ae. aegypti formosus* may still be occurring in West Africa, where the subspecies enters huts to feed on humans. Both *Ae. aegypti* and *Ae. aegypti formosus* occur in Senegal with the former occurring mostly in coastal urban environments (Sylla et al., 2009; Moore et al., 2009; Dickson, et al., 2014). *Ae. aegypti* in coastal West Africa may have resulted from a reintroduction of *Ae. aegypti* or may be the harbinger of a new domestication of *Ae. aegypti formosus* (Brown et al., 2013). Either scenario poses increased threats of arbovirus urbanization in West Africa.

Domestication of *Ae. aegypti* has also resulted in changes in vector competence for YFV and DENV. Vector competence is a component of the vectorial

TABLE A3-1 *Aedes aegypti*—Behavioral and Biological Factors Contributing to the Extraordinary Vectorial Capacity for Arboviruses

Anthropophily:	Feeds preferentially on humans, and when humans are available, zoophily is minimal. In addition, sugar feeding is very limited.
Endophily:	Prefers to live and feed indoors in homes and other structures. Extraordinarily close association with humans. Will oviposit in homes/structures if larval development sites are available. Readily lays eggs in cans, tires, refuse, and other manmade larval development sites surrounding homes. Endophily protects mosquitoes from outdoor insecticide-based space spraying in settings where the typical housing type is "closed" and thus prevents ingress of the spray.
Interrupted feeding:	May only take a partial blood meal before being disturbed and may complete feeding on other hosts. Evolved defensive behavior of the mosquito may promote mechanical and biological transmission of arboviruses.
Multiple feeding:	May feed multiple times during a gonadotrophic cycle, greatly promoting the potential for arbovirus transmission.

SOURCES: Selected papers documenting the behavioral and biological factors that contribute to the vectorial capacity of *Ae. aegypti*: Edman et al., 1992; Harrington et al., 2001, 2014; Gubler, 2011; Garcia-Rejon et al., 2008, 2011; Hemingway et al., 2006; Reiter et al., 2003; Reiter and Gubler, 1997; Scott et al., 1993, 2000; Scott and Takken, 2012.

capacity of a mosquito population; it is more narrowly defined as the permissiveness of a mosquito for infection, replication, and subsequent transmission of a pathogen (Black et al., 2002). Genetic and environmental determinants of *Flavivirus* infection and transmission by *Ae. aegypti* and of the distribution of vector competence in natural populations have been reviewed elsewhere (Black et al., 2002; Tabachnick, 2013). Early studies revealed that *Ae. aegypti formosus* is less able to become infected with and to transmit YFV than *Ae. aegypti* (Beaty and Aitken, 1979; Tabachnick et al., 1985). Subsequent studies also revealed that *Ae. aegypti formosus* is also a less effective vector for DENV; however, this is dependent upon the origin of the DENV isolates used to challenge the mosquitoes (e.g., Sylla et al., 2009; Dickson et al., 2014). There also is considerable genetic variability among *Ae. aegypti* populations in their vector competence for DENV (Bennett et al., 2002). This will be addressed in detail below in the context of *Ae. aegypti* vector competence for DENV in Mexico.

Urbanization of Arboviruses

Arboviruses have the potential to spill out of their enzootic or sylvatic transmission cycles into cycles in which humans become the vertebrate amplifying hosts for the virus; such spillovers can have devastating public health consequences (Weaver and Reisen, 2010; Weaver, 2013). Spillover can take many forms; for example, humans may simply encroach upon new environments and become more frequently exposed to enzootic vectors that are willing to take human blood. Urbanization of sylvatic arboviruses is by far the greatest threat. In such scenarios, humans may become the dominant vertebrate host, eliminating the need for amplifying sylvatic hosts. YFV, DENV, and CHIKV have emerged from their respective zoonotic sylvatic cycles involving forest mosquitoes and nonhuman primates into transmission cycles involving *Ae. aegypti* and humans, resulting in global pandemics. The super nidus with its hyperabundant *Ae. aegypti* and burgeoning susceptible human populations provides unprecedented receptivity to arbovirus spillover into the urban cycle and also promotes opportunities for spillback into sylvatic cycles. Spillback is epidemiologically significant; establishment of the virus in a sylvatic cycle in a newly invaded region effectively limits opportunities for virus eradication in that region.

Yellow fever virus YFV is the archetypical virus in terms of spillover and urbanization. YFV originated in Africa and was maintained in cycles involving principally canopy dwelling mosquitoes and nonhuman primates (Mutebi and Barrett, 2002; Beck et al., 2013). Spillover of YFV into the urban transmission cycle involving humans and *Ae. aegypti* resulted in yellow fever epidemics that decimated cities, especially port cities, in the Americas and Africa (Weaver and Reisen, 2010). YFV is also the archetypical example of arbovirus spillback into a sylvatic cycle involving nonhuman primates and forest mosquitoes. YFV spilled

back into sylvatic cycles in the Americas, posing an ongoing threat of urbanization of YFV from these sylvatic foci. The lack of reurbanization of YFV from sylvatic cycles in South America is a mystery (Barrett and Higgs, 2007). Another great mystery has been the lack of emergence of YFV in Asia, which would be a public health catastrophe. Early studies revealed that the vector competence of Asian *Ae. aegypti* mosquitoes was lower than that of Caribbean populations of the vector, which may in part condition the lack of emergence in Asia (Tabachnick et al., 1985). However, dengue hyperendemicity first in Asia and now in the New World could provide cross-protective herd immunity in humans and ironically thereby restrict urbanization of YFV in both regions.

Dengue virus DENV originated in Southeast Asia where the four DENV serotypes (DENV1–4) diverged and are maintained in cycles involving canopy-dwelling mosquitoes and nonhuman primates (Hanley et al., 2013; Messina et al., 2014). Spillover of all four DENV serotypes from sylvatic cycles has occurred and continues to occur into human cycles with anthropophilic vectors, including *Ae. aegypti* and *Ae. albopictus*. There is apparently no need for adaptation for human transmission and virulence (Vasilakis et al., 2007, 2010; Weaver, 2013). Clearly the expanding super nidus is even more receptive to emergence and urbanization of DENV from sylvatic cycles. Spillback of DENV-2 into a sylvatic nonhuman primate and forest mosquito cycle has occurred in West Africa (Weaver and Reisen, 2010; Weaver, 2013), but thus far, spillback of DENV into sylvatic cycles in Latin America has not been detected. Epidemic dengue and DHF/DSS emerged in Southeast Asia following the introduction of *Ae. aegypti* and the rapid urbanization following World War II, highlighting the importance of the urban transmission cycle for dengue hyperendemicity (Smith, 1956; Gubler, 2011).

Dengue is the most important arthropod-borne viral disease of humans with more than 3 billion people living in dengue endemic areas (Guzman and Harris, 2014). Worldwide, more than 390 million infections, 100 million DF cases, and 500,000 cases of the more severe DHF occur each year (Bhatt et al., 2013). Infection with one of the four antigenically related DENV serotypes confers long-term protection to that serotype but no or very short-lived cross-protection to the other serotypes. Dengue disease severity ranges from asymptomatic to fatal (Srikiatkhachorn et al., 2011). Most symptomatic cases are classified as dengue fever (DF), an acute and self-limited condition characterized by fever, generalized pains, rash, lymphadenopathy, and minor hemorrhages. Even the asymptomatic infections are likely to be epidemiologically significant; these silent infections can prime patients for the more serious forms of the disease. People who experience secondary infections with a heterologous serotype of the virus are primed for the more serious forms of the disease—DHF/DSS, which is characterized by hemostatic disorders, hepatic involvement, and plasma leakage resulting from increased vascular permeability. DSS is potentially fatal. In Mexico and much of Latin America, almost 30 percent of patients are now progressing to severe

dengue disease (see Figure A3-1). The surge of patients experiencing severe dengue disease has overwhelmed the public health infrastructure in many cities and countries. The emergence of epidemic dengue and DHF/DSS in the pantropical world has been a public health disaster.

Chikungunya virus CHIKV has emerged from its sylvatic nidus in Africa multiple times, causing major epidemics in Africa, Asia, and Latin America. Historically, CHIKV emerged from its enzootic cycle involving forest mosquitoes and nonhuman primates and was transported around the world in sailing ships with *Ae. aegypti*, as with YFV and DENV (Weaver and Reisen, 2010; Carey, 1971). In the 1950s, epidemics of chikungunya were reported in India and Southeast Asia, but the virus disappeared in India. The situation changed dramatically in recent years. Major epidemics caused by different strains of CHIKV have occurred with millions of cases reported (Nasci, 2014, Weaver, 2014). The recent reintroduction of CHIKV into the Caribbean (Halstead, 2015) and its explosive spread throughout Latin America are the latest manifestations of urbanization and spread of the virus. Considering the size of the super nidus in Latin America, it is likely that CHIKV will become endemic in the tropical Americas as it has in Asia following its emergence there (Nasci, 2014; Weaver, 2014). It will be of great interest to see if CHIKV spills back into a sylvatic cycle in the Americas. In Southeast Asia, the virus has been endemic for many years in the *Ae. aegypti*–human cycle, yet there is no evidence of spillback into a sylvatic cycle.

Virus infection can cause a febrile disease with severe, debilitating arthritis, which can last for several weeks, and in some cases may become chronic. Historically, chikungunya was thought to be a serious but self-limiting disease. However, mortality rates in the range of 1 per 1,000 cases have been reported in epidemics in India and Asia, likely due to comorbidities (Renault et al., 2008; Tandale et al., 2009). CHIKV exerts a major socioeconomic burden during epidemics (Soumahoro et al., 2011). Following CHIKV introduction into India in 2006, millions of cases occurred and transmission continues (Mohan et al., 2010). The explosive nature of the epidemic of chikungunya in the New World has been amazing. More than 850,000 cases have been reported since its introduction early in 2013 (http://www.cdc.gov/chikungunya), and it is likely that all of pantropical America will soon experience the burden of chikungunya.

Other arbovirus threats Arboviruses maintained in cycles involving nonhuman primates clearly pose a great threat for urbanization. Mayaro virus, a relative of CHIKV, is maintained in a forest mosquito–nonhuman primate cycle in the Americas and Zika virus, which is presumably maintained in enzootic cycles involving nonhuman primates and forest mosquitoes in Africa and Asia recently caused an outbreak of febrile disease in a Pacific Island in which humans were the amplifying hosts, are clear candidates for urbanization (Weaver and Reisen, 2010; Weaver, 2013). Other non-*Ae. aegypti* vectored viruses have the potential

to adapt to *Ae. aegypti* and to become urbanized. The dramatic expansion of the super nidus and its increasing encroachment on sylvatic cycles of other important arboviruses provides unprecedented opportunities for arbovirus urbanization. For example, *Ae. aegypti* has been demonstrated to be a competent vector of Venezuelan equine encephalitis virus (VEEV) in laboratory studies, and humans develop a significant viremia (Weaver and Reisen, 2010). The expanding super nidus in South America ensures that VEEV and *Ae. aegypti* will overlap; urbanization of VEEV would be a public health catastrophe.

Comment We are entering new territory in terms of urbanization of arboviruses; the pantropical super nidus and globalization combine to provide (1) unprecedented opportunities for spillover of arboviruses from sylvatic cycles, (2) extraordinary potential for viruses and vectors to be transported rapidly throughout the world, and (3) major threats of ongoing epidemics in the super nidus, with enormous public health and economic consequences. Some of the causes, threats, and needs for control of the super nidus will be addressed in the context of our studies of epidemic dengue and DHF/DSS and control of *Ae. aegypti* in Mexico.

Epidemic Dengue and Dengue Hemorrhagic Fever in Mexico

Epidemic DHF/DSS emerged in the Americas in the 1980s (Gubler, 2005; Guzman and Harris, 2014). Since then dengue has emerged as a major public health problem and is considered to be hyperendemic in most of Latin America. In Mexico, *Ae. aegypti* has resurged and is hyperabundant in most urban areas in the country (with the exception of those at higher altitudes in the central plateau), all four serotypes of DENV now cocirculate in endemic areas, more virulent viruses and genotypes have been introduced, and the number of secondary infections and severe disease manifestations are greatly increased (see Figure A3-1). Indeed, dengue epidemics are annual occurrences and can overwhelm public health capacity in the affected areas. As mentioned above, CHIKV has exploded throughout much of Latin America since its introduction in 2013. The Ministry of Health of Mexico reported the first autochthonous (locally contracted) case of chikungunya on November 7, 2014, in Chiapas State (http://www.cenaprece.salud.gob.mx/programas/interior/emergencias/descargas/pdf/Declaratoria_Emergencia_Chiapas_Chikungunya.pdf). There is great concern about a major epidemic of chikungunya in Mexico in 2015. In the following, we will focus upon selected factors that have resulted in *Ae. aegypti* hyperabundance in Mexico and contributed to the dengue hyperendemicity.

Collaborative Studies of Dengue Hyperendemicity and Control in Mexico

In the early 1990s, before the emergence of DHF/DSS, we embarked upon collaborative studies with Mexican scientists that focused upon viral, vector,

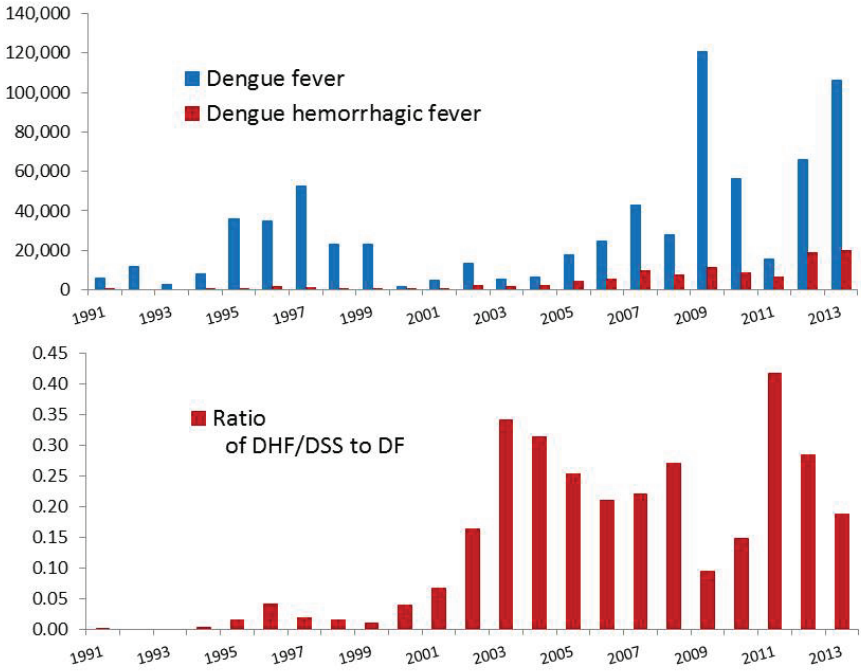

FIGURE A3-1 Dengue fever and dengue hemorrhagic fever and shock syndrome in Mexico.
Top: Laboratory-confirmed dengue fever cases in Mexico.
Bottom: Ratio of DHF/DSS (dengue hemorrhagic fever/dengue shock syndrome) to DF (dengue fever) laboratory-confirmed cases.
SOURCE: Beaty et al. Data from Mexican Ministry of Health (http://www.epidemiologia.salud.gob.mx/dgae/infoepid/inicio_anuarios.html).

and epidemiological determinants of the lack of DHF/DSS in Mexico, which was occurring elsewhere in the Americas. The collaborations then evolved to develop innovative approaches to control *Ae. aegypti* and DENV transmission to humans in homes. Mérida City in Yucatán State became a major field site and focus of many of our studies. Some of the studies and lessons learned will be briefly reviewed here.

Viral Determinants of Dengue Hyperendemicity

Molecular epidemiological studies were conducted to investigate the viral determinants of the emergence of epidemic dengue and DHF/DSS as major public health problems in Mexico (Diaz et al., 2006). Phylogenetic analyses were

conducted to determine the origin, persistence, and geographical dispersion of the four serotypes of DENV isolated in Mexico between 1980 and 2002. Changes in the incidence and severity of dengue were temporally associated with the introduction and circulation of different serotypes and more virulent genotypes of DENV into the Yucatán State of Mexico in 2002 (Loroño-Pino et al., 2004). This was associated with increased incidence of DHF/DSS. Nucleotide sequencing and phylogenetic analyses identified isolates from patients with severe disease as DENV-2 viruses of the American-Asian genotype, which was the first report of this genotype in Yucatán State. Ominously, 31 percent of the patients met the World Health Organization criteria for DHF. The majority (77 percent) of the patients experienced secondary infections in this epidemic. The new virus genotypes supplanted the DENV-2 American genotype viruses in Mexico. The reasons for these genetic sweeps remain to be determined, and this is an important area of research. The introduction of new virulent virus genotypes resulted in a dramatic increase in severe dengue cases (see Figure A3-1). Increased surveillance for such introductions is critical to allow public health authorities to intervene in impending epidemics.

Vector Determinants of Dengue Hyperendemicity

Many entomological factors have contributed to dengue hyperendemicity and *Ae. aegypti* resurgence in Mexico:

1. The collapse of sustainable vector control
2. Introduction and trafficking of vectors
3. Presence and distribution of highly competent vectors
4. The extreme endophily of the vector in concrete housing and buildings typical of Latin America
5. The emergence of the throw-away society providing inexhaustible breeding sites for the vector
6. Behavioral changes permitting *Ae. aegypti* to more effectively exploit the urban environment

Selected investigations and lessons learned concerning these entomological factors follow to illustrate the threats and complexities of controlling *Ae. aegypti*-transmitted arboviral diseases in the super nidus.

Ineffective or nonsustainable *Ae. aegypti* control *Ae. aegypti* control in the super nidus is a major challenge to public health. It is noteworthy that in the mid-20th century, countries in the Western Hemisphere including Mexico as well as the Pan American Health Organization (PAHO) waged an effective campaign to control *Ae. aegypti*. In the early 1900s, *Ae. aegypti* and dengue were widely distributed, but a successful hemispheric campaign against yellow fever led by

Fred Soper of PAHO that was initiated in 1947 and continued to the early 1970s resulted in *Ae. aegypti* and DENV being eliminated from most of Central and South America. This campaign was based on spraying of larval development sites and indoor environments with DDT. The campaign was quite effective, and *Ae. aegypti* presence and/or abundance was dramatically reduced in the Americas (Gubler, 2005; Gratz, 1999). Ironically, the success of the program led to its demise. The resources devoted to vector control, which is quite expensive, were diverted to other programs. This resulted in an astounding reemergence of *Ae. aegypti* and also of DENV with dengue outbreaks and the emergence of DHF/DSS across the Americas in the following decades (Gubler, 2005, 2011). The measures used in Soper's campaign would likely not be as effective today (IOM, 2008). The urban super nidus vastly increases the difficulty of controlling *Ae. aegypti,* and current vector control programs have not stemmed the rising tide of the dengue pandemic.

These control programs typically include activities to control both immature and adult stages of *Ae. aegypti*. Chemical or biological larviciding and physical source reduction are widely used to control immatures and to try to maintain mosquito populations below threshold levels thought to interrupt DENV transmission (Gubler, 2005; Eisen et al., 2009). This overall strategy has not proven to be sustainable. Programs were often poorly funded or did not receive long-term support by government agencies. Indeed, source reduction may no longer be a practically sustainable control strategy because of the emergence of the "throw-away society" where breeding sites for *Ae. aegypti* accumulate rapidly and are almost ubiquitous. Chemical or biological larviciding can be effective, but it is tremendously labor intensive and costly, and locating breeding sites can be difficult as described below. The extreme endophily of female *Ae. aegypti* complicates efforts to control adults (Edman et al., 1992; Harrington et al., 2001; Bonds 2012). Outdoor spraying of insecticides during dengue outbreaks is likely to be ineffective in most situations because of poor penetration of the insecticide into cement housing, but it is still routinely used in many control programs (Gubler, 2005). Ironically, exposure of mosquitoes to sublethal doses of insecticides during outdoor spraying may increase evolution of resistance. Indoor space spraying can be an effective dengue outbreak intervention strategy; recent studies in Iquito, Peru, have demonstrated that indoor space spraying three times reduces *Ae. aegypti* populations and the number of dengue cases (T. Scott, personal communication). Although effective, the approach is laborious, expensive, and needs to be targeted to be cost-effective.

Thus despite the tremendous efforts and resources expended by public health organizations in dengue-endemic countries, it has proven difficult to achieve sustainable control of *Ae. aegypti* and to prevent or disrupt dengue outbreaks (Eisen et al., 2009). *Ae. aegypti* control in the super nidus is very difficult and complex as illustrated by some of the following examples.

Trafficking of vectors Globalization is predicated upon commerce and rapid and efficient transport of goods and people, and it provides great threats of trafficking of *Ae. aegypti* between and within super nidus regions (IOM, 2003a; Gubler, 2011). The mosquito can be transported as eggs in tires and other containers into new regions and hatch upon exposure to water, or as adults in vehicles and even in airplanes (Lounibus, 2002; Whelan et al., 2012). Such trafficking potential threatens sustainability of vector control. Understanding the breeding structure and trafficking potential of *Ae. aegypti* in a country is critical for developing effective vector surveillance, monitoring, and control strategies and for understanding spatial heterogeneities in DENV transmission. To characterize the breeding structure of *Ae. aegypti* in Mexico, we conducted a population genetic analysis of 38 collections of *Ae. aegypti* from throughout coastal regions of Mexico (Gorrochotegui-Escalante et al., 2002). Single-strand conformation polymorphism analysis was used to screen for variation in a 387-bp region of the ND4 mitochondrial gene, and 25 haplotypes were detected. Northeastern Mexico collections were genetically differentiated from and had lower genetic diversity than southern Yucatán and western coastal Pacific collections (see Figure A3-2). Yucatán and Pacific collections were genetically homogeneous. Regression analysis of geographic and genetic distances indicated that collections were genetically isolated by distance in the Pacific and the Yucatán, but not among collections in the northeast. Free gene flow occurred among all collections within 130 km of one another in the northeast and within 180 km in the Yucatán. F(ST) values were never large among Pacific collections, suggesting extensive gene flow along the Pacific coast (see Figure A3-2).

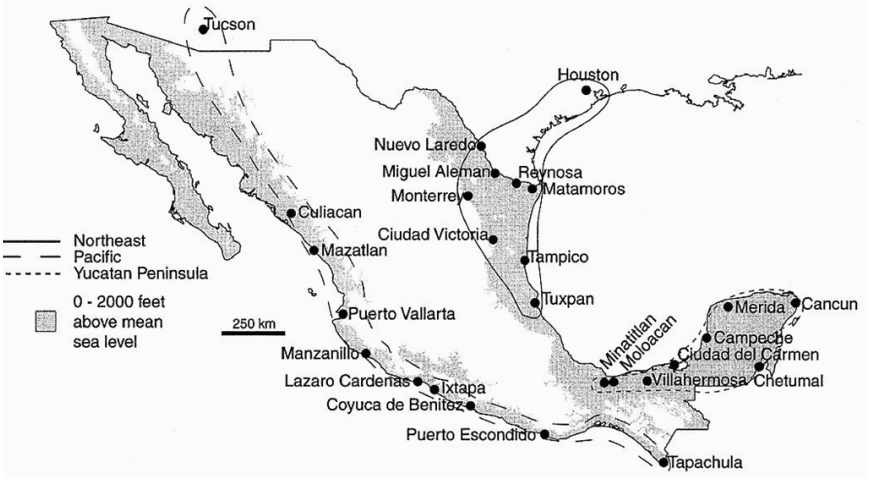

FIGURE A3-2 Breeding structure of *Aedes aegypti* in Mexico and the United States. SOURCE: Gorrochotegui-Escalante et al., 2002, with permission from *American Journal of Tropical Medicine and Hygiene*.

The extraordinary gene flow in *Ae. aegypti* populations along the western coast of Mexico was presumed to be attributable to trafficking of *Ae. aegypti* in the extensive commerce and tourism trade routes located there. The reasons for limited gene flow between *Ae. aegypti* populations in the northeast of Mexico and the Yucatán in the south were investigated (Lozano-Fuentes et al., 2009). Targeted population genetic studies revealed that the intersection of the neovolcanic axis (NVA) with the Gulf of Mexico coast in the state of Veracruz acts as a discrete barrier to gene flow among *Ae. aegypti* populations north and south of the NVA, presumably because of the lack of commerce and tourism routes through this region of eastern Mexico. The breeding structure of *Ae. aegypti* in Mexico is complex and dynamic. Vector (and vector gene) trafficking into previously controlled areas will clearly complicate sustainable control efforts by public health officials.

Competence of vectors for virus transmission Vector competence is a critical component of the transmission potential of an arbovirus and may contribute significantly to the heterogeneities seen in DENV transmission. Concomitantly with our breeding structure studies, we characterized heterogeneities in vector competence of Mexican populations of *Ae. aegypti* from 24 collections in Mexico and the United States (see Figure A3-3). Mosquitoes were challenged orally with DENV-2 JAM1409, an American-Asian genotype isolated during one of the initial epidemics of DHF/DSS in the New World (Bennett et al., 2002). The presence or absence of a midgut infection barrier (MIB) and a midgut escape barrier (MEB) was determined for mosquitoes in each population. The percentage of mosquitoes exhibiting a MIB ranged from 14 percent to 59 percent, and those exhibiting a MEB ranged from 4 percent to 43 percent in the collections. Midgut infection rates were dose dependent. Thus new, more virulent genotypes

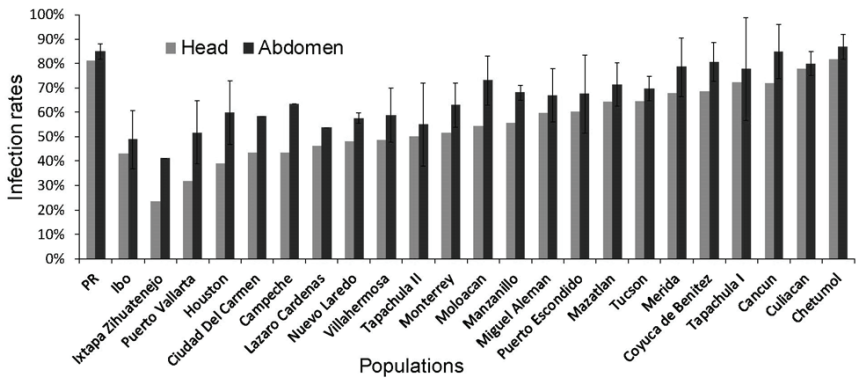

FIGURE A3-3 Infection rates of *Aedes aegypti* populations after *per os* challenge with DENV-2 JAM 1409 virus.
SOURCE: Adapted from Bennett et al., 2002.

of DENV that cause higher titered viremias in humans would likely be more infectious for mosquitoes. The vector competence rate of examined collections (i.e., the number of mosquitoes with disseminated DENV infections/number of mosquitoes orally challenged with the virus) ranged from 24 percent to 83 percent (see Figure A3-3). Considerable genetic variability in vector competence for DENV occurs in *Ae. aegypti* collections in Mexico, with mosquitoes from the Yucatán Peninsula being highly competent vectors (Bennett et al., 2002).

The mosquito populations north and south of the NVA also differed in their vector competence for DENV-2 (Lozano-Fuentes et al., 2009). The average vector competence rate for *Ae. aegypti* from populations north of the NVA was 55 percent, as compared with 20 percent south of the NVA. Most of this variation was attributable to midgut infection and escape barriers. In *Ae. aegypti* north of the NVA, 22 percent failed to develop midgut infections and 30 percent of those with an infected midgut failed to develop a disseminated infection. In contrast, 45 percent of the mosquitoes from south of the NVA failed to develop a midgut infection, and 63 percent of those with an infected midgut failed to develop a disseminated infection.

Mexican vector and virus interactions We also conducted studies to understand heterogeneities in dengue prevalence and mechanisms conditioning the genetic sweeps of virus genotypes. For example, *Ae. aegypti* from Chetumal were orally challenged with American and American-Asian genotype viruses isolated from severe dengue patients in Mérida City (Loroño-Pino et al., 2004). The American-Asian DENV-2 isolates were much more fit in their ability to be transmitted by *Ae. aegypti* (see Figure A3-4). The American genotype virus was dramatically less efficient than the American-Asian genotypes in escaping the midgut to infect

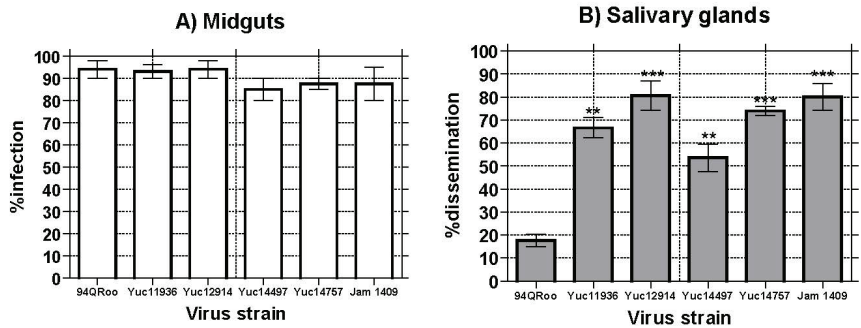

FIGURE A3-4 Dengue 2 American-Asian genotype viruses disseminate in *Aedes aegypti* much more efficiently than an American genotype virus.
NOTES: American-Asian genotype viruses—Yuc11936, Yuc12914, Yuc14497, Yuc14757; American genotype virus—94QRoo.
SOURCE: Salazar et al., 2010. Reproduced with permission from *Revista Biomédica*.

salivary glands. The American-Asian genotype DENVs could be detected in salivary glands of the Chetumal strain of *Ae. aegypti* as early as 4 days post oral challenge (Salazar-Sanchez et al., 2007), a dramatically shorter incubation than previously reported. The infection and replication efficiency of the American-Asian viruses was attributable in part to mutations in the 3′UTR of the virus (see Figure A3-5; Salazar et al., 2010). The 3′UTR contains motifs that are critical for translation and RNA synthesis, and secondary structure of the 3′UTR is a determinant of virus replication efficiency. Efficient and rapid productive infection of vectors could be major determinants of the genetic sweep of the newly introduced genotypes. Identification of genetic markers for vector competence in mosquitoes could be exploited as a marker for risk assessment and surveillance programs for improved and targeted vector control.

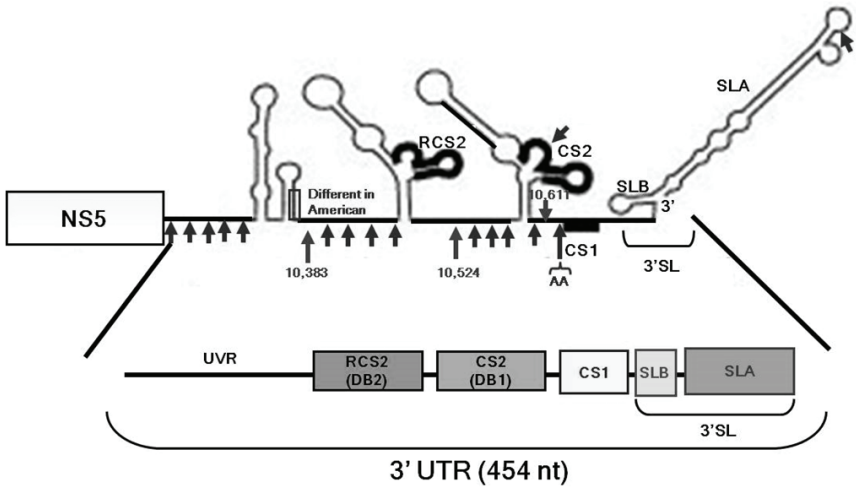

FIGURE A3-5 DENV-2 American and American-Asian genotype viruses differ in 3'UTR sequences.

NOTES: The secondary structure of the 3′UTR and the different functional domains are shown. Arrows point out the sites where the principal secondary structures were affected by mutations. The 3′UTR consists of: (1) a variable region (VR) adjacent to the stop codon of the viral polyprotein that encloses 2 hairpin structures (HP), (2) a core region containing two predicted secondary structures, the DB1 (containing CS2) and DB2 (containing RCS2), and (3) a 3′-terminal region enclosing the CS1 and the 3′SL (formed by SLA and SLB).

SOURCE: Salazar et al., 2010. Reproduced with permission from *Revista Biomédica*.

Extreme Endophily and Hyperabundance of Ae. aegypti in Human Structures

The domicile Domestication of *Ae. aegypti* and its consequent close association with humans in their domiciles is one of the major factors promoting efficient DENV transmission. A classic epidemiological investigation of dengue in Laredo, Texas, and Nuevo Laredo, Mexico, revealed the seminal importance of protecting the home to reduce DENV transmission (Reiter et al., 2003). In the U.S. city there were minimal DENV infections, but *Ae. aegypti* breeding sites were common. In contrast, in the Mexican City, there were many DENV infections, but mosquito breeding site control was more effective than in the U.S. city. The reduction in dengue in the United States was attributed to screens and air conditioning protecting the home from *Ae. aegypti* and thus dramatically reducing indoor DENV transmission.

Following publication of these results and in anticipation of initiating mosquito control efforts, we investigated the abundance of *Ae. aegypti* and presence of DENV in females collected from within and around homes of laboratory-confirmed dengue patients over a 12-month period in Mérida City (Garcia-Rejón et al., 2008). Backpack aspiration from 880 homes produced 1,836 females indoors (predominantly from bedrooms) and 102 females (< 5 percent of the females collected) from patios or backyards. The mean weekly indoor catch rate per home peaked at 8 females in late August. In some homes, up to 40 *Ae. aegypti* females were collected in one visit (see Table A3-2). Clearly these highly infested homes are threats to both the occupants and visitors for transmission of DENV and CHIKV. Other important outcomes of these investigations are provided in Table A3-3. DENV-infected *Ae. aegypti* females were recovered from 34 homes, and up to 7 DENV-infected females were collected in a home. DENV-infected females were collected from homes of dengue patients up to 27 days after the onset of symptoms. The epidemiological significance of the long-term persistence of DENV-infected mosquitoes in homes cannot be overstated. *Ae. aegypti* feeds multiple times during a gonadotrophic cycle, is an efficient interrupted feeder, and can survive for more than a month in the protective confines of the home. Obviously, all the members of the household, visitors, and nearby neighbors are

TABLE A3-2 *Ae. aegypti* and *Culex quinquefasciatus* Females in Dengue Patient Homes

Species	No. Collected	% of Total	Range for Individual Homes	No. (%) of Homes with Females
Aedes aegypti	1,836	41	0–40	332 (37.7)
Culex quinquefasciatus	2,641	59	0–59	312 (35.5)
Total	4,477	100		

SOURCE: Adapted from Garcia-Rejon et al., 2008.

TABLE A3-3 The Critical Epidemiological Need to Control *Aedes aegypti* in the Indoor Environment

- 38 percent of homes yielded *Ae. aegypti* females based on a very limited collection effort; up to 40 females per home were collected.
- Mosquito pools from 34 dengue patient homes were positive for DENV.
- 60 percent of *Ae. aegypti* females were collected in bedrooms, even though *Ae. aegypti* is a daytime feeder.
- Sick individuals are more likely to be in bedrooms where they can infect more mosquitoes that can then infect other home occupants and visitors.
- The DENV serotype from the mosquito pool matched the patient serotype in all five cases where the patient serotype was known (4 DENV-1; 1 DENV-2).
- Dengue virus-infected *Ae. aegypti* females were collected from homes of dengue patients up to 27 days after the onset of symptoms in index case.

SOURCE: Adapted from Garcia-Rejon et al., 2008.

at great risk for being infected by these mosquitoes. Clearly the domicile is one of the most epidemiologically significant points of contact between infected vectors and humans, making it a key target for interventions. For example, indoor insecticide application in homes of suspected dengue patients could be used to reduce intradomiciliary transmission of DENV and prevent their homes from becoming sources for dispersal of DENV by persons visiting and being bitten by infected mosquitoes.

The school Following the results of the domicile studies, we decided to determine the abundance of *Ae. aegypti* mosquitoes and presence of DENV in females collected from schools in Mérida City (Garcia-Rejón et al., 2011b). Backpack aspiration from 24 schools produced 468 *Ae. aegypti* females and 1,676 *Culex quinquefasciatus* females, another human biter (see Figure A3-6). *Ae. aegypti* females were collected most commonly from classrooms, followed by offices and bathrooms. DENV RNA was detected in 19 of 118 pools (16 percent) of *Ae. aegypti* females (total of 415 females). The overall rate of DENV infection per 100 *Ae. aegypti* females was 4.8. DENV-infected pools were detected from 11 of 24 schools (46 percent) and came from different room types, including classrooms, offices, and bathrooms (see Figure A3-6). Clearly, schools in Mérida City and elsewhere in tropical areas are a risky environment for students, teachers, and other personnel to be exposed to DENV-infected *Ae. aegypti* females. Children infected at school could in turn introduce the virus into their respective domiciles, infect *Ae. aegypti*, and initiate an intradomiciliary cycle. Schools are clearly an important target for dengue vector control.

Based upon the results of the home and school studies, we have proposed a new model of dengue epidemiology in urban areas in Mexico (see Figure A3-7). There is an intradomiciliary cycle in which DENV is transmitted in the home or peridomestic environment. There is also an extradomiciliary cycle in which

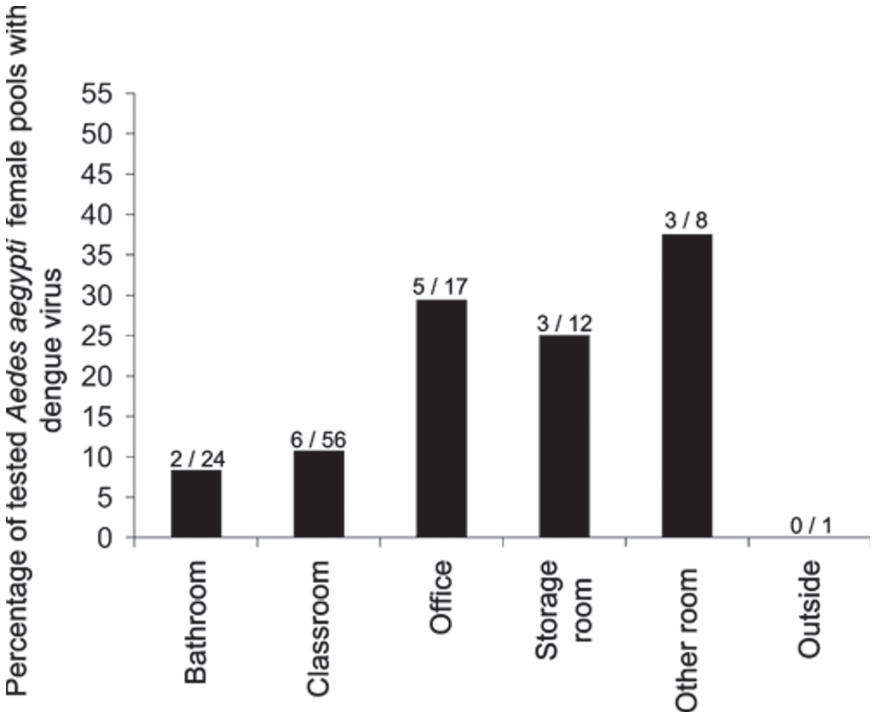

FIGURE A3-6 Percentages of tested pools of *Ae. aegypti* females with dengue virus RNA from different environments in Mérida schools during 2008 and 2009.
NOTE: Numbers above the bars indicate numbers of positive pools per total tested pools.
SOURCE: Garcia-Rejon et al., 2011b. Reproduced with permission from *American Journal of Tropical Medicine and Hygiene*.

individuals can be infected outside of the home, such as in schools and work places. DENV can be introduced into and amplified in either cycle by transmission to susceptible individuals and noninfected mosquitoes. Effective dengue control will require reducing *Ae. aegypti* abundance in both cycles.

Because the home is such a critical environment for transmission of DENV between humans and mosquito vectors and because community-wide distribution of insecticide-treated curtains (ITCs) showed promise in reducing DENV infections (Kroeger, et al., 2006), we conducted a Casa Segura study in Mérida City to determine the potential to reduce intradomiciliary DENV transmission through ITC use in individual homes (Loroño-Pino et al., 2013). Windows of homes were covered with ITCs, and humans and mosquitoes were monitored for DENV infections. DENV infections in mosquitoes and in humans were reduced in homes with ITCs in one of two study subareas. Overall, ITCs reduced intradomiciliary DENV transmission. DENV-infected *Ae. aegypti* females were

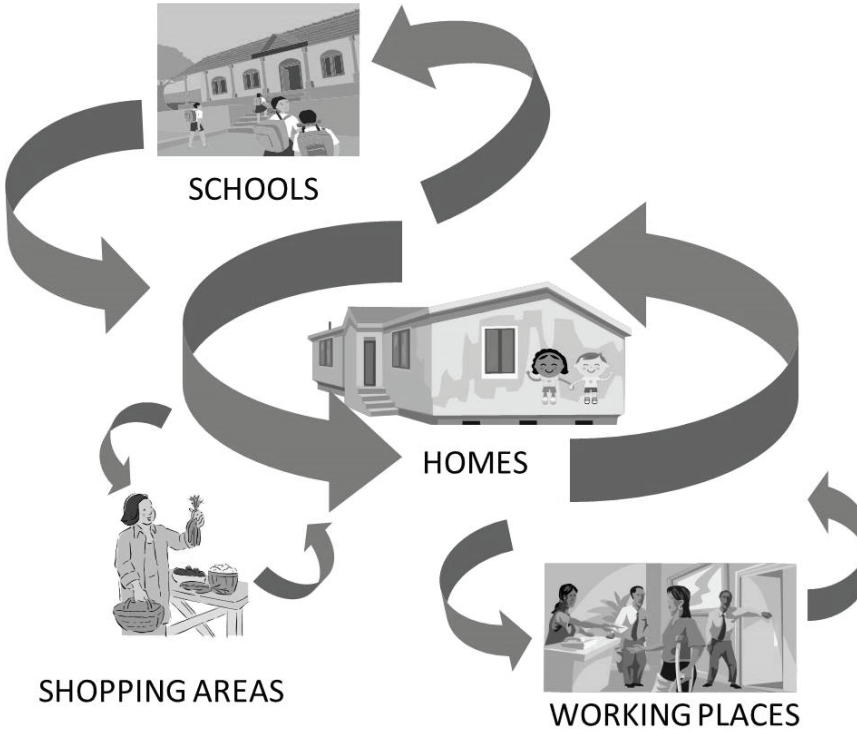

FIGURE A3-7 Model of dengue epidemiology in Mexico—intradomiciliary and extradomiciliary transmission cycles.

reduced within the ITC homes where the treated curtains were used most appropriately (see Table A3-4). Indeed, no infected *Ae. aegypti* were detected in homes where the curtains were used most appropriately (4th quartile use index). In these homes, curtains were present, covered the window, and not tied up or covered by a privacy curtain (nontreated), all of which would aid mosquitos in entering or exiting homes (Loroño-Pino et al., 2013). ITC homes were also significantly less likely to experience multiple DENV infections in humans than control homes (see Table A3-5), indicating interruption of the intradomiciliary transmission cycle. Some homes yielded up to nine infected *Ae. aegypti* females, emphasizing again the potential importance of highly infested homes in DENV transmission (Loroño-Pino et al., 2013). The studies were promising and revealing of best practices for protecting the homes from intradomiciliary transmission. However,

TABLE A3-4 Proper Usage of Insecticide-Treated Curtains Reduces the Number of DENV-Infected *Aedes aegypti* Females Detected in Homes

Curtain Use Index (CUI)	Mean DENV-Infected *Ae. aegypti* Females per Home	
CUI quartile	Nontreated Curtain Homes	Insecticide-Treated Curtain Homes
1st to 3rd quartile (limited, low and medium use)	0.13 (34)*	0.09 (16)
4th quartile (high use)	0.23 (16)	0.00 (0)

*Total number of infected females collected.
SOURCE: Adapted from Loroño-Pino et al., 2013.

TABLE A3-5 Homes with Insecticide-Treated Curtains Experience Fewer Multiple Human DENV Infections (Reduced Intradomiciliary Transmission) Than Homes with Nontreated Curtains

Dengue Infections in Humans by Individual Home	Percentage of Homes with Single or Multiple DENV Infections in Humans	
	Nontreated Curtain Homes	Insecticide-Treated Curtain Homes
Single infection	51 (40/78)	49 (38/78)
Multiple infection	71 (24/34)	29 (10/34)

SOURCE: Adapted from Loroño-Pino et al., 2013.

the potential for ITCs for DENV vector control remain to be determined, especially in the face of emerging insecticide resistance, which could reduce ITC efficacy (see Table A3-4).

Breeding site identification and source reduction The emergence of our throwaway society and rapid urbanization have greatly complicated vector control, especially in urban areas. Larval development sites, such as tires, cans, bottles, and other water-holding containers are now ubiquitous breeding sites for *Ae. aegypti*. In addition, the urban environment provides multiple other breeding sites that *Ae. aegypti* may exploit and that sometimes are difficult to locate and to control. Detection of *Ae. aegypti* breeding in sewer systems (Barrera et al., 2008) and demonstration that the mosquitoes obtained from these cryptic breeding sites were genetically the same as those collected from conventional breeding sites, is clear evidence of the plasticity of *Ae. aegypti* behavior in the super nidus (Somers et al., 2011).

In our Casa Segura studies, breeding sites around and near premises in Mérida City were identified, using the classification scheme of Servicios de Salud de Yucatán, and characterized for productivity (Garcia-Rejón et al., 2011a). The

most productive breeding sites for *Ae. aegypti* immatures included small and larger discarded water-holding containers, tires, and so on. The importance of different container types varied between dry and wet periods. Such information is important for targeting productive containers in source reduction campaigns. Entomological investigations also revealed the presence of two categories of extremely productive but uncontrolled breeding sites in the city: storm water drains and vacant lots.

Storm water drains Storm water drains near some of the homes contained large numbers of mosquito immatures. This prompted a survey of storm water drains and catch basins in Mérida City for production of *Ae. aegypti* and *Cx. quinquefasciatus* (Arana-Guardia et al., 2014). We examined 1,761 storm water drains located in 45 different neighborhoods spread across the city over dry and wet seasons; 262 (14.9 percent) held water and 123 yielded mosquito immatures. In total, we collected 64,560 immatures representing nine species, including 39,269 *Cx. quinquefasciatus* and 23,313 *Ae. aegypti*. Clearly storm water drains produce massive numbers of potential vector mosquitoes across Mérida City, both in the wet and dry seasons, and are nonresidential development sites that should be included in mosquito surveillance and control programs.

Vacant lots We also assessed the potential for vacant lots and other nonresidential settings to serve as source environments for *Ae. aegypti* (Baak-Baak et al., 2014). Mosquito immatures were collected from residential premises (n = 156 site visits) and nonresidential settings represented by vacant lots, parking lots, and streets or sidewalks. Collections totaled 46,025 mosquito immatures of 13 species. *Ae. aegypti* was the most commonly encountered species accounting for 81.0 percent of total immatures, followed by *Cx. quinquefasciatus* (12 percent). Site visits to vacant lots (74 percent) were more likely to result in collection of *Ae. aegypti* immatures than residential premises (36 percent). Tires accounted for 75.5 percent of *Ae. aegypti* immatures collected from vacant lots. Vacant lots should be included in mosquito surveillance and control efforts; they often are located near homes and frequently harbor numerous small and large discarded water-holding containers that serve as development sites for immature mosquitoes.

Comment Vacant lots, storm water drains and sewer systems, and other cryptic breeding sites clearly must be included in the efforts to control *Ae. aegypti* in source reduction and larviciding control programs. The plethora and rapid accumulation of breeding sites in the throw-away society certainly complicate vector control. Even if homeowners clear their own patios and surroundings of breeding sites (for example as in the Patio Limpio program in Mexico and/or in the *Recicla por tu Bienestar* program in the state of Yucatán; Mendoza-Mezquita et al., 2014), their homes can be inundated by mosquitoes from sites on uncontrolled storm drains, vacant lots, or neighboring patios and yards.

Human mobility complicates *Ae. aegypti* and dengue control studies Human mobility in the urban environment can confound dengue control studies targeting the home or other indoor environments. Human movement is extensive, thus complicating identification of where individuals become infected with DENV (Stoddard et al., 2013; Vazquez-Prokopec et al., 2013). In the Casa Segura study (Loroño-Pino et al., 2013) and other studies involving protection of the domicile, it is difficult to ascertain whether or not the individual was infected in the home or outside of the home during movement around the city. Thus, if epidemiological outcomes are being measured (e.g., human infections or seroconversions), it is difficult to determine if that infection occurred in the home or outside the home. Consequently, we feel that monitoring homes for DENV-infected *Ae. aegypti*, which are more restricted to the specific home environment than humans, may be a better measure of the protective effect of the intervention than human infection, because the latter may well have occurred elsewhere.

The Threat of Emerging Insecticide Resistance for *Ae. Aegypti* Control

Insecticides are critical now and for the foreseeable future to control *Ae. aegypti* and the pathogens it transmits. Pyrethroids and temephos for adult and larval control, respectively, are the cornerstones of *Ae. aegypti* control in Mexico and much of the pantropical world. Numerous studies have now documented resistance in *Ae. aegypti* to these commonly used pesticides, most notably to pyrethroids (e.g., Flores et al., 2013). The increases in insecticide resistance are of great concern. Although the operational significance of these forms of resistance need to be determined, there is the frightening possibility that these tools may be removed from the armamentarium used by mosquito control officials to control dengue. Here the main focus will be upon one form of resistance, "knock-down resistance" (kdr), which has exploded in *Ae. aegypti* in Mexico and the Yucatán, a pattern that is being documented throughout the pantropical super nidus.

Knock Down Resistance

In mosquitos, kdr is caused by mutations in the voltage-gated sodium channel transmembrane protein (*para*) that reduce pyrethroid binding (Kasai et al., 2014). Insect sodium channels contain four homologous repeats (domains I–IV) each with six transmembrane segments (S1–S6); interestingly, the majority of pyrethroid-resistance associated mutations occur in the IIS5, IIS6, and IIIS6 segments (see Figure A3-8). In *Ae. aegypti*, many point mutations associated with pyrethroid resistance had been identified in different geographical mosquito populations (see Figure A3-8). We screened the IIS6 segment in *para* in 1,318 mosquitoes in 30 strains from throughout Latin America (Saavedra-Rodriguez et al., 2007) and identified two alternate nonsynonymous mutations in codon Ile1,011 in exon 20, and one nonsynonymous mutation at codon Val1,016 in

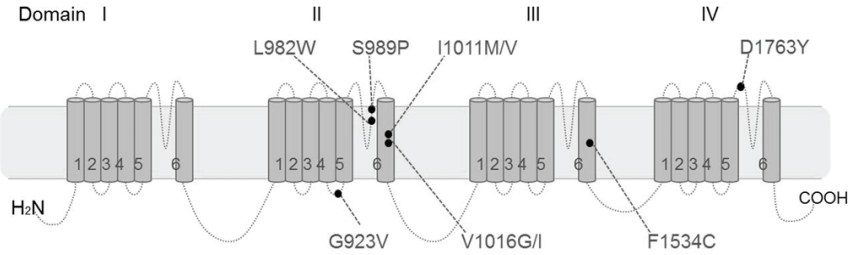

FIGURE A3-8 Voltage-gated sodium channel kdr alleles in *Aedes aegypti*.
NOTES: Pyrethroid resistance-associated mutations identified in the voltage-gated sodium channel of *Aedes aegypti*. Point mutations are designated based on the house fly sodium channel gene (GenBank accession number X96668). Replacements G923V, L982W, I1011M and V1016G were first identified by Brengues et al., 2003; S989P was first described by Srisawat et al., 2010; Replacements I1011V and V10161 are described in Saavedra-Rodriguez et al., 2007; F1534 was identified by Yanola et al., 2011; and D1763Y mutation was identified in Chang et al., 2009.

exon 21 of *Ae. aegypti*. From these point mutations, a transition in the first position of codon 1,016 encoding an Ile replacement (Ile1,016) rapidly increased in frequency in two separate selection experiments, one with deltamethrin on a field strain from Santiago de Cuba and another with permethrin on a strain from Isla Mujeres in Mexico.

The frequency of the kdr-conferring allele, Ile1,016, was then determined in Mexico (Garcia et al., 2009; see Figure A3-9). A total of 81 field collections containing 3,951 *Ae. aegypti* were made throughout Mexico from 1996 to 2009. These mosquitoes were analyzed for the frequency of the 1,016 mutation using a melting-curve PCR assay. Dramatic increases in frequencies of Ile1,016 were recorded from the late 1990s to 2006–2009 in several states including Nuevo León in the north, Veracruz on the central Atlantic coast, and Yucatán, Quintana Roo, and Chiapas in the south. From 1996 to 2000, the overall frequency of Ile1,016 was 0.04 percent. The earliest detection of Ile1,016 was in Nuevo Laredo on the U.S. border in 1997. By 2003–2004 the overall frequency of Ile1,016 had increased approximately 100-fold to 3 percent. When checked again in 2006, the frequency had increased slightly to 4 percent. This was followed in 2007–2009 by a sudden jump in Ile1,016 frequency to 33 percent. There was spatial heterogeneity in Ile1,016 frequencies among 2007–2008 collections, which ranged from 46 percent in the state of Veracruz to 51 percent in the Yucatán Peninsula and 15 percent in and around Tapachula in the state of Chiapas. Spatial heterogeneity was also evident at smaller geographic scales. For example, within the city of Chetumal, Quintana Roo, Ile1,016 frequencies varied from 38 to 88 percent. This dramatic and rapid increase in kdr frequencies has also been documented in *Ae. aegypti* populations from throughout the world. This may be related to heavy

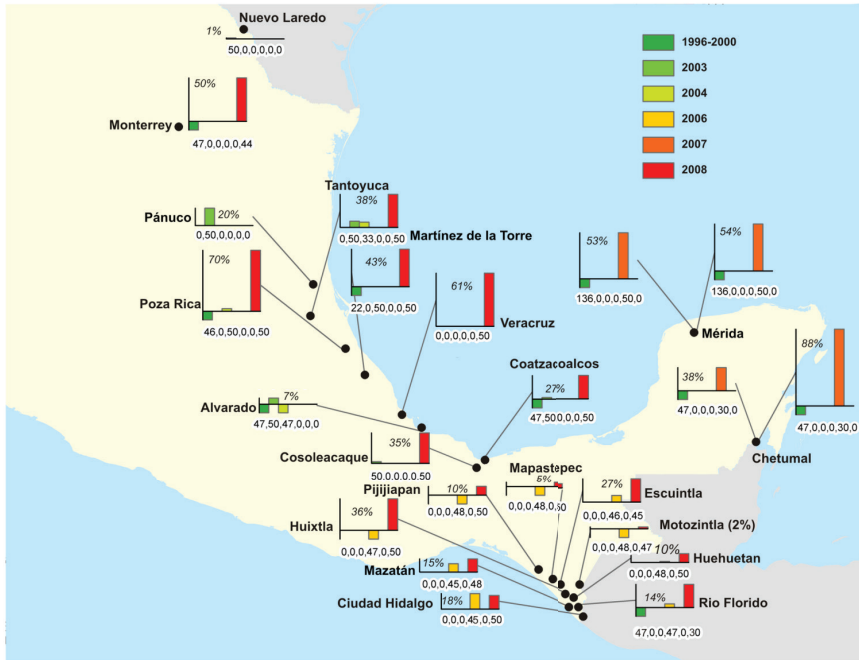

FIGURE A3-9 Recent rapid rise of a permethrin kdr allele in *Aedes aegypti* in Mexico. SOURCE: Garcia et al., 2009. Available from *PLoS Neglected Tropical Disease* under Creative Commons license.

use of permethrin-based insecticides in mosquito control programs. A simple model of positive directional selection predicted rapid fixation of Ile1,016 unless there is negative fitness associated with Ile1,016 in the absence of permethrin. If so, then spatial refugia of susceptible *Ae. aegypti* or rotational schedules of different classes of adulticides could be established to slow or prevent fixation of Ile1,016 (Garcia et al., 2009).

There was also a dramatic increase in kdr-conferring allele frequencies in Mérida City during the last decade. Previous analyses (Saavedra-Rodriquez et al., 2007) revealed that the kdr allele was absent in *Ae. aegypti* in Mérida City in 1999. By 2010 the kdr allele was approaching fixation in mosquitoes in the urban center of Mérida City (see Table A3-6). Our model had predicted this rapid fixation in the absence of refugia (Garcia et al., 2009). The operational significance of fixation of the kdr allele remains to be determined. In *An. gambiae*, kdr alleles have become fixed and have had limited impact upon vector control; however, there continues to be evolution of metabolic resistance mechanisms in the mosquito, which may increase resistance by 1,000 fold (Hemingway, 2014). The same may be true for metabolic resistance in *Ae. aegypti* (Donnelly et al., 2009).

TABLE A3-6 Temporal Increase in kdr in *Aedes aegypti* in Mérida City

Year	Generation	Ile/Ile AA	Ile/Val AG	Val/Val GG	Ile1,016 n	Ile1,016 Allele Frequency
1999	F1	0	0	0	272	0.000
2007	F2	26	55	19	100	0.535
2010	F1	147	77	7	231	0.803
2011	F1	309	135	10	454	0.829
2013	F1	91	22	1	114	0.895

Metabolic Resistance

We have also characterized metabolic insecticide resistance mechanisms in *Ae. aegypti* from the Yucatán using biochemical assays (Flores et al., 2006). The activities of *alpha* and *beta* esterases, mixed-function oxidases (MFO), glutathione-S-transferase (GST), acetylcholinesterase (AChE), and insensitive acetylcholinesterase (iAChE) were assayed in microplate assays. Elevation of *alpha* and *beta* esterases in some of the populations suggested potential insecticide-resistance mechanisms against organophosphate, carbamate, and pyrethroid insecticides.

Metabolic resistance gene expression before, during, and after five generations of permethrin laboratory selection were monitored in *Ae. aegypti* from the Yucatán Peninsula (Saavedra-Rodriguez et al., 2012). Changes in expression of 290 metabolic detoxification genes were measured using the Aedes Detox microarray. Selection simultaneously increased the LC(50), KC(50), and Ile1,016 frequency. Very few of the same genes were differentially transcribed among field strains, but 10 cytochrome P(450) genes were upregulated in more than one strain. Identification of one or a few metabolic genes that are predictably associated with permethrin adaptation may very be difficult, but such information would be invaluable for incorporation into mosquito surveillance and control programs.

Potential Factors Conditioning the Increase in Knockdown Resistance

Public health insecticide usage The selective pressures that produced the observed dramatic increase in Ile1,016 frequencies in Mexico remain a subject of discussion. Clearly major insecticidal control efforts have been expended to control *Ae. aegypti* in Mexico, especially with the emergence of DHF/DSS. Pyrethroids are applied in and around dengue case households and intensive space spraying of areas with dengue cases may promote evolution of resistance. We demonstrated local adaptation to pyrethroids by comparing patterns of variation among 27 *Ae. aegypti* collections at 13 single nucleotide polymorphisms

(SNPs): Ile1,016 and Cys1,534 in the voltage-gated sodium channel gene (see Figure A3-8), three in detoxification genes previously associated with resistance, and eight in putatively neutral loci. The SNPs in *para* varied greatly in frequency among collections, whereas SNPs at the remaining 11 loci showed little variation supporting previous evidence for extensive local gene flow. Thus, local adaptation to pyrethroids appears to offset the homogenizing effects of gene flow (Saavedra-Rodriguez et al., 2014). Such control efforts are much less extensive in rural areas and villages, and the importance of vector control in selection for resistance is likely reflected in the fact that kdr frequencies are significantly less in rural areas surrounding Mérida City, where public health vector control is much less intensive than in Mérida City. This is also evidence for negative fitness associated with the Ile1,016 and Cys1,534 alleles in the absence of selection in rural areas.

Consumer product insecticide usage Extensive usage of insecticide consumer products for indoor mosquito control has emerged as a potential additional source of kdr in *Ae. aegypti*. We conducted a study to evaluate the household use of insecticide consumer products to kill mosquitoes and the expenditures for using these products in Mérida City (Loroño-Pino et al., 2014). A questionnaire was administered to 441 households; 382 (86.6 percent of) surveyed households took action to kill insect pests with consumer products. The most commonly used product types were insecticide aerosol spray cans (74 percent), electric plug-in insecticide emitters (37 percent), and mosquito coils (28 percent) (see Table A3-7). Mosquitoes were targeted by 90 percent of households using insecticide aerosol spray cans, and more than 99 percent of households used electric plug-in insecticide emitters or mosquito coils. Products were used daily or every 2 days in most of the households. For all products used to kill insect pests, the median annual estimated expenditure per household that took action was approximately US$31. These numbers are suggestive of an annual market in excess of US$5.7 million

TABLE A3-7 Consumer Usage of Mosquito Control Products in Homes

Methods Used to Control Insect Pests*	All Households Number and (percent)	Urban Area Number and (percent)	Rural Area Number and (percent)
Interviewed households	382	300 (79)	82 (21)
Insecticide aerosol spray can	281 (74)	238 (79)	43 (52)
Electric plug-in insecticide emitter	143 (37)	99 (33)	44 (54)
Mosquito coil	108 (28)	82 (27)	26 (32)
Smoke	19 (5)	15 (5)	4 (5)
Electric insect racquet	11 (3)	10 (3)	1 (1)

SOURCE: Adapted from Loroño-Pino et al., 2014.

for Mérida City alone. Homeowners spent substantial amounts of money on insecticide consumer products. Clearly, there is a large market and incentive for companies to provide effective consumer products for vector control.

The constant exposure to pyrethroid-based insecticides resulting from public health control efforts and the use of consumer products could promote insecticide resistance in the mosquito populations.

The Global Threat of Pyrethroid Resistance for Control of VBDs

Pyrethroid resistance also may affect control of other globally important VBDs by insecticides. Pyrethroids are by far the most commonly used mosquito adulticides, and evolution of resistance to these compounds is a major threat to public health. Pyrethroid resistance, both kdr and metabolic resistance, has now been documented in vectors of most globally important pathogens (Hemingway et al., 2006). This resistance threatens some significant advances that have been made in control of VBDs in the last decade. The situation is potentially most grave for control of the *Anopheles spp*. vectors of malaria in Africa. The use of long-lasting insecticide-treated bed nets (LLIN) and indoor residual spraying (IRS) has reduced malaria deaths by a third (Hemingway, 2014). All LLINs and most IRS have pyrethroid active ingredients. Pyrethroids are relatively safe for use around humans, are easy to formulate, and cheap to produce. Other classes of insecticides do not share these attributes, making pyrethroids the current insecticides of choice (Hemingway, 2014). Some insecticides also have been lost to the armamentarium for vector control. The discovery of DDT and its use to control VBDs was a landmark achievement in public health. However indiscriminant usage of DDT to control insect pests led to detrimental effects on nontarget organisms, and DDT was banned even for public health use in IRS programs. The widespread termination of DDT usage coincided with a resurgence in malaria, leishmaniasis, dengue, and other diseases that are transmitted principally indoors in the Americas and elsewhere (Attaran et al., 2000). Indoor use of DDT disrupted the close association between the human host and important anthropophilic and endophilic vectors, such as *Ae. aegypti* and *An. gambiae*, thereby reducing transmission and disease. Pyrethroids have largely taken the place of DDT in vector control, especially in IRS and LLINs. But now this important tool for vector control is in jeopardy.

Although kdr has now been demonstrated in most of the major vectors of important human pathogens, its operational significance remains to be determined. Pyrethroid resistance in *An. gambiae* in Africa has occurred in waves. Pyrethroid resistance was relatively rare in these species until the start-up of massive vector control programs using LLINs and IRS. Initial kdr resistance was not associated with obvious operational impact, but subsequent waves of resistance have involved relatively new metabolic mechanisms, such as P450-based metabolic enzymes. Metabolic resistance can result in mosquitoes that are 1,000 fold more

resistant to pyrethroids than kdr mosquitoes (Hemingway, 2014). If a similar scenario occurs with *Ae. aegypti*, pyrethroids may also be lost to the armamentarium for dengue and chikungunya control. The same can be said for control programs for vectors of Chagas, leishmaniasis, filariasis, and other VBDs. The emerging resistance to pyrethroids is a potential public catastrophe on the order of emerging resistance to antibiotics in bacteria. These subjects were addressed in an IOM workshop that for the first time gathered experts in resistance in vectors, bacteria, parasites, and viruses to discuss common mechanisms, threats, and opportunities for mitigating resistance in their respective systems (IOM, 2003b).

Clearly, there is a public health imperative to develop new, environmentally sensitive insecticides and formulations with the efficacy of DDT or pyrethroids to augment existing and future control programs. The development of new insecticides is not trivial; no new chemical insecticides have been brought to market for decades (Hemingway et al., 2006). To help address this public health threat, the Innovative Vector Control Consortium (IVCC) was formed in 2005 to facilitate the development and applications of new insecticides for vector control. The IVCC partners with industry to develop new insecticides of different classes with different modes of action to replace or complement pyrethroids. The new insecticides will provide the potential for rotational or mosaic applications to minimize development of insecticide resistance and thereby provide improved stewardship of existing and new pesticides and sustainable vector control.

Needs and Opportunities

The pantropical urban super nidus for *Ae. aegypti*-transmitted diseases is a major threat to public health and is proving to be intractable to classical vector control measures. Unprecedented population growth, unplanned urbanization, and the throw-away society are major factors contributing to the creation of the super nidus and a dramatic global increase in *Ae. aegypti*-transmitted diseases. The situation is exacerbated by globalization and the extraordinary movement of humans, vectors, and viruses throughout the pantropical world. Control measures that proved successful in the past are unlikely to be as successful in the super nidus. The situation is dire. Conventional vector control measures, even when applied most efficiently, as in Singapore (Ooi et al., 2006), are not sufficient to control dengue hyperendemicity. New approaches are sorely needed to augment insecticidal control of *Ae. aegypti* (Morrison et al., 2008).

Despite the gravity of the situation, new and innovative tools evolving from the digital, genomic, and molecular revolutions are available to augment and enhance control of *Ae. aegypti* and dengue; some are being developed and tested for efficacy in *Ae. aegypti* control currently. A major breakthrough occurred with the transfer of *Wolbachia*, an insect endosymbiont, into *Ae. aegypti*. *Wolbachia* infection causes cytoplasmic incompatibility in *Ae. aegypti*, and thus the bacteria traffics efficiently in laboratory and wild populations, rapidly infecting most of

the target mosquitoes (Hoffman et al., 2011). Most remarkably the *Wolbachia*-infected mosquitoes are resistant to DENV (and a variety of other pathogens transmitted by mosquitoes), thereby reducing DENV transmission to humans (Moreira et al., 2009). *Wolbachia* control trials are ongoing in many parts of the world. RIDL (release of insects carrying a dominant lethal) technology is another innovative approach being tested for *Ae. aegypti* control in field trials. This is more akin to but differs from sterile insect technology (Wise de Valdez et al., 2011; Black et al., 2011). Transgenic males propagated in the presence of tetracycline are innundatively released to mate with wild females (Carvalho et al., 2014). Progeny of these mosquitoes die, thus suppressing the *Ae. aegypti* population. Field trials are ongoing or planned in other parts of the world. A variety of other genetic approaches are being explored for vector control (Franz et al., 2014). Exciting new chemical interventions for *Ae. aegypti* control are also being tested. For example, pyriproxyfen (a synthetic juvenile hormone) is being tested for mosquito control. The active ingredient can be used to coat nets and other surfaces. When female mosquitoes contact these nets, they transfer the chemical back to oviposition sites, thereby stopping larval development. Treating LLINs with pyrethroids and pyriproxyfen potentially provides a negative cross-selection resistance-blocking mechanism for vector control (Devine et al., 2009). These strategies are environmentally sensitive and particularly suited to dengue vector control in the super nidus, as in using mosquitoes to find cryptic breeding sites and deliver the control agent. New and effective traps also are being designed that offer exciting new potential to control vector populations (Barrera et al., 2014; Hiscox et al., 2014).

Vector control programs would benefit by not only having new insecticides and innovative control interventions in their armamentarium but also from application of modern management tools. Failure of some vector programs can be attributable to inconsistent implementation of vector control strategies (Hemingway et al., 2006). The digital revolution has provided unprecedented computational power for modeling of control interventions and for developing surveillance and decision support systems, which could enhance the efficacy of control programs (IOM, 2008). Decision support systems can provide improved vector control through rapid and efficient monitoring of entomological and epidemiological parameters related to pathogen transmission; provide more effective vector control through prompt, timely, and focused application of the appropriate insecticides, which can mitigate insecticide resistance; and provide efficient and effective use of resources (Eisen et al., 2011). Many tools with potential to improve vector control are now freely available on the web (e.g., Lozano-Fuentes et al., 2008). It is likely that all of these new technologies and innovative approaches will be needed to control *Ae. aegypti* in the super nidus. Finally, although not the subject of this contribution, significant advances are being made in the development of a new generation of vaccines for DENV and CHIKV (e.g., da Costa et al., 2014; Powers, 2014).

Conclusion

The burden of *Ae. aegypti*-transmitted diseases is too great to bear, and new approaches and technologies are critical. It is clearly time to declare "war" on *Ae. aegypti* and to control this enemy of humankind!

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References

Arana-Guardia, R., C. M. Baak-Baak, M. A. Loroño-Pino, C. Machain-Williams, B. J. Beaty, L. Eisen, and J. E. García-Rejón. 2014. Stormwater drains and catch basins as sources for production of *Aedes aegypti* and *Culex quinquefasciatus*. *Acta Tropica* 134:33-42.

Attaran, A., D. R Roberts, C. F. Curtis, and W. L. Kilama. 2000. Balancing risks on the backs of the poor. *Nature Medicine* 6(7):729-731.

Baak-Baak, C. M., R. Arana-Guardia, N. Cigarroa-Toledo, M. A. Loroño-Pino, G. Reyes-Solis, C. Machain-Williams, B. J. Beaty, L. Eisen, and J. E. García-Rejón. 2014. Vacant lots: Productive sites for *Aedes* (Stegomyia) *aegypti* (Diptera: Culicidae) in Mérida City, Mexico. *Journal of Medical Entomology* 51(2):475-483.

Barrera, R., M. Amador, V. Acevedo, R.R. Hemme, and G. Félix. 2014. Sustained, area-wide control of *Aedes aegypti* using CDC autocidal gravid ovitraps. *American Journal of Tropical Medicine and Hygiene* 15. pii: 14-0426.

Barrera, R., M. Amador, A. Diaz, J. Smith, J. L. Munoz-Jordan, and Y. Rosario. 2008. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. *Medical Veterinary Entomology* 22(1):62-69.

Barrett, A. D., and S. Higgs. 2007. Yellow fever: A disease that has yet to be conquered. *Annual Review of Entomology* 52:209-229.

Beck, A., H. Guzman, L. Li, B. Ellis, R. B. Tesh, and A. D. Barrett. 2013. Phylogeographic reconstruction of African yellow fever virus isolates indicates recent simultaneous dispersal into east and west Africa. *PLoS Neglected Tropical Diseases* 7(3):e1910.

Beaty, B. J., and T. H. G. Aitken. 1979. *In vitro* transmission of yellow fever virus by geographic strains of *Aedes aegypti*. *Mosquito News* 39:232238.

Bennett, K. E., K. E. Olson, L. Muñoz, I. Fernandez-Salas, J. A. Farfán-Alé, S. Higgs, W. C. Black 4th, and B. J. Beaty. 2002. Variation in vector competence for dengue 2 virus among 24 collections of *Aedes aegypti* from Mexico and the United States. *American Journal of Tropical Medicine and Hygiene* 67(1):85-92.

Bhatt, S., P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, O. Sankoh, M. F. Myers, D. B. George, T. Jaenisch, G. R. Wint, C. P. Simmons, T. W. Scott, J. J. Farrar, and S. I. Hay. 2013. The global distribution and burden of dengue. *Nature* 496(7446):504-507.

Black, W. C., 4th, K. E. Bennett, N. Gorrochótegui-Escalante, C. V. Barillas-Mury, I. Fernández-Salas, M. de Lourdes Muñoz, J. A. Farfán-Alé, K. E. Olson, and B. J. Beaty. 2002. Flavivirus susceptibility in *Aedes aegypti*. *Archives of Medical Research* 33(4):379-388.

Black, W. C., 4th, L. Alphey, and A. A. James. 2011. Why RIDL is not SIT. *Trends in Parasitology* 27(8):362-370.

Bonds, J. A. S. 2012. Ultra-low-volume space sprays in mosquito control: A critical review. *Medical Veterinary Entomology* 26:121-130.

Brengues, C., N. J. Hawkes, F. Chandre, L. McCarroll, S. Duchon, P. Guillet, S. Manguin, J. C. Morgan, and J. Hemingway. 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Medical Veterinary Entomology* 17(1):87-94.

Brown, J. E., C. S. McBride, P. Johnson, S. Ritchie, C. Paupy, H. Bossin, J. Lutomiah, I. Fernandez-Salas, A. Ponlawat, A. J. Cornel, W. C. Black 4th, N. Gorrochotegui-Escalante, L. Urdaneta-Marquez, M. Sylla, M. Slotman, K.O. Murray, C. Walker, and J. R. Powell. 2011. Worldwide patterns of genetic differentiation imply multiple domestications' of *Aedes aegypti*, a major vector of human diseases. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 278(1717):2446-2454.

Carey, D.E. 1971. Chikungunya and dengue: A case of mistaken identity? *Journal of the History of Medicine and Allied Sciences* 26:243-262.

Carvalho, D. O., D. Nimmo, N. Naish, A. R. McKemey, P. Gray, A. B. Wilke, M. T. Marrelli, J. F. Virginio, L. Alphey, and M. L. Capurro. 2014. Mass production of genetically modified *Aedes aegypti* for field releases in Brazil. *Journal of Visualized Experiments* 4(83):e3579.

Chang, C., W. K. Shen, T. T. Wang, Y. H. Lin, E. L. Hsu, and S. M. Dai. 2009. A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* 39(4):272-278.

da Costa, V. G., A. C. Marques-Silva, V. G. Floriano, and M. L. Moreli. 2014. Safety, immunogenicity and efficacy of a recombinant tetravalent dengue vaccine: A meta-analysis of randomized trials. *Vaccine* 32(39):4885-4892.

Díaz, F. J., W. C. Black 4th, J. A. Farfán-Alé, M. A. Loroño-Pino, K. E. Olson, and B. J. Beaty. 2006. Dengue virus circulation and evolution in Mexico: A phylogenetic perspective. *Archives of Medical Research* 37(6):760-773.

Devine, G. J., E. Z. Perea, G. F. Killeen, J. D. Stancil, S. J. Clark, and A. C. Morrison. 2009. Using adult mosquitoes to transfer insecticides to *Aedes aegypti* larval habitats. *Proceedings of the National Academy of Sciences* 106(28):11530-11534.

Dickson, L. B., I. Sanchez-Vargas, M. Sylla, K. Fleming, and W. C. Black 4th. 2014. Vector competence in West African *Aedes aegypti* is flavivirus species and genotype dependent. *PLoS Neglected Tropical Diseases* 8(10):e3153.

Donnelly, M. J., V. Corbel, D. Weetman, C. S. Wilding, M. S. Williamson, and W. C. Black 4th. 2009. Does kdr genotype predict insecticide-resistance phenotype in mosquitoes? *Trends in Parasitology* 25(5):213-219.

Edman, J. D., D. Strickman, P. Kittayapong, and T. W. Scott. 1992. Female *Aedes aegypti* (Diptera: Culicidae) in Thailand rarely feed on sugar. *Journal of Medical Parasitology* 29(6):1035-1038.

Eisen, L., B. J. Beaty, A. C. Morrison, and T. W. Scott. 2009. Proactive vector control strategies and improved monitoring and evaluation practices for dengue preventional strategies and improved monitoring and evaluation practices for dengue prevention. *Journal of Medical Entomology* 46(6):1245-1255.

Eisen, L., M. Coleman, S. Lozano-Fuentes, N. McEachen, M. Orlans, and M. Coleman. 2011. Multi-disease data management system platform for vector-borne diseases. *PLoS Neglected Tropical Diseases* 5:e1016.

Franz, A. W., R. J. Clem, and A. L. Passarelli. 2014. Novel genetic and molecular tools for the investigation and control of dengue virus transmission by mosquitoes. *Current Tropical Medicine Reports* 1(1):21-31.

Flores, A. E., J. S. Grajales, I. F. Salas, G. P. Garcia, M. H. Becerra, S. Lozano, W. G. Brogdon, W. C. Black 4th, and Beaty, B. 2006. Mechanisms of insecticide resistance in field populations of *Aedes aegypti* (L.) from Quintana Roo, Southern Mexico. *Journal of the American Mosquito Control Association* 22(4):672-677.

Flores, A. E., G. Ponce, B. Silva, S. Gutierrez, C. Bobadilla, B. Lopez, R. Mercado, and I. W. C. Black. 2013. Wide spread cross resistance to pyrethroids in *Aedes aegypti* (Diptera: Culicidae) from Veracruz state Mexico. *Journal of Economic Entomology* 106(2013):959-969.

Garcia, G. P., A. E. Flores, I. Fernández-Salas, K. Saavedra-Rodríguez, G. Reyes-Solis, S. Lozano-Fuentes, J. Guillermo Bond, M. Casas-Martínez, J. M. Ramsey, J. García-Rejón, M. Domínguez-Galera, H. Ranson, J. Hemingway, L. Eisen, and W. C. Black IV. 2009. Recent rapid rise of a permethrin knock down resistance allele in *Aedes aegypti* in Mexico. *PLoS Neglected Tropical Disease* 3(10):e531.

Garcia-Rejón, J., M. A. Loroño-Pino, J. A. Farfán-Alé, L. Flores-Flores, E. Del Pilar Rosado-Paredes, N. Rivero-Cardenas, R. Najera-Vazquez, S. Gomez-Carro, V. Lira-Zumbardo, P. Gonzalez-Martinez, S. Lozano-Fuentes, D. Elizondo-Quiroga, B. J. Beaty, and L. Eisen. 2008. Dengue virus-infected *Aedes aegypti* in the home environment. *American Journal of Tropical Medicine and Hygiene* 79(6):940-950.

García-Rejón, J. E., M. P. López-Uribe, M. A. Loroño-Pino, J. A. Farfán-Alé, M. R. Del Najera-Vazquez, S. Lozano-Fuentes, B. J. Beaty, and L. Eisen. 2011a. Productive container types for *Aedes aegypti* immatures in Mérida, Mexico. *Journal of Medical Entomology* 48(3):644-650.

García-Rejón, J. E., M. A. Loroño-Pino, J. A. Farfán-Alé, L. F. Flores-Flores, M. P. López-Uribe, R. Najera-Vazquez, G. Nuñez-Ayala, B. J. Beaty, and L. Eisen. 2011b. Mosquito infestation and dengue virus infection in *Aedes aegypti* females in schools in Merida, Mexico. *American Journal of Tropical Medicine and Hygiene* 84(3):489-496.

Gorrochotegui-Escalante, N., C. Gomez-Machorro, S. Lozano-Fuentes, L. Fernandez-Salas, M. De Lourdes Munoz, J. A. Farfán-Alé, J. Garcia-Rejón, B. J. Beaty, and W. C. Black 4th. 2002. Breeding structure of *Aedes aegypti* populations in Mexico varies by region. *American Journal of Tropical Medicine and Hygiene* 66(2):213-22.

Gratz, N. G. 1999. Emerging and resurging vector-borne diseases. *Annual Review of Entomology* 44:51-75.

Gubler, D. 2005. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: A case of failed public health policy. *Revista Panamericana de Salud Pública* 17(4):221-224.

Gubler, D. J. 2011. Dengue, urbanization, and globalization: The unholy trinity of the 21(st) century. *Tropical Medicine and Health* 39:3-11.

Guzman, M. G., and E. Harris. 2015. Dengue. *Lancet* 85(9966):453-465.

Halstead, S.B. 2015. Reappearance of chikungunya, formerly called dengue, in the Americas. *Emerging Infectious Diseases* 21:557-561.

Hanley, K. A., T. P. Monath, S. C. Weaver, S. L. Rossi, R. L. Richman, and N. Vasilakis. 2013. Fever versus fever: The role of host and vector susceptibility and interspecific competition in shaping the current and future distributions of the sylvatic cycles of dengue virus and yellow fever virus. *Infectious, Genetics, and Evolution* 19:292-311.

Harrington, L. C., J. D. Edman, and T. W. Scott. 2001. Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *Journal of Medical Entomology* 38(3):411-422.

Harrington, L. C., A. Fleisher, D. Ruiz-Moreno, F. Vermeylen, C. V. Wa, R. L. Poulson, J. D. Edman, J. M. Clark, J. W. Jones, S. Kitthawee, and T. W. Scott. 2014. Heterogeneous feeding patterns of the dengue vector, *Aedes aegypti*, on individual human hosts in rural Thailand. *PLoS Neglected Tropical Diseases* 8(8):e3048. doi: 10.1371/journal.pntd.0003048.

Hemingway, J. 2014. The role of vector control in stopping the transmission of malaria: Threats and opportunities. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 369(1645):20130431.

Hemingway, J., B. J. Beaty, M. Rowland, T. W. Scott, and B. L. Sharp. 2006. The Innovative Vector Control Consortium: Improved control of mosquito-borne diseases. *Trends in Parasitology* 22(7):308-312.

Hemingway, J., J. Vontas, R. Poupardin, J. Raman, J. Lines, C. Schwabe, A. Matias, and I. Kleinschmidt. 2013. Country-level operational implementation of the global plan for insecticide resistance management. *Proceedings of the National Academy of Sciences of the United States of America* 110(23):9397-9402.

Hiscox, A., B. Otieno, A. Kibet, C. K. Mweresa, P. Omusula, M. Geier, A. Rose, W. R. Mukabana, and W. Takken. 2014. Development and optimization of the Suna trap as a tool for mosquito monitoring and control. *Malaria Journal* 13:257.

Hoffmann, A. A., B. L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P. H. Johnson, F. Muzzi, M. Greenfield, M. Durkan, Y. S. Leong, Y. Dong, H. Cook, J. Axford, A. G. Callahan, N. Kenny, C. Omodei, E. A. McGraw, P. A. Ryan, S. A. Ritchie, M. Turelli, and S. L. O'Neill. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476(7361):454-457.

Kasai, S., O. Komagata, K. Itokawa, T. Shono, L. C. Ng, M. Kobayashi, and T. Tomita. 2014. Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: Target site insensitivity, penetration, and metabolism. *PLoS Neglected Tropical Diseases* 8(6):e2948.

IOM (Institute of Medicine). 2003a. *Microbial threats to health*. Washington, DC: The National Academies Press.

IOM. 2003b. *The resistance phenomenon in microbes and infectious disease vectors. Implications for human health and strategies for containment*. Washington, DC: The National Academies Press.

IOM. 2008. *Vector-borne diseases: Understanding the environmental, human health, and ecological connections*. Washington, DC: The National Academies Press.

Kroeger, A., A. Lenhart, M. Ochoa, E. Villegas, M. Levy, N. Alexander, and P. J. McCall. 2006. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: Cluster randomised trials. *British Medical Journal* 332(7552): 1247-1252.

Loroño-Pino, M. A., J. A. Farfán-Alé, A. L. Zapata-Peraza, E. P. Rosado-Paredes, L. F. Flores-Flores, J. E. García-Rejón, F .J. Díaz, B. J. Blitvich, M. Andrade-Narváez, E. Jiménez-Ríos, C. D. Blair, K. E. Olson, W. Black 4th, and B. J. Beaty. 2004. Introduction of the American/Asian genotype of dengue 2 virus into the Yucatan State of Mexico. *American Journal of Tropical Medicine and Hygiene* 71(4):485-492.

Loroño-Pino, M. A., J. E. García-Rejón, C. Machain-Williams, S. Gomez-Carro, G. Nuñez-Ayala, R. Nájera-Vázquez, A. Losoya, L. Aguilar, K. Saavedra-Rodriguez, S. Lozano-Fuentes, M. K. Beaty, W. C. Black 4th, T. J. Keefe, L. Eisen, and B. J. Beaty. 2013. Towards a Casa Segura: A consumer product study of the effect of insecticide-treated curtains on *Aedes aegypti* and dengue virus infections in the home. *American Journal of Tropical Medicine and Hygiene* 89(2):385-397.

Loroño-Pino, M. A., Y. N. Chan-Dzul, R. Zapata-Gil, C. Carrillo-Solís, A. Uitz-Mena, J. E. García-Rejón, T. J. Keefe, B. J. Beaty, and L. Eisen. 2014. Household use of insecticide consumer products in a dengue-endemic area in Mexico. *Tropical Medicine & International Health* 19(10):1267-1275.

Lounibos, L. P. 2002. Invasions by insect vectors of human disease. *Annual Review of Entomology* 47:233-266.

Lozano-Fuentes, S., D. Elizondo-Quiroga, J. A. Farfán-Alé, M. A. Loroño-Pino, J. Garcia-Rejón, S. Gomez-Carro, V. Lira-Zumbardo, R. Najera-Vazquez, I. Fernandez-Salas, J. Calderon-Martinez, M. Dominguez-Galera, P. Mis-Avila, N. Morris, M. Coleman, C. G. Moore, B. J. Beaty, and L. Eisen. 2008. Use of Google Earth to strengthen public health capacity and facilitate management of vector-borne diseases in resource-poor environments. *Bulletin of the World Health Organization* 86(9):718-725.

Lozano-Fuentes, S., Fernandez-Salas, I., M. de Lourdes Munoz, J. Garcia-Rejón, K. E. Olson, B. J. Beaty, and W. C. Black 4th. 2009. The neovolcanic axis is a barrier to gene flow among *Aedes aegypti* populations in Mexico that differ in vector competence for dengue 2 virus. *PLoS Neglected Tropical Diseases* 3(6):e468.

Mendoza-Mezquita, J. E., N. Torres-Arcila, M. M. Tec-Kumul, W. A. Páez-Cantón, J. L. Fraga Moreno, J. R. Torres-Castro, S. M. Vázquez-Narváez, and M. R. Nájera-Vázquez. 2014. Recicla por tu bienestar para prevenir el dengue. Poster session presented at: LXVIII Reunión Anual de Salud Pública en Mexico. November 19-22, Mérida, Yucatán, Mexico.

Messina, J. P., O. J. Brady, T. W. Scott, C. Zou, D. M. Pigott, K. A. Duda, S. Bhatt, L. Katzelnick, R. E. Howes, K. E. Battle, C. P. Simmons, and S. I. Hay. 2014. Global spread of dengue virus types: Mapping the 70 year history. *Trends in Microbiology* 22(3):138-146.

Mohan, A., D. Kiran, I. Manohar, and Kuman D. 2010. Epidemiology, clinical manifestations, and diagnosis of chikungunya fever: Lessons learned from the re-emerging epidemic. *Indian Journal of Dermatol*ogy 55(1):54-63.

Moore, M., M. Sylla, L. Goss, M. W. Burugu, R. Sang, L. W. Kamau, E. U. Kenya, C. Bosio, L. Munoz, M. Sharakova, and W. C. Black. 2013. Dual African origins of global *Aedes aegypti* s.l. populations revealed by mitochondrial DNA. *PLoS Neglected Tropical Diseases* 7(4):e2175.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, L. E. Hugo, K. N. Johnson, B. H. Kay, E. A. McGraw, A. F. van den Hurk, P. A. Ryan, and S. L. O'Neill. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* 139(7):1268-1278.

Morrison, A. C., E. Zielinski-Gutierrez, T. W. Scott, and R. Rosenberg. 2008. Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Medicine* 5(3):e68. doi: 10.1371/journal.pmed.0050068.

Mutebi, J. P., and A. D. Barrett. 2002. The epidemiology of yellow fever in Africa. *Microbes and Infection* 4(14):1459-1468.

Nasci, R. 2014. Movement of chikungunya virus into the Western Hemisphere. *Emerging Infectious Diseases* 20(8):1394-1395.

Ooi, E. E., K. T. Goh, and D. J. Gubler. 2006. Dengue prevention and 35 years of vector control in Singapore. *Emerging Infectious Diseases* 12(6):887-893.

Petersen, L. R., A. C. Brault, and R. S. Nasci. 2013. West Nile virus: Review of the literature. *JAMA* 310(3):308-315.

Powell, J. R., and W. J. Tabachnick. 2013. History of domestication and spread of *Aedes aegypti*—A review. *Memórias do Instituto Oswaldo Cruz* 108(Suppl 1):11-17.

Powers, A. M. 2014. Chikungunya virus control: Is a vaccine on the horizon? *Lancet* S0140-6736(14)61290-61293.

Reiter P., and D. J. Gubler. 1997. Surveillance and control of urban dengue vectors. In: D. J. Gubler and G. Kuno, editors. *Dengue and dengue hemorrhagic fever.* New York: CAB International. Pp. 425-462.

Reiter, P., S. Lathrop, M. Bunning, B. Biggerstaff, D. Singer, T. Tiwari, L. Baber, M. Amador, J. Thirion, J. Hayes, C. Seca, J. Mendez, B. Ramirez, J. Robinson, J. Rawlings, V. Vorndam, S. Waterman, D. Gubler, G. Clark, and E. Hayes. 2003. Texas lifestyle limits transmission of dengue virus. *Emerging Infectious Diseases* 9(1):86-89.

Renault, P., L. Josseran, and V. Pierre. 2008. Chikungunya-related fatality rates, Mauritius, India, and Reunion Island. *Emerging Infectious Diseases* 14(8):1327.

Saavedra-Rodriguez, K., L. Urdaneta-Marquez, S. Rajatileka, M. Moulton, A. E. Flores, I. Fernandez-Salas, J. Bisset, M. Rodriguez, P. J. McCall, M. J. Donnell, H. Ranson, J. Hemingway, and W. C. Black 4th. 2007. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. *Insect Molecular Biology* 16(6):785-798.

Saavedra-Rodriguez, K., A. F. Suarez, I. F. Salas, C. Strode, H. Ranson, J. Hemingway, and W. C. Black 4th. 2012. Transcription of detoxification genes after permethrin selection in the mosquito *Aedes aegypti*. *Insect Molecular Biology* 21(1):61-77.

Saavedra-Rodriguez, K., M. Beaty, S. Lozano-Fuentes, S. Denham, J. Garcia-Rejón, G. Reyes-Solis, C. Machain-Williams, M. A. Loroño-Pino, A. Flores-Suarez, G. Ponce-Garcia, B. Beaty, L. Eisen, and W. C. Black 4th. 2014. Local evolution of pyrethroid resistance offsets gene flow among *Aedes aegypti* collections in Yucatan State, Mexico. *American Journal of Tropical Medicine and Hygiene* 92(1):201-209.

Salazar, M. I., M. A. Loroño Pino, J. A. Farfán Alé, K. E. Olson, and B. J. Beaty. 2010. American and American/Asian genotypes of dengue virus differ in mosquito infection efficiency: Candidate molecular determinants of productive vector infection. *Revista Biomédica* 21(3):121-135.

Salazar-Sanchez, I., I. Sanchez-Vargas, J. H. Richardson, K. E. Olson, and B. J. Beaty. 2007. Dengue virus type 2: Virogenesis and tropisms in orally infected *Aedes aegypti* mosquitoes. *BMC Microbiology* 7:9.

Scott, T. W., G. G. Clark, L. H. Lorenz, P. H. Amerasinghe, P. Reiter, and J. D. Edman. 1993. Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique. *Journal of Medical Entomology* 30(1):94-99.

Scott, T. W., P. H. Amerasinghe, A. C. Morrison, L. H. Lorenz, G. G. Clark, D. Strickman, P. Kittayapong, and J. D. Edman. 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: Blood feeding frequency. *Journal of Medical Entomology* 37(1):89-101.

Scott, T. W., and W. Takken. 2012. Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission. *Trends in Parasitology* 28(3):114-121. doi: 10.1016/j.pt.2012.01.001.

Smith, C. 1956. The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *Journal of Tropical Medicine and Hygiene* 59:3-11.

Somers, G., J. E. Brown, R. Barrera, and J. R. Powell. 2011. Genetics and morphology of *Aedes aegypti* (Diptera: Culicidae) in septic tanks in Puerto Rico. *Journal of Medical Entomology* 48(6):1095-1102.

Soumahoro, M., P. Boelle, B. Gaüzere, K. Atsou, C. Pelat, B. Lambert, G. Ruche, M. Gastellu-Etchegorry, P. Renault, M. Sarazin, Y. Yazdanpanah, A. Flahault, D. Malvy, and T. Hanslik. 2011. The chikungunya epidemic on La Réunion Island in 2005–2006: A cost-of-illness study. *PLoS Neglected Tropical Diseases* 5(6):e1197.

Srikiatkhachorn, A., A. L. Rothman, R. V. Gibbons, N. Sittisombut, P. Malasit, F. A. Ennis, S. Nimmannitya, and S. Kalayanarooj. 2011. Dengue—How best to classify it. *Clinical Infectious Diseases* 53(6):563-567.

Srisawat, R., N. Komalamisra, C. Apiwathnasorn, P. Paeporn, S. Roytrakul, Y. Rongsriyam, and Y. Eshita. 2012. Field-collected permethrin-resistant *Aedes aegypti* from central Thailand contain point mutations in the domain IIS6 of the sodium channel gene (KDR). *Southeast Asian Journal of Tropical Medicine and Public Health* 43(6):1380-1386.

Stoddard, S. T., B. M. Forshey, A. C. Morrison, V. A. Paz-Soldan, G. M. Vazquez-Prokopec, H. Astete, R. C. Reiner Jr., S. Vilcarromero, J. P. Elder, E. S. Halsey, T. J. Kochel, U. Kitron, and T. W. Scott. 2013. House-to-house human movement drives dengue virus transmission. *Proceedings of the National Academy of Sciences of the United States of America* 110(3):994-999.

Strode, C., S. Donegan, P. Garner, A. A. Enayati, and J. Hemingway. 2014. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: Systematic review and meta-analysis. *PLoS Medicine* 11(3):e1001619.

Sylla, M., C. Bosio, L. Urdaneta-Marquez, M. Ndiaye, and W. C. Black 4th. 2009. Gene flow, subspecies composition, and dengue virus-2 susceptibility among *Aedes aegypti* collections in Senegal. *PLoS Neglected Tropical Diseases* 3(4):e408.

Tabachnick, W. 2013. Nature, nurture, and evolution of intra-species variation in mosquito arbovirus transmission competence. *International Journal of Environmental Research and Public Health* 10:249-277.

Tabachnick, W. J., G. P. Wallis, T. H. G. Aitken, B. R. Miller, G. D. Amato, L. Lorenz, J. R. Powell, and B. J. Beaty. 1985. Oral infection of *Aedes aegypti* with yellow fever virus: Geographic variation and genetic considerations. *American Journal of Tropical Medicine and Hygiene* 34:12191224.

Tandale, B. V., P. S. Sathe, V. A. Arankalle, R. S. Wadia, R. Kulkarni, S. V. Shah, S. K. Shah, J. K. Sheth, A. B. Sudeep, A. S. Tripathy, and A. C. Mishra. 2009. Systemic involvements and fatalities during chikungunya epidemic in India, 2006. *Journal of Clinical Virology* 46(2):145-149.

Vasilakis, N., E. J. Shell, E. B. Fokam, P. W. Mason, K. A. Hanley, D. M. Estes, and S. C. Weaver. 2007. Potential of ancestral sylvatic dengue-2 viruses to re-emerge. *Virology* 358(2):402-412.

Vasilakis, N., J. Cardosa, M. Diallo, A. A. Sall, E. C. Holmes, K. A. Hanley, S. C. Weaver, J. Mota, and R. Rico-Hesse. 2010. Sylvatic dengue viruses share the pathogenic potential of urban/endemic dengue viruses. *Journal of Virology* 84(7):3726-3728.

Vazquez-Prokopec, G. M., D. Bisanzio, S. T. Stoddard, V. Paz-Soldan, A. C. Morrison, J. P. Elder, J. Ramirez-Paredes, E. S. Halsey, T. J. Kochel, T. W. Scott, and U. Kitron. 2013. Using GPS technology to quantify human mobility, dynamic contacts and infectious disease dynamics in a resource-poor urban environment. *PLoS One* 8(4):e58802.

Weaver, S. C. 2013. Urbanization and geographic expansion of zoonotic arboviral diseases: Mechanisms and potential strategies for prevention. *Trends in Microbiology* 21(8):360-363.

Weaver, S. C. 2014. Arrival of chikungunya virus in the new world: Prospects for spread and impact on public health. *PLoS Neglected Tropical Diseases* 8(6):e2921.

Weaver, S. C., and W. K. Reisen. 2010 Present and future arboviral threats. *Antiviral Research* 85(2):328-345.

Whelan, P., H. Nguyen, K. Hajkowicz, J. Davis, D. Smith, A. Pyke, V. Krause, and P. Markey. 2012. Evidence in Australia for a case of airport dengue. *PLoS Neglected Tropical Disease* 6(9):e1619.

Wise de Valdez, M. R., D. Nimmo, J. Betz, H. F. Gong, A. A. James, L. Alphey, and W. C. Black 4th. 2011. Genetic elimination of dengue vector mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America*. 108(12):4772-4775.

Yanola, J., P. Somboon, C. Walton, W. Nachaiwieng, P. Somwang, and L. A. Prapanthadara. 2011. High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Tropical Medicine and International Health* 16(4):501-509.

A4

DENGUE, CHIKUNGUNYA, AND OTHER VECTOR-BORNE DISEASES (VBDs): SURVEILLANCE AND RESPONSE IN LATIN AMERICA AND THE CARIBBEAN: THE ROLE OF THE PAN AMERICAN HEALTH ORGANIZATION

Luis Gerardo ***Castellanos***[1]

Introduction

To better understand the role of the Pan American Health Organization (PAHO) in support of the prevention, control, and elimination of vector-borne diseases in the American continents, it is useful to briefly describe what PAHO is, what has been done, and where the organization is today in its practice.

The Pan American Health Organization

During the 19th century, four international sanitary conferences that included participation of countries from the Americas were held in Europe with unclear

[1] Pan American Health Organization.

results. Later in the 1870s, an epidemic of yellow fever spread in several countries of South America, and from there it reached the United States of America through maritime contacts, resulting in a major epidemic with more than 20,000 cases and deaths.

The countries of the Americas resolved to take action with an international perspective; thus a 5th International Conference was arranged to be held in the Americas for the "purpose of securing an international system of notification as to the actual sanitary situation of ports and places." Around the same time, inter-American cooperation was beginning to grow, and during the 1890s a first international conference was also organized to establish the International Union of American Republics (today known as the Organization of American States).

In 1901, during the 2nd Conference of the International Union (in Mexico) a recommendation was made to call a general convention of representatives of health from the different American republics, with the purpose of proposing sanitary agreements and regulations. The First General International Sanitary Convention of the American Republics, to assure effective cooperation in promoting health in the Americas, was held in Washington, DC, in December 1902. This meeting gave birth to the Pan American Sanitary Bureau.

Originally called the Pan American Sanitary Bureau (PASB), PAHO today is the world's oldest international public health agency continuously working.

PAHO can be considered a coalition encompassing 30 percent of Earth's land mass and 14 percent of the world's current population. With 28 country offices in 35 countries, PAHO's scope has also continued to grow. The initial focus on controlling epidemic diseases has broadened to noncommunicable diseases, better health education, health systems and services, essential medications, mental health, and other fields that include environmental improvements designed to help all populations, especially communities in need.

PAHO's current vision is to serve as the major catalyst for ensuring that all the peoples of the Americas enjoy optimal health, and contribute to the well-being of their families and communities. PAHO's current mission is to lead strategic collaborative efforts among member states and other partners to promote equity in health, to combat disease, and to improve the quality of, and lengthen, the lives of the peoples of the Americas.

The Fight Against Vector-Borne Diseases in Latin America and the Caribbean

Yellow Fever

PAHO was the first international health organization to organize a united front against the spread of yellow fever in what today is a key shipping route connecting the Atlantic and Pacific Oceans. Founded by 11 countries, PAHO's first task was to eliminate yellow fever and malaria in the Panama Canal Zone.

The 2nd International Sanitary Convention, which took place in 1905 in Washington, DC, continued to emphasize the importance of yellow fever, noting the success of control campaigns in Cuba, the Panama Canal Zone, and Mexico. Setting an important precedent, the convention resolved that, in event of epidemics, *national health authorities would be responsible for quarantine and disease control campaigns*.

Despite successful achievements, yellow fever has continued to be a public health concern in the Americas, and it is a reportable disease according to the International Health Regulations coordinated by the World Health Organization (WHO). Currently, between 16 and 60 cases are reported every year, despite the millions of vaccines applied yearly to prevent its spread, mostly in South America where a dozen countries remain as endemic territories or are under permanent threat.

PAHO's role is to support countries to keep up-to-date capacity in prevention, control, diagnosis, adverse event management, and risk communication. In addition, PAHO periodically collaborates with WHO in the reviewing of guidelines and recommendations for endemic or at-risk countries.

Malaria

The efforts to eradicate malaria worldwide were spurred on by the successes seen through use of DDT to kill anopheline vectors of the disease. The global launch to eradicate malaria was held in Mexico City in 1955. After World War II, WHO helped countries put together programs of DDT spraying to combat malaria transmission. PAHO coordinated these efforts in the Americas. These campaigns partially interrupted malaria transmission, and it was reflected in dramatic reductions in infection and number of cases in a relatively short time between the 1960s and 1980s.

With more than one million cases in the year 2000 to less than 430,000 malaria cases in 2013, the Americas have earned a first place in steadily decreasing the incidence and mortality (82 deaths in 2013) due to malaria in the last decade.

One of PAHO's roles has been concentrated in maintaining political and financial interest from governments and international stakeholders in supporting their national malaria programs and efforts towards control and elimination. For this, permanent consultation with countries has allowed PAHO to properly analyze and map the technical needs and keen efforts necessary to advance the agenda of malaria elimination in the region.

Resolution CD51.R09, approved by PAHO's member states in 2011, described the strategy and plan of action elaborated to aggressively pursue control and advance towards the elimination of malaria in the Americas. In the year 2015, a new regional strategy and plan of action will be presented considering the advances reached by the countries, including the potential use of newly developed and available tools and in concordance with recommendations of the forthcoming

new WHO Global Malaria Strategy 2016–2030. Among the main areas of action, PAHO supports countries in the following ways:

- Intensify efforts directed toward malaria prevention, surveillance, early detection, and outbreak containment in various program contexts (including malaria elimination)
- Integrated vector management by promoting, strengthening, and optimizing mechanisms and tools for judicious and cost-effective vector management
- Malaria diagnosis and treatment by strengthening efforts to achieve universal access to prompt, accurate, and quality malaria diagnosis, followed by rapid treatment with effective antimalarial medicines
- Advocacy, communications, partnerships, and collaborations through specific actions that foster an environment that promotes sustainability and supports collaborative efforts and best practices to combat the disease
- Health systems strengthening, strategic planning, operational research, and country-level capacity building
- Optimize efforts to strengthen health systems (including strategic planning, monitoring and evaluation, operations research) and the countries' capacities to address their respective malaria challenges both relevantly and adequately

Dengue Virus

Similar to malaria, PAHO also has played a role in the history of (attempted) *Aedes aegypti* eradication or control as described in the PAHO Director's Report from 1958, but outcomes so far are different. In the Americas, dengue incidence has increased 30 fold in the last 50 years, and between 2008 and 2012 more than 1.2 million cases of dengue were notified annually, including 28,233 severe cases and 1,000 deaths. Furthermore, 2013 had the highest burden of disease ever registered, with the largest epidemic in the history of the Americas, with a total of 2.3 million cases, 37,898 severe cases, and 1,318 deaths.[2] This disease has a high social and economic impact, affecting not just the patient, but also families and the community as a whole. The estimated economic cost of the disease in the region supersedes US$2.1 billion per year.

Dengue and its main vector in the Americas have continued to spread geographically, and its unusual capacity to survive in cold climates and temperatures has increased. The United States is not exempt from this threat, as documented in 2013 by Añez and collaborators from the U.S. Food and Drug Administration. The geographic spread of these potentially harmful vectors has already invaded a

[2] PAHO. Number of Reported Cases and Severe Dengue (SD) in the Americas, by Country. Available at: http://www.paho.org/dengue. Accessed on June 5, 2014.

significant portion of North American soil. Currently all four serotypes of dengue virus (I, II, III, and IV) are known to be circulating in the Americas, and simultaneous circulation of all four types are documented in at least eight countries.

PAHO/WHO, through the Dengue Regional Program, supports member states in the implementation of the Integrated Management Strategy for the Prevention and Control of Dengue (IMS-Dengue). This strategy was adopted by the countries of the Americas through the Resolution of PAHO's governing bodies CD44.R9 in 2003; since then, 22 countries of the Americas have developed national IMS-Dengue prevention and control plans. In addition, 20 of the countries have completed an assessment of their IMS-Dengue strategy, with the support of experts from the International Technical Group on Dengue (ITG-Dengue), following the recommendations of Resolution CSP27.R15, adopted in 2007 by the 27th Pan American Health Organization Sanitary Conference.

The current efforts of the Regional Dengue Program include the following:

- Strengthen epidemiological surveillance of dengue through the development of a generic model of an integrated epidemiological surveillance system.
- Strengthen laboratory networks in the management of effective practices in the diagnosis of dengue through the Dengue Laboratory Network of the Americas (RELDA, acronym is from the Spanish name of the network).
- Strengthen vector monitoring and control in entomology, integrated vector management, and monitoring of insecticide resistance.
- Improve clinical management of patients through the adaptation for the Americas of the WHO clinical guidelines published in 2009, a second edition of which is currently in progress.
- Strengthen social communication by use of communication planning methodologies to improve behaviors of populations facing the dengue problem, including political leaders, health officials, residents, and other stakeholders.

Chikungunya Virus

As the Americas evolve along with the rest of the world, communication and international trade facilitates the travel or transport of individuals and goods, and with them, the spread of diseases and their vectors. On December 2013, PAHO/WHO received confirmation of the first cases of autochthonous transmission of chikungunya virus (CHIKV) in the Americas. Yet, since 2012, PAHO/WHO and the U.S. Centers for Disease Control and Prevention (CDC) collaborated together and with countries in the region, anticipating and preparing for the risk of introduction of the virus. As a result of these efforts, new joint guidelines were published that same year for preparedness and response on CHIKV introduction. These guidelines were aimed to help countries throughout the Americas improve

their ability to detect the virus and be prepared to monitor, prevent, and control the disease.

Ever since the first cases were reported, PAHO has officially acknowledged the reporting of over 900,000 cases (over 15,000 laboratory diagnosed), and close to 150 deaths related to CHIKV in more than 30 countries and territories of the Americas.

In addition to the already developed capacity in countries to properly respond to any chikungunya-related threat in the Americas, PAHO has from the very beginning supported countries to (a) formulate evidence-based outbreak management plans and effectively manage cases and outbreaks, and (b) improve their reporting systems and technical skills to properly diagnose cases, and improve their capacity to assess and implement vector control activities. PAHO also publishes guidelines and handbooks for surveillance, case management, laboratory detection, and vector control for its member states and receives support from a network of referral laboratories located in Argentina, Brazil, Cuba, French Guyana, and the United States.

Current Challenges

Countries in the Americas have a history of success in achieving public health goals, and elimination of diseases has been in the agenda for more than 50 years. Ever since the eradication of smallpox, initiated in the Americas in the 1950s, the region grew its reputation for tackling vaccine-preventable diseases and showing the world that it was possible to eliminate them. After smallpox, polio, measles and rubella have followed the same path, as the Americas proudly reflects their achievements in the health of all children and adults. Nowadays, the Americas are convinced that elimination of diseases can be expanded to conquer diseases that cannot be prevented with immunizations; and as examples of such possibilities, in 2013 Colombia became the first country in the world to be verified by WHO as having eliminated onchocerciasis transmission, followed recently by Ecuador in September 2014.

Conclusions

Understanding the role of the Pan American Health Organization in the public health history of the Americas, we note the following:

1. The Pan American Health Organization (PAHO) is the world's oldest international public health agency continuously working for the public health and well-being of the Americas.
2. Vector-borne diseases have been a historical public health challenge to the Americas, and they continue to be a significant threat.

3. Countries in the Americas have also historically been leaders in preventing, controlling, and eliminating vector-borne diseases as public health problems. Great examples of this are malaria in the Caribbean, yellow fever in the region, and most recently onchocerciasis from Colombia and Ecuador.
4. PAHO has been instrumental, supporting countries in preparedness, prevention, control and elimination of vector-borne diseases, always in collaboration with governments and partners.
5. Vector-borne diseases will continue to be a dynamic public health threat to countries in the Americas; therefore, the commitment and financial support from governments and international stakeholders to prevent further spread and strive for elimination is essential.

A5

VECTOR-BORNE DISEASES: ANIMALS AND PATTERNS

Margot Stuchin,[1] *Catherine C. Machalaba,*[2] *William B.* ***Karesh***[2]

We are in the early stages of understanding patterns of vector-borne disease (VBD) in animals in the United States and globally. While the enormous impacts of VBD to human and economic health have been well studied, there are unique challenges associated with assessing and controlling VBDs for which an animal host is a major component and even more so when multiple host species can play epidemiologically significant roles.

The scope, relevance, and evaluation of vector-borne pathogens are highly dependent on organizational priorities. No individual list or organization focuses on all VBDs that pose a risk to animals; however, many do cover a portion of viral, bacterial, or parasitic illnesses of relevance. For example, the National Institute of Allergy and Infectious Diseases (NIAID) categorizes infectious agents by their threat to public health and national security. These agents are prioritized and divided into three categories (A, B, and C) based on their transmissibility, potential to cause social disruption, and impact to human health, although many of these pathogens affect animal health as well. Forty percent of the NIAID priority pathogens are vector borne and also widely regarded to infect or cause disease in animals (4 of 18 in Category A, 9 of 24 in Category B, and 13 of 23 in Category C) (NIAID, 2014) (see Table A5-1).

[1] Colorado University.
[2] EcoHealth Alliance, 460 West 34th Street, New York, USA.

TABLE A5-1 Vector-Borne NIAID Priority Pathogens (Categories A, B, C) That Affect Animals and Humans, OIE-Reportable Terrestrial Mammalian Pathogens, and Viral Families with Novel Primate, Bat, Rodent, and Shrew Viruses Discovered Through the PREDICT Project

OIE	NIAID		PREDICT
Bluetongue virus Crimean Congo hemorrhagic fever Heartwater Eastern equine enchephalomyelitis Japanese encephalitis Rift Valley fever Surra (*Trypanosoma evansi*) *Tularemia tularensis* West Nile virus Nairobi sheep disease African swine fever Bovine anaplasmosis Bovine babesiosis Theileriosis Trypanosomosis Western equine encephalomyelitis Equine infectious anemia Equine piroplasmosis Infection with African horse sickness virus Venezuelan equine encephalomyelitis	Category A	*Yersinia pestis* *Franciscella tularensis* Rift Valley fever Crimean Congo hemorrhagic fever	*Flavivirus* *Orbivirus* *Rhabdovirus* *Seadornavirus* *Phlebovirus*
	Category B	Typhus fever (*Rickettsia prowazekii*) West Nile virus (WNV) LaCrosse encephalitis (LACV) California encephalitis Venezuelan equine encephalitis (VEE) Eastern equine encephalitis (EEE) Western equine encephalitis (WEE) Japanese encephalitis virus St. Louis encephalitis virus (SLEV)	
	Category C	Severe fever with thrombocytopenia Syndrome virus (SFTSV) Heartland virus Omsk hemorrhagic fever virus Alkhurma virus Kyasanur Forest virus Tick-borne encephalitis complex flaviviruses • Tick-borne encephalitis viruses • European subtype • Far Eastern subtype • Siberian subtype • Powassan/deer tick virus Yellow fever virus Other rickettsias Chikunguya virus	

SOURCES: NIAID, 2014; OIE, 2014; PREDICT Consortium, 2014.

Several VBDs are of importance to international trade and are listed as notifiable diseases. One-quarter of the terrestrial vertebrate pathogens of concern to the World Organisation for Animal Health (OIE) are vector borne (OIE, 2014). The goal of the OIE's list is to promote global transparency and awareness of the condition of animal health to prevent disease introduction or spread.

As these different listings highlight, known VBDs are of great importance and concern to both federal and international organizations for their existing

or potential burden to human and animal health. However, there is no single resource for assessing or prioritizing these VBDs. With the threat of potentially unidentified vector-borne pathogens or pathogens yet to emerge, it is important to recognize this as a shortcoming in our current classifications systems. Over a 5-year period, the PREDICT project, supported by USAID's Emerging Pandemic Threats program, has identified an additional 36 viruses from taxa that are known to encompass VBDs, suggesting that unknown vector-borne diseases may represent a burgeoning threat to both human and animal health. PREDICT seeks to identify novel zoonotic pathogens before their spread to humans (PREDICT Consortium, 2014), and tests samples from wildlife based on their risk for zoonotic transmission given the ecological setting. Literature review suggests that approximately 40 percent of emerging zoonotic viruses are vector borne (Johnson et al., 2015). Combined, these results point to the importance of VBDs in both animals and humans.

Vector-borne viruses account for 29 percent of the 593 known mammalian viruses (Olival et al., in review). These pathogens have three times the host range compared to nonvector-borne viruses (Johnson et al., 2015) meaning that multiple animal species may act as hosts or reservoirs for any particular VBD. Additionally, individual vector-borne viruses can be transmitted by multiple, related vector species. Not only does this mean that VBDs may broadly affect animal health over a range of species, but it also poses challenges for disease control that targets hosts rather than vectors.

When a VBD affects both people and animals, humans are typically an incidental host and do not serve an important role in transmitting the disease to additional vectors. However, this does not exclude humans from being affected both directly and indirectly by VBDs for which they are not the primary host. VBDs can have serious effects on human and animal health as well as significant economic implications.

While climate change is commonly cited as a major contributor to increasing VBD prevalence and distribution, it is important to recognize that numerous human and ecological factors play a major role in disease emergence and spread. Patterns of VBDs can be attributed to a wide range of variables that vary by disease, location, and circumstance (see Figure A5-1). Additionally, identifying the drivers that are associated with VBD emergence and spread presents an opportunity for prevention, education, and control. Changes in land use, war and famine, breakdown of public health measures, global trade and travel, and human behavior are all associated with VBD emergence (Loh et al., 2015) (see Figure A5-2). By identifying situations where we anticipate VBD emergence, we can more effectively target prevention and intervention strategies.

Recent VBD emergence events have highlighted the important role of animal hosts or reservoirs. We examine four examples, Schmallenberg virus, West Nile virus, tick-borne illness, and Rift Valley fever virus, for their trends and implications in terms of animal health.

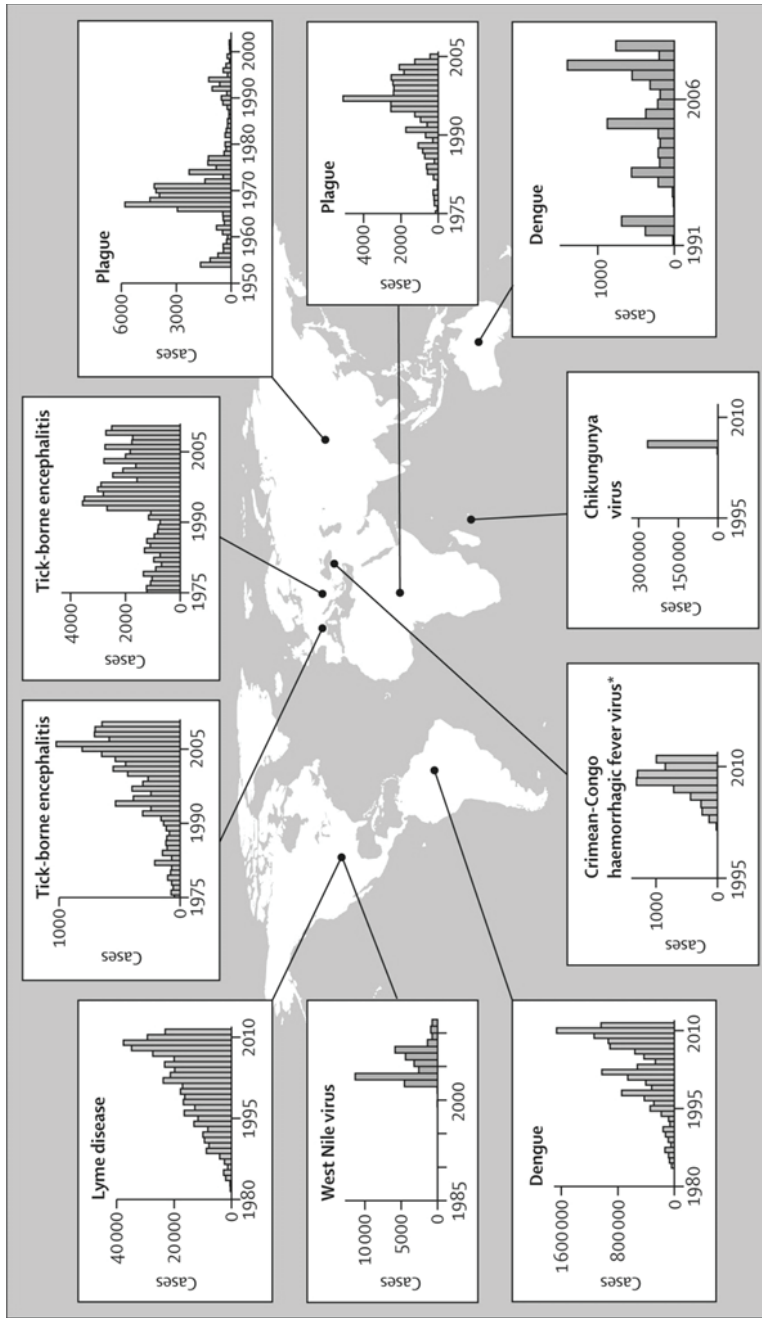

FIGURE A5-1 Temporal patterns of select vector-borne disease emergence.
SOURCE: Kilpatrick and Randolph, 2012. Reprinted with permission from Elsevier.

FIGURE A5-2 Scaled number of zoonotic EID events (n = 180) per transmission route categorized by the primary driver of disease emergence for each pathogen from Loh et al., 2015. Vector-borne diseases are shown in pink.
SOURCE: Loh et al., 2015. Reproduced with permission from Mary Ann Liebert, Inc. on behalf of *Vector-Borne and Zoonotic Diseases*.

Schmallenberg Virus

Schmallenberg virus (SBV) is a novel nonzoonotic virus in the Bunyaviridae family that emerged in Germany and the Netherlands in 2011 and is now reported in most European nations. It primarily affects domestic ruminants and has been detected serologically in dogs and a number of wildlife and zoo species including alpaca, water buffalo, elk, bison, red deer, fallow deer, roe deer, muntjac, and chamois (Sailleau et al., 2013; EFSA, 2014). Biting midges, *Culucoides* spp., are the primary vectors (EFSA, 2014), which likely dispersed throughout Europe via wind-mediated spread (Sedda and Rogers, 2013). Although midges do not easily acquire SBV from infected sheep, and the prevalence of the virus in midges is low at 0.25 percent (Elbers et al., 2013), biting midges are efficient at transmitting the virus to animals, with a 0.76 probability of transmission from an infected vector to host (EFSA, 2014).

The basic reproduction number (R_0) of Schmallenberg is 5–7 per infected animal, which peaks at 21°C (see Figure A5-3). This high value for R_0 follows

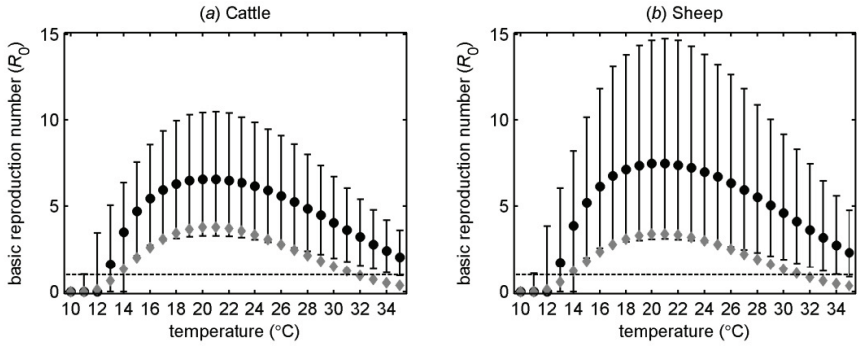

FIGURE A5-3 Basic reproduction number (R_0) for Schmallenberg virus in (a) cattle and (b) sheep, indicating a temperature-dependent relationship.
NOTE: The posterior median is shown by the black circles, surrounded by 95 percent CI error bars. The black dotted line represents the $R_0 = 1$ threshold.
SOURCE: EFSA, 2014.

suit with similar VBDs; however, the temperature for optimal transmission is relatively mild. Indeed, warmer conditions are not universally optimal for all vector-borne diseases, and additional factors must be considered when addressing VBD spread on a whole.

SBV does not generally kill sheep, and currently it is not a notifiable disease to the OIE. However, non-OIE related international trade restrictions due to SBV have had major implications for the EU's live animal and bovine semen trade, resulting in serious economic consequences. For example, in 2012, SBV was responsible for an 11–26 percent decline in bovine semen exports to non-EU countries and a 20 percent decline in breeding animal exports from €590 million to €475 million (EFSA, 2014).

While symptoms in affected cattle and sheep are generally rare, clinical signs of acute SVB infection can cause fever, reduced milk yield, diarrhea, and abortion. The animal typically recovers in 4–6 days, after which it is immune (Meroc et al., 2014). The rate of abortions in SBV infected flocks is double compared to uninfected flocks, with a five-fold increase in malformations (Saegerman et al., 2014). Obstructed labor in domestic ruminants imposes additional draining of resources from farmers and veterinary professionals as a result of the work in assisting with birth. Fifteen percent of SBV infected pregnant ewes have obstructed labor, and 2 percent die as a result (Dominguez et al., 2012). This loss can impose a major burden on affected farmers who operate on small profit margins.

Current strategies for mitigation of midge-borne viruses include vector control, timed breeding, and vaccination. Midge control through the use of pesticides is largely impractical both for the individual farmer and for large-scale

prevention of disease. Breeding before or after the midge season is also uncertain, as expansions in vector range or longer peak midge season may have implications for the usefulness of this method (Wittmann et al., 2002; Wernike et al., 2013a,b). Available vaccines suggest promise for SBV control. However, the marketability of these immunizations is questionable. There is marginal incentive for livestock owners to purchase the vaccines and hence for pharmaceutical companies to promote them. Given the overall mild symptoms, short duration of illness in domestic ruminants, and the gain of immunity postrecovery it may not be economically viable to vaccinate. Individual livestock owners will most likely have to live with the burden of disease unless improved control strategies become available.

West Nile Virus: Shifts in Surveillance

Patterns of West Nile virus (WNV) emergence and transmission are highly dependent on a wide range of variables, many of which are stochastic or unpredictable. While landscape and weather factors do play a role in transmission dynamics, it is impossible to discuss patterns of WNV emergence without addressing the dramatic changes in surveillance throughout the history of the virus in the United States. From 2004 to 2012 there was a 61 percent reduction of CDC epidemiology and laboratory capacity (ELC) funding which affected state- and county-level WNV surveillance in their early detection capacity and ability to determine and monitor patterns of the virus in animals and humans (Hadler et al. 2014) (see Figure A5-4).

To function effectively, consistent support is needed in surveillance activities, as geographically patchy surveillance limits our ability to draw conclusions on trends or correlations with factors that may affect disease prevalence (see Figure A5-5). WNV is far from the only vector-borne disease for which lapses in testing and reporting leaves gaps in our understanding of pathogen dynamics. Lyme disease in dogs, discussed later in this report, is also a valuable example of how gaps in surveillance affect our ability to monitor disease trends.

When addressing WNV control in domestic animals, our competitive marketing strategies may interfere with optimal surveillance. There are currently two WNV vaccines available for veterinary use in the United States; however, vaccine use in the United States is confidential information, and sales data are regarded as proprietary. We are therefore lacking critical knowledge regarding disease control in domestic animals. It would be of benefit to the public health community to have information regarding the geographic distribution and volume of vaccines used to detect potential trends or changes over time. A network for immunization use across the United States would aid the public and animal health community in our understanding of where disease control is being implemented and our ability to take action in emerging areas for control.

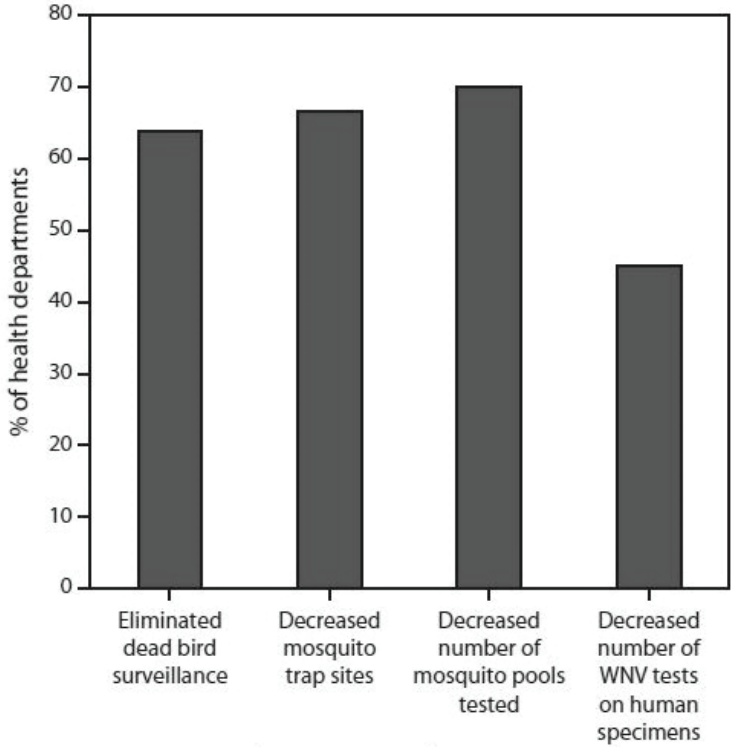

FIGURE A5-4 Changes in WNV surveillance as of 2013 reported by 50 states and 6 county or city CDC-funded jurisdictions.
SOURCE: Hadler et al., 2014 (CDC).

Tick-Borne Diseases in Companion Animals and Livestock

Pathogens transmitted via tick bite, or tick-borne diseases (TBDs), broadly affect domestic animals, livestock, and wildlife worldwide. Ticks feed on a wide range of animal taxa including mammals, reptiles, amphibians and birds, often using different hosts throughout their life cycle, creating multiple opportunities for disease spread between species.

Geographic patterns of Lyme disease (*Borrelia burgdorferi*) prevalence in dogs have closely followed those in humans, with the highest regions of seroprevalence occurring in the United States northeast and Midwest, where some clusters have seroprevalence as high as 44.1 percent. A comparison of studies of Lyme disease seroprevalence in domestic dogs in the United States showed an increase from 11.2 percent in the 2001–2007 study period to 13.3 percent in the 2010–2012 study period (Bowman et al., 2009; Little et al., 2014) (see

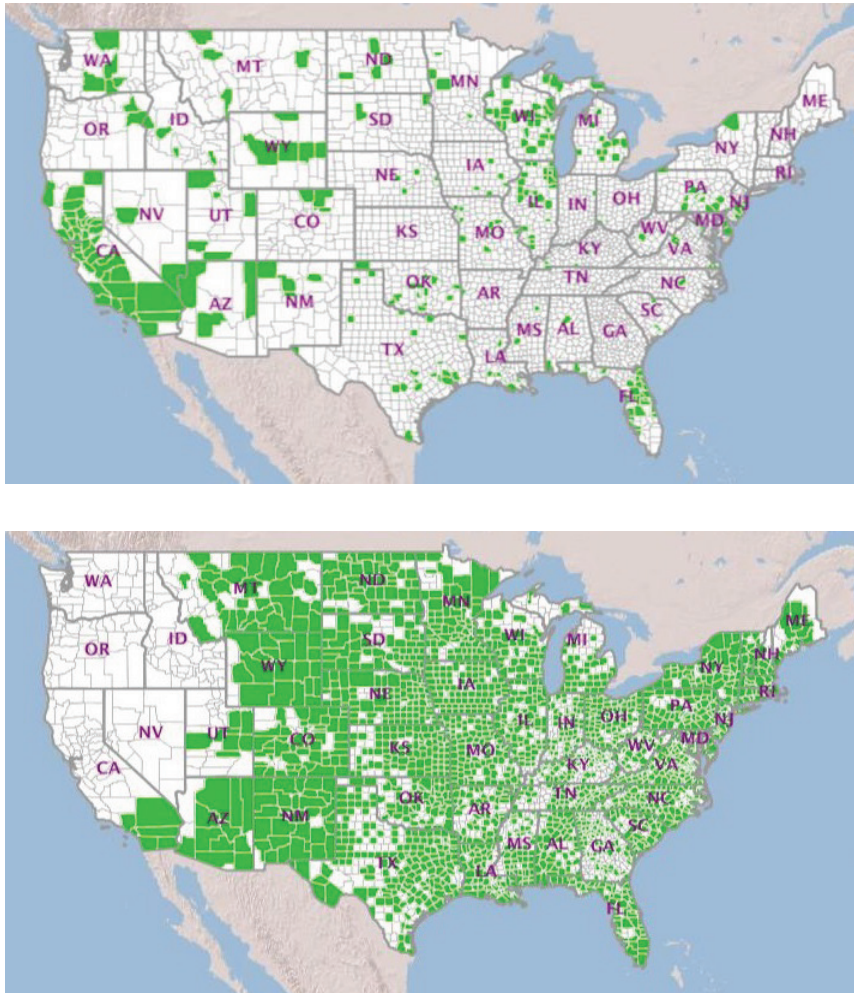

FIGURE A5-5 Nonhuman (avian, sentinel, and veterinary) reported WNV infections for 2003 (top) and 2014 (bottom).
SOURCE: USGS, 2014.

Figure A5-6). Because of the interconnectedness of humans and domestic dogs, it can be expected that patterns for Lyme disease would be similar among both. Human activity and other ecological drivers are likely responsible for these increases of disease prevalence; however, the role of inadequate surveillance in our ability to perceive these patterns must be addressed. A lack of centralized reporting for canine Lyme disease makes it difficult to discern whether these parallel increases

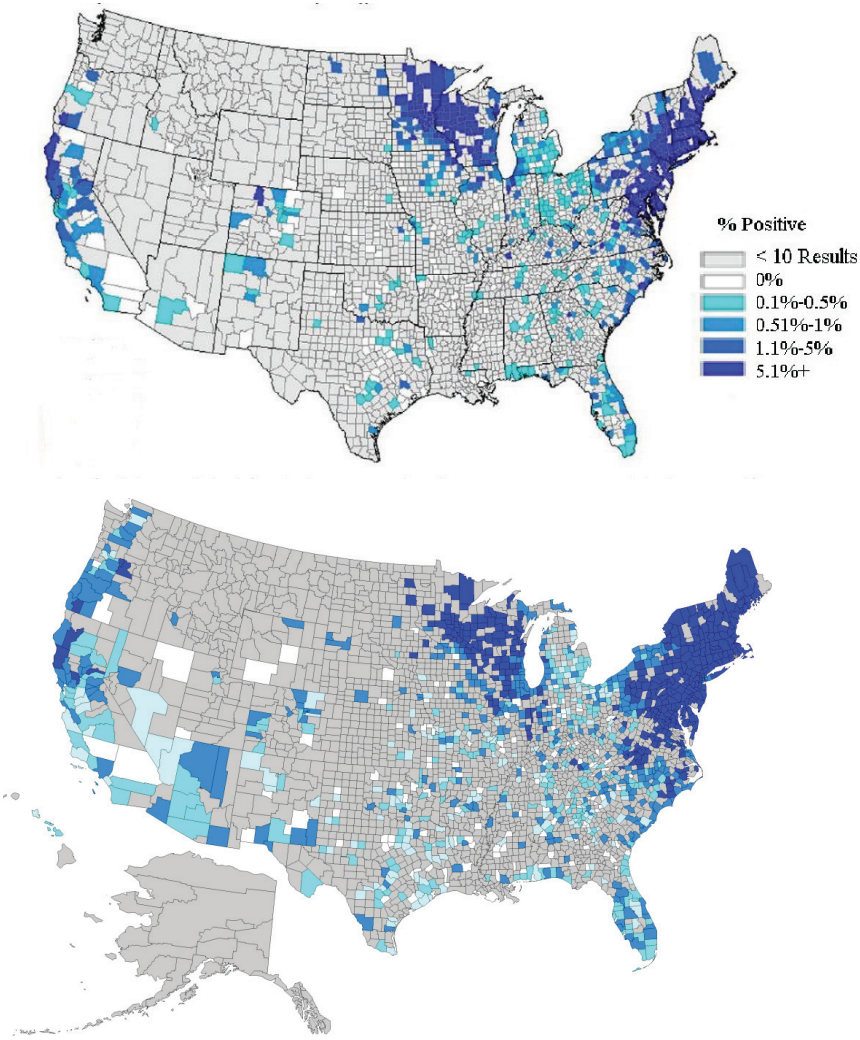

FIGURE A5-6 Lyme disease (*Borrelia burgdorferi*) seroprevalence in dogs 2001–2007 (top) and 2010–2012 (bottom).
SOURCE: Bowman et al., 2009, reproduced with permission from Elsevier (top); Little et al., 2014, available from *Parasites & Vectors* under Creative Commons license (bottom).

are a function of a change in the amount of diagnostic testing or actual shift in disease range. Gaps in uniform surveillance of TBD in companion animals impede accurate and integral epidemiologic monitoring, particularly in nonendemic regions (see Figure A5-6).

Whereas ectoparasiticides are relatively easy to administer to companion animals for tick control, managing ticks and TBD in the livestock industry is a major challenge both to individual farmers and on the global scale. TBD affects 80 percent of the world's livestock holdings, and the economic cost of TBD is $13.9 billion to $18.7 billion annually (Minjauw and McLeod, 2003). This economic burden can be substantial in resource-poor tropical and subtropical regions, particularly to small-scale livestock owners (Minjauw and McLeod, 2003). The cost of controlling TBD in some areas exceeds animal production costs, as is seen with theileriosis in Tanzania (see Figure A5-7). The significant burden is especially pertinent when adherence to international standards for vector control at national levels is a trade requirement.

A One Health Approach to Vector-Borne Diseases—RVF as an Example

Rift Valley fever (RVF) is an emerging mosquito-borne zoonotic disease and has been recognized as a pathogen of significant concern by the WHO, OIE, FAO, U.S. CDC, U.S. DoD, and USDA, with broad relevance for both human health and livestock production. The virus has caused large epizootics in Africa, and has recently led to outbreaks in the Middle East. RVF outbreaks are devastating to domestic ruminants, in which it causes widespread abortions and high mortality (> 90 percent in some cases) in juveniles (Murphy et al., 1999). Infection in humans can occur through the bite of an infected mosquito or via contact with tissues or bodily fluids from an infected animal.

RVF outbreaks have been extensively studied in Kenya, where it was first discovered in 1931. East African RVF outbreaks appear to occur periodically (in cycles every 7–15 years), with little to no activity during interepidemic periods, and are associated with heavy floods. This RVF virus cycle involves a sylvatic cycle with transmission between *Aedes* mosquitos and wild and domestic ruminants; the mosquitoes can transmit it transovarially. Wild and domestic ruminants typically experience subclinical infections in interepidemic periods, but heavy rains increase *Aedes* populations, leading to amplification in domestic ruminants, and increasing potential for outbreaks in domestic ruminants and transmission to humans.

While this weather-dependent cycle is well established in Kenya, outbreaks appear to be less cyclical, or have different determinants, in South Africa. A recent analysis of outbreaks showed RVF outbreaks of varying scales and in different regions reported in South Africa each year from 2008–2011 (Metras et al., 2012). The scale of infection and spread may be a major consideration for immunity, with apparent low immunity in interepidemic periods, and potential herd immunity established during outbreaks. However, despite potential mixing of wildlife and domestic animals at some ranch sites (whether through presence of farmed wildlife or via free-ranging wildlife at the periphery of farms), the role of wildlife—if any—in the infection cycle and resulting immunity for wildlife and domestic animals has not been widely studied. The potential role of

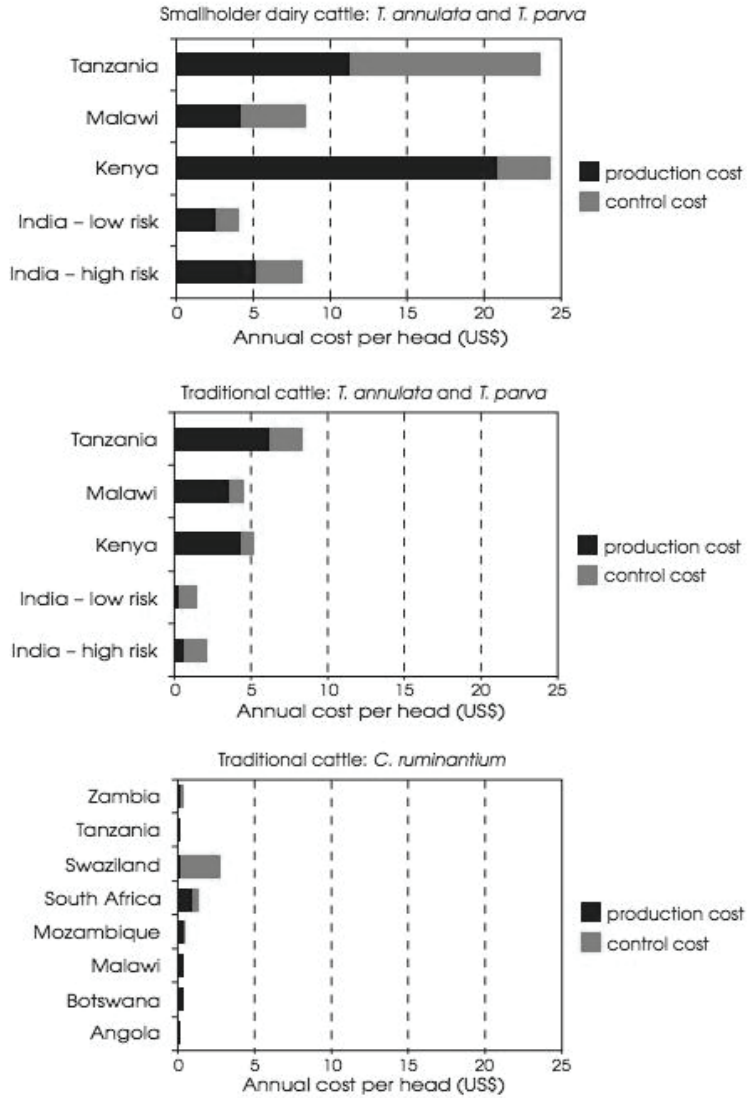

FIGURE A5-7 Annual costs per head of different tick-borne diseases in cattle systems. SOURCE: Minjauw and McLeod, 2003.

burden on mosquitos, domestic animals, wildlife, humans, and ecological factors (e.g., climate) in RVF show the complexity of some VBDs. Thus, an integrated approach is needed to better understand interspecies and other transmission dynamics.

A One Health approach that considers the links between humans, animals, and the environment can thus provide a more robust view around causes and possible solutions to VBDs such as RVF. A unique U.S. Department of Defense (DoD) DTRA-supported partnership between EcoHealth Alliance, the South African National Institute for Communicable Diseases, South African National Parks, the Free State Province Department of Economic Development, Tourism and Environmental Affairs, Republic of South African Department of Defence, University of Pretoria, and NASA/Universities Space Research Association has been established under a 5-year comprehensive study of RVF in South Africa. The project, which has a strong capacity-building component, will allow for a greater epidemiological understanding of RVF dynamics through four central aims: (1) Determine how immunity to RVFV changes over time in sheep; (2) determine the herd immunity in wildlife and domestic animals; (3) understand the ecology of the virus in the mosquito vector; and (4) determine the immunity level in people working on the study farms and detect new infections. By employing different vaccination scenarios in flocks to study herd immunity, studying prevalence of natural infection and epidemiological risk factors, investigating mosquito abundance and succession, and using climate, vegetation and soil data, we will gain a greater ability to better predict potential outbreaks in the future. Additionally, by enhancing knowledge on herd immunity at individual, population, and metapopulation levels, information obtained from this project will enable more targeted vaccination and mitigation methods for RVFV. This project will involve a 40,000 square km study site in the Free State and the Northern Cape province. The study will monitor humans, cattle, sheep, goats, and selected species of wildlife; assess the presence of RVFV throughout the life cycle of multiple mosquito species; and analyze mosquito blood meals to determine which species the vectors prefer. Additionally, the project will link patterns of human, animal, and mosquito occurrence with weather and vegetation cycles. This broad-based approach will hopefully provide a more comprehensive epidemiological understanding of RVFV as it pertains to wild and domestic animals, vectors, people, and the environment.

Conclusions

For the future, we can say with confidence that known VBDs will continue to be a significant disease burden for animals and people, and new VBDs will continue to be identified. We are even witnessing VBD affecting endangered species recovery programs; for example, *Yersinia pestis*, has been a major barrier to the recovery of black-footed ferret populations (Godbey et al., 2006). There is no indication that vector-borne disease is going to be eliminated in the near future. Current methods of VBD classification are highly dependent on organizational priorities with a strong focus on direct and indirect impacts to human health and a segregation of VBDs of animal importance.

The ability to discern patterns of VBD in animals hinges on consistent surveillance, prioritization, and integrative strategies. It would be an immense and inappropriate undertaking to attempt to eliminate ticks, mosquitoes, fleas, and midges in order to prevent VBDs. While vaccines present an opportunity at the individual animal or herd level, the associated cost-benefit relationships poses additional challenges. To better understand and control VBDs, we need more than molecular diagnostics and new or better vaccines. A fundamental quality of VBDs, their dependence on the ecology of vectors and hosts, points to the need for the earnest engagement of the ecological sciences. Skilled medical entomologists are critical for future work, and the number in this field are dwindling. There is an urgent need for ongoing support and training in medical entomology to meet emerging demands.

The possibility and opportunity for introduction of VBDs on a global scale cannot be ignored. As was seen with the emergence of WNV in South Dakota, the favorable ecological conditions for disease emergence cannot always be predicted. Controlling VBDs can be expensive and labor intensive. With the 10-year anniversary of One Health behind us, it is important to pull together thinking on human, animal, and plant vector-borne illness to find synergistic collaborative interventions to benefit health as a whole.

References

Bowman, D., S. E. Little, L. Lorentzen, J. Shields, M. P. Sullivan, and E. P. Carlin. 2009. Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: Results of a national clinic-based serologic survey. *Veterinary Parasitology* 160(1-2):138-148.

Dominguez, M., P. Hendrikx, S. Zientara, D. Calavas, M. Jay, A. Touratier, J. Languille, and A. Fediaevsky. 2012. Preliminary estimate of Schmallenberg virus infection impact in sheep flocks—France. *Veterinary Record* 171(17):426.

EFSA (European Food Safety Authority). 2014. Schmallenberg virus: State of art. *EFSA Journal* 12(5):3681.

Elbers, A. R., R. Meiswinkel, E. van Weezep, M. M. Sloet van Oldruitenborgh-Oosterbaan, and E. A. Kooi. 2013. Schmallenberg virus in *Culicoides* spp. biting midges, the Netherlands, 2011. *Emerging Infectious Diseases* 19(1):106-109.

Godbey, J. L., D. E. Biggins, and D. Garelle. 2006. Exposure of captive black-footed ferrets (*Mustela nigripes*) to plague (*Yersinia pestis*) and implications for species recovery. *US Geological Survey Scientific Investigations Report* 5293:233-237.

Hadler, J. L., D. Patel, K. Bradley, J. M. Hughes, C. Blackmore, P. Etkind, L. Kan, J. Getchell, J. Blumenstock, J. Engel, and Centers for Disease Control and Prevention. 2014. National capacity for surveillance, prevention, and control of West Nile virus and other arbovirus infections—United States, 2004 and 2012. *Morbidity and Mortality Weekly Report* 63(13):281-284.

Johnson, C.K., P. L. Hitchens, T. Smiley Evans, T. Goldstein, K. Thomas, A. Clements, D. O. Joly, N. D. Wolfe, P. Daszak, W. B. Karesh, and J. K. Mazet. 2015. Spillover and pandemic properties of zoonotic viruses with high host plasticity. *Scientific Reports* 5:14830.

Kilpatrick, A. M., and S. E. Randolph. 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* 380(9857):1946-1955.

Little, S. E., M. J. Beall, D. D. Bowman, R. Chandrashekar, and J. Stamaris. 2014. Canine infection with *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. in the United States, 2010-2012. *Parasites and Vectors* 7:257.

Loh, E. H., K. J. Olival, C. Zambrana-Torrelio, T. L. Bogich, C. K. Johnson, J. A. K. Mazet, and W. Karesh. 2015. Targeting transmission pathways for emerging zoonotic disease surveillance and control. *Vector-Borne and Zoonotic Diseases*. July 2015, 15(7): 432-437.

Meroc, E., N. De Regge, F. Riocreux, A. B. Caij, T. van den Berg, and Y. van der Stede. 2014. Distribution of Schmallenberg virus and seroprevalence in Belgian sheep and goats. *Transboundary Emerging Diseases* 61(5):425-431.

Metras, R., T. Porphyre, D. U. Pfeiffer, A. Kemp, P. N. Thompson, L. M. Collins, and R. G. White. 2012. Exploratory space-time analyses of Rift Valley fever in South Africa in 2008-2011. *PLoS Neglected Tropical Diseases* 6(8).

Minjauw, B., and A. McLeod. 2003. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine. University of Edinburgh, UK.

Murphy, F. A., E. P. J. Gibbs, M. C. Horzinek, and M. J. Studdert. 1999. *Veterinary virology*, 3rd ed. San Diego, CA: Academic Press.

NIAID (National Institute on Allergy and Infectious Diseases). 2014. Biodefense and emerging diseases. http://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Pages/CatA.aspx (accessed September 10, 2014).

OIE (World Organisation for Animal Health). 2014. OIE-listed diseases, infections and infestations in force 2014. http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2014 (accessed September 10, 2014).

Olival, K. J., P. R. Hosseini, C. Zambrana-Torrelio, T. L. Bogich, and P. Daszak (In Review). Host and viral traits predict zoonotic spillover from mammals. *Nature*.

PREDICT Consortium. 2014. *Reducing pandemic risk, promoting global health*. Davis, CA: One Health Institute, University of California, Davis.

Saegerman, C., L. Martinelle, F. Dal Pozzo, and N. Kirschvink. 2014. Preliminary survey on the impact of Schmallenberg virus on sheep flocks in South of Belgium. *Transboundary and Emerging Diseases* 61(5):469-472.

Sailleau, C., C. Boogaerts, A. Meyrueix, E. Laloy, E. Breard, C. Viarouge, A. Desprat, D. Vitour, V. Doceul, C. Boucher, S. Zientara, A. Nicolier, and D. Grandjean. 2013. Schmallenberg virus infection in dogs, France, 2012. *Emerging Infectious Diseases* 19(11):1896-1898.

Sedda, L., and D. J. Rogers. 2013. The influence of the wind in the Schmallenberg virus outbreak in Europe. *Scientific Reports* 3:3361.

USGS (U.S. Geological Survey). 2014. West Nile virus dynamic map viewer. http://diseasemaps.usgs.gov (accessed December 22, 2014).

Wernike, K., M. Eschbaumer, H. Schirrmeier, U. Blohm, A. Breithaupt, B. Hoffmann, and M. Beer. 2013a. Oral exposure, reinfection and cellular immunity to Schmallenberg virus in cattle. *Veterinary Microbiology* 165(1-2):155-159.

Wernike, K., B. Hoffmann, E. Breard, A. Botner, C. Ponsart, S. Zientara, L. Lohse, N. Pozzi, C. Viarouge, P. Sarradin, C. Leroux-Barc, M. Riou, E. Laloy, A. Breithaupt, and M. Beer. 2013b. Schmallenberg virus experimental infection of sheep. *Veterinary Microbiology* 166(3-4):461-466.

Wittmann, E. J., P. S. Mello, and M. Baylis. 2002. Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Medical and Veterinary Entomology* 16(2):147-156.

A6

DRIVERS, DYNAMICS, AND CONTROL OF EMERGING VECTOR-BORNE ZOONOTIC DISEASES[1]

*A. Marm **Kilpatrick**[2] and Sarah E. Randolph*[3]

Summary

Emerging vector-borne diseases are an important issue in global health. Many vector-borne pathogens have appeared in new regions in the past two decades, while many endemic diseases have increased in incidence. Although introductions and emergence of endemic pathogens are often considered to be distinct processes, many endemic pathogens are actually spreading at a local scale coincident with habitat change. We draw attention to key differences between dynamics and disease burden that result from increased pathogen transmission after habitat change and after introduction into new regions. Local emergence is commonly driven by changes in human factors as much as by enhanced enzootic cycles, whereas pathogen invasion results from anthropogenic trade and travel where and when conditions (eg, hosts, vectors, and climate) are suitable for a pathogen. Once a pathogen is established, ecological factors related to vector characteristics can shape the evolutionary selective pressure and result in increased use of people as transmission hosts. We describe challenges inherent in the control of vector-borne zoonotic diseases and some emerging non-traditional strategies that could be effective in the long term.

Introduction

In the past three decades, many vector-borne pathogens (VBPs) have emerged, creating new challenges for public health (Weaver and Reisen, 2010). Some are exotic pathogens that have been introduced into new regions, and others are endemic species that have greatly increased in incidence or have started to infect local human populations for the first time. Here, we review the drivers of these processes. Of particular interest are zoonoses that are maintained by transmission in wildlife but also affect people who have been bitten by infected vectors. Additionally, we draw from lessons learned from diseases that now use only people as transmission hosts, such as malaria and dengue.

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BOX A6-1
Key Messages

- Many vector-borne zoonotic diseases have emerged in the past three decades.
- Emergence in new regions is caused primarily by pathogen movement due to trade and travel, whereas local emergence is driven by a combination of environmental changes that affect vectors and wildlife hosts and social changes (eg, poverty and conflict) that affect human exposure to vectors.
- Pathogens introduced into novel regions often cause explosive epidemics followed by declining incidence, whereas pathogens that emerge locally because of land use or social changes usually show consistent increases.
- Vector-borne diseases are highly sensitive to climate, but the past and future effects of climate change on vector-borne disease will probably be less than will those of changes in land use and social factors.
- Land use and increasing human populations exert selective pressure on vector-borne pathogens to be able to infect and be transmitted by people and vectors associated with human development.
- Control of vector-borne zoonotic diseases needs combined efforts by clinicians and public health officials to treat patients and promote behaviour likely to minimise risk of infection, and by disease ecologists, urban planners, and medical entomologists to advise on development, restoration of ecological communities, and vector control to reverse the ecological drivers of transmission.

Clinicians have an important role alongside disease ecologists and epidemiologists in the study of the causes of an outbreak and minimisation of the burden of disease, because the effectiveness of control is improved by rapid identification (Lloyd-Smith et al., 2003; Ferguson et al., 2006). In many cases, clinicians are on the first line of detection of these epidemics because clusters of patients present with novel sets of symptoms; evidence of new outbreaks then has to be passed to public health agencies for appropriate management. New high-throughput technologies for detection and identification of novel genetic material in samples taken from patients can greatly aid this process (Gaynor et al., 2007; Lipkin, 2010). Additionally, data obtained via mobile phones and online social networks checked against expert assessment of plausibility offer the potential to detect changes in spatial and temporal patterns of illness in real time so that new epidemics can be detected early (Brownstein et al., 2009).

West Nile virus and chikungunya virus are among the best understood zoonotic VBPs to have emerged in the past two decades and show just how explosive epidemics can be in new regions (see Figure A6-1). In 1999, the New York City Department of Health (NY, USA) reported a cluster of patients with meningoencephalitis associated with muscle weakness; epidemiological evidence suggested that an arbovirus (ie, a virus transmitted by arthropod vectors) was a probable

cause (Nash et al., 2001). Clinicians and veterinarians collaborated to identify the aetiological agent as West Nile virus, but unfortunately identification and initial control efforts did not prevent the virus spreading from the east to the West Coast of North America within 4 years (CDC, 2012a; Kilpatrick, 2011), causing national epidemics in 2002 and 2003.

Similarly, on the Indian Ocean island of Réunion in 2005, hundreds of patients had painful and disabling polyarthralgia, and a subset presented with neurological signs or fulminant hepatitis (Pialoux et al., 2007). A second wave of such symptoms in 2006 exceeded all expectations, eventually affecting more than a third of the entire population of 770,000 people (Pialoux et al., 2007). The causative agent was identified as chikungunya virus, which is also causing continuing epidemics in India, with several million cases so far (Pialoux et al., 2007; Yergolkar et al., 2006; Schuffenecker et al., 2006). Other introductions of VBPs have caused smaller outbreaks but have been important in the expansion of the range of human populations at risk. For example, dengue virus has spread to Hawaii (Effler et al., 2005), Zika virus to the Micronesian island of Yap (Duffy et al., 2009), and chikungunya virus to Europe (Rezza et al., 2007). Whether the outbreak of chikungunya in Europe died out naturally because of the arrival of the temperate autumn or was interrupted by mosquito control efforts is unclear.

These past experiences—together with increases in the known drivers of pathogen introduction—suggest that future introductions are likely (see Table A6-1). A key challenge arises from the non-specificity and similarity of symptoms caused by many of these viruses, especially Zika virus, dengue, and chikungunya virus that all present with acute fever similar to many diseases endemic in the tropics, such as malaria (San Martin et al., 2010; Duffy et al., 2009). This difficulty makes rapid identification methods (Gerstl et al., 2010) and high-quality laboratory-based diagnoses necessary for accurate surveillance and appropriate treatment. Recent advances in identification of unknown pathogens with deep sequencing and microarrays should enable rapid identification of novel or introduced pathogens (Yozwiak et al., 2012). A key need is to develop diagnostics for point-of-care use for infection and exposure to allow for proper assessments of case fatality ratios and disease burden.

The emergence of endemic VBPs is usually thought to be a qualitatively different process from the arrival of exotic ones, but in some cases increases in incidence of endemic VBPs result more from spread into new areas than increases in local transmission. A combination of local spread and an increase in transmission potential in situ is also possible. Lyme disease is perhaps the best understood example of a mixed emergence. Reported cases (and estimated illnesses) have roughly tripled since 1990 in the USA (see Figure A6-1), appeared increasingly in Canada (Ogden et al., 2009), and apparently increased by between two and ten times in various parts of Europe where diagnosis and reporting are more variable. Evidence for the importance of local invasion in the USA comes from counties in the states of Wisconsin and Virginia, where Lyme cases have

TABLE A6-1 Important Pathogen Threats for Introduction into New Regions and Range Extensions within Endemic Regions, and Probable Sources and Pathways for Introduction

	Regions at Risk	Endemic Region	Pathways for Introduction*
Japanese encephalitis virus	Americas	Asia	Infected livestock
Rift Valley fever virus	Americas, southern Europe	Africa, Asia	Infected livestock
Venezuelan equine encephalitis virus	Europe, Asia, Africa	Americas	Infected livestock
Chikungunya virus	Europe, Americas, Australia	Africa, Asia	Infected people
Mayaro virus	Africa, Asia, Europe	South America	Infected people
Zika virus	Europe, Americas	Africa, Asia	Infected people
Crimean-Congo haemorrhagic fever	North Africa, east Asia, central and western Europe	Africa, Asia, Europe	Infected livestock
Dengue virus	Southern Europe	Southern hemisphere	Infected people
West Nile virus	Central Europe, Turkey	Africa, Asia, Europe, Australia	Migratory or dispersing birds
Sindbis virus	Northern Europe	Africa, Asia, Australia	Migratory or dispersing birds

* Infected mosquitoes transported via aeroplanes are a potential pathway for all these pathogens (except Crimean-Congo haemorrhagic fever which is tick borne) in addition to pathways listed.
SOURCE: Adapted from Kilpatrick et al., 2006a.

only been reported in the past decade and few if any cases occurred previously (CDC, 2012). By contrast, in the state of Connecticut—where the first cases of Lyme were detected 30 years ago—incidence of the disease has hardly risen in the past decade (CDC, 2012b).

In Europe and Eurasia, the substantial rise in cases of Lyme disease and other tick-borne diseases, including babesiosis, ehrlichiosis, and rickettsiosis, and tick-borne encephalitis, is due as much or more to upsurges within pre-existing ranges of the vector ticks (principally *Ixodes ricinus* and *Ixodes persulcatus*) as to the establishment of enzootic cycles in new places. Zoonotic VBPs with other types of vectors also represent an important and growing threat in some places, such as those that cause Chagas disease, plague, and leishmaniasis (Hotez et al., 2008). Strong evidence suggests that ecological and human factors have had important roles in establishment of the differential patterns of increased incidence of all these diseases, while increasing awareness and testing by clinicians has contributed to improved reporting of cases.

Differences in the drivers of emergence of exotic and endemic VBPs have important implications for their subsequent dynamics, where they will emerge,

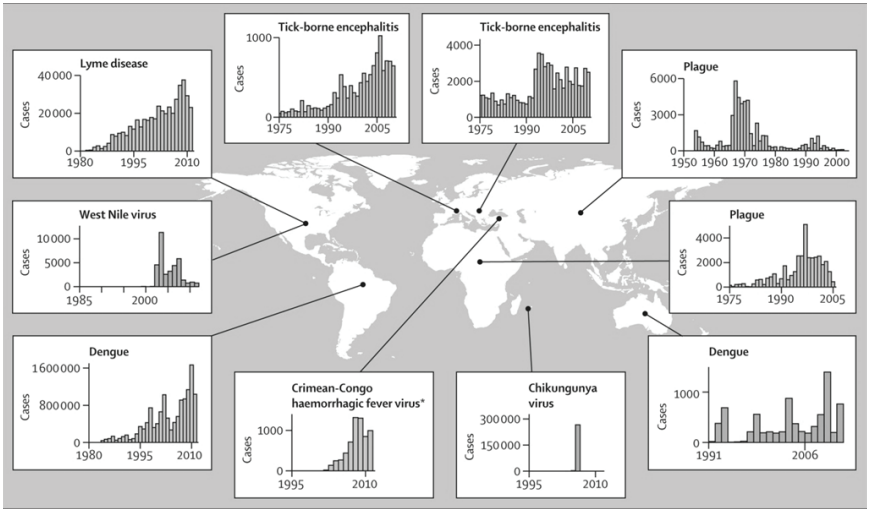

FIGURE A6-1 Temporal patterns of reported cases for selected introduced vector-borne pathogens (red) and endemic or long-established diseases (blue). Introduced pathogens can cause notable epidemics followed by a decreased incidence (eg, West Nile virus in the USA [CDC, 2012] and chikungunya virus in Réunion [Effler et al., 2005]), or sporadic epidemics from repeated introductions and local transmission (dengue in Australia [Duffy et al., 2009]). The incidence of some endemic or long-established zoonotic vector-borne diseases has increased greatly in the past several decades (Lyme disease in North America [Rezza et al., 2007], plague in Africa [Kilpatrick et al., 2006b], and dengue in South America [San Martin et al., 2010]), but could show different trajectories (plague in Africa *vs* plague in Asia [Stenseth et al., 2008]), even in neighbouring regions (tick-borne encephalitis in eastern [ex-communist] and western [historically free market] Europe) because of socioeconomic differences.
* Crimean-Congo haemorrhagic fever virus is shown as endemic to Turkey because there is evidence of its presence there many years before its appearance in people.

and the efforts that can be made to control or eliminate them. We consider each of these aspects in turn, illustrated by some of the more notable examples across the globe. We argue that viewing emerging endemic pathogens as invading at a local scale can be used to take a prospective approach to prevention and control.

Emergence of Exotic Versus Endemic Pathogens

Arrival of Exotic Pathogens

The main driver of pathogen introductions in the past five decades—the accelerating increase in trade and travel—is well known. What is less discussed

is that four centuries of trade and travel set the stage for many present pathogen introductions. In the 17th to 19th centuries, shipping traffic resulted in the transport of larvae of several important mosquito species, such as *Aedes aegypti* (a vector of dengue, yellow fever, chikungunya virus, and others), *Culex pipiens* (a vector of West Nile virus) and *Culex quinquefasciatus* (a vector of West Nile virus and filariasis) (Fonseca et al., 2006; Bryant et al., 2007; Farajollahi et al., 2011).

Some pathogens (eg, *Plasmodium vivax*) were introduced to new continents and became established coincident with or shortly after these early vector introductions because they cause chronic infections in people that are still infectious after weeks or months of travel (Mendis et al., 2001). Other pathogens that have only short periods of infectiousness in people, including yellow fever virus and dengue virus, could also reach distant regions centuries ago because pathogen transmission cycles could occur aboard ships in which vectors were present and could reproduce (Farajollahi et al., 2011).

The growth in air travel enabling global transit in a single day (see Figure A6-2) has accelerated introductions because it has allowed many pathogens that cause acute infectiousness (eg, chikungunya and West Nile viruses) to reach other continents within the few days that hosts are infectious, and even during the latent period for some diseases (Kilpatrick et al., 2006a). Several of

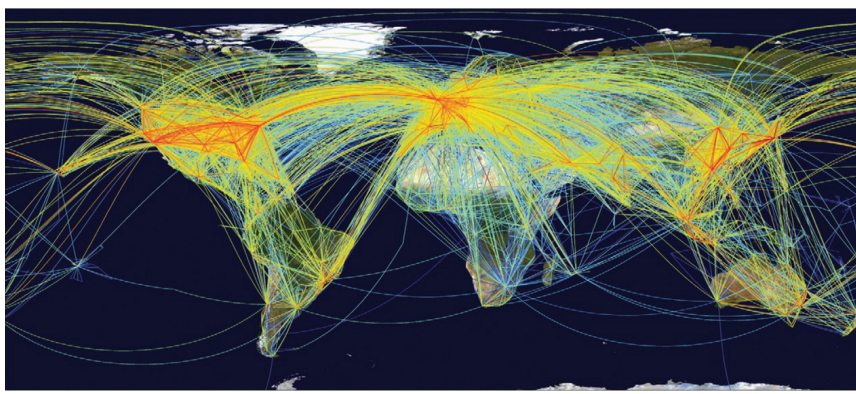

FIGURE A6-2 The global aviation network. Lines show direct links between airports, and the colour indicates passenger capacity in people per day (thousands [red]; hundreds [yellow]; tens [blue]). Routes linking regions at similar latitudes (in the northern or southern hemisphere) represent pathways that pathogens can move along to reach novel regions. Notably, air traffic to most places in Africa, regions of South America, and parts of central Asia is low. If travel increases in these regions, additional introductions of vector-borne pathogens are probable.
SOURCE: Adapted from Hufnagal et al., 2004.

these pathogens were also aided by the 20th century introductions of another key vector, *Aedes albopictus* (Reiter, 2010; Tatem et al., 2006). Thus, the most recent wave of pathogen introductions, and those likely to occur in the near future, take place against the backdrop of centuries of vector introductions that enable establishment.

A key result of an already well established vector population and a highly suitable environment (including hosts and climate) is that many introduced pathogens cause explosive epidemics in which a large fraction of the population at risk is infected in the first few years after introduction (see Figure A6-1). High vector populations (relative to host abundance) result in a high basic reproduction number (R_0) of the pathogen, and if the host population is immunologically naive to the introduced pathogen, as is usually the case, then the effective pathogen reproduction number (R_{eff}) is close to the maximum R_0. This high R_{eff} leads to another common pattern, which is that the intense and rapid initial spread of a novel pathogen is frequently followed by a substantial decrease in case burden shortly after introduction, especially on a local scale, as the fraction of the population that is immune to infection rises (Kilpatrick, 2011). This pattern both contrasts with, and has similarities to, the emergence of endemic diseases.

Emergence of Endemic Pathogens

Emergence of endemic VBPs is frequently associated with changes in land use (Lambin et al., 2010) or socioeconomic conditions (Randolph, 2010), and these transitions control the dynamics of disease emergence. For pathogens affected by land-use change, the rise in case numbers is often gradual (see Figure A6-1), paralleling changes in the pathogen's abiotic and biotic environment. By contrast, the increased incidence of endemic disease driven by changes in socioeconomic conditions can be abrupt if the shift is rapid, such as that caused by political upheavals, military conflicts, or natural disasters (see Figure A6-1).

Changes in land use affect VBPs by altering the interactions and abundance of wildlife and domestic hosts, vectors, and people, with some diseases better understood than are others (Lambin et al., 2010). In the Amazon and east Africa, deforestation increases standing water and sunlight and enhances the breeding success of some mosquito species, which can increase risk of malaria. Further increases in urbanisation frequently eliminate anopheline mosquito habitat and have reduced malaria elsewhere (Yasuoka and Levins, 2007). In northeastern North America, reforestation during the 20th century is thought to have allowed recolonisation by deer and the consequent expansion of the range of ticks (*Ixodes scapularis*), underpinning the emergence of Lyme disease in the mid-20th century (Barbour and Fish, 1993). Deer (*Odocoileus virginianus* in the USA and *Capreolus* in Europe) have a key role in feeding adult *Ixodes* ticks, although they are actually incompetent hosts for the Lyme disease bacterial spirochaetes. Additionally, in the past three decades, fragmentation of forests in eastern regions

of Canada and the USA and changes in predator communities (Levi et al., 2012) have altered the host community for ticks and the Lyme bacterium *Borrelia burgdorferi*, and might have increased relative abundance of small mammals (white-footed mice [*Peromyscus leucopus*], eastern chipmunks [*Tamias striatus*], and shrews [*Sorex* spp and *Blarina brevicauda*]) that are the principal transmission hosts for Lyme disease spirochaetes. These changes in the host community can result in increased spirochaete infection prevalence in nymphal ticks (Logiudice et al., 2008). A key remaining question is how fragmentation and hunting-induced changes in the host community affect the abundance of infected nymphal ticks, which is the key metric for disease risk.

Changes in land use might also be responsible for recent emergent foci of Crimean-Congo haemorrhagic fever virus within its large range through parts of Africa, Asia, southeastern Europe, and the Middle East. By contrast with typical sporadic outbreaks of only a few cases, an exceptional epidemic occurred in Turkey, starting with about 20 cases in 2002, and rising to nearly 1,400 cases by 2008 (see Figure A6-1). Most infections occurred in agricultural and animal husbandry workers via tick bites and direct contamination from infected animals. Changes in land cover associated with political unrest and reduced agricultural activities might have allowed colonisation by wildlife and subsequent tick population growth, as is thought to have precipitated the first recorded epidemic of Crimean-Congo haemorrhagic fever in Crimea in 1944–45 (Hoogstraal, 1979). The case fatality rate (5 percent) in Turkey has been much lower than is usually observed (20–30 percent) (Hoogstraal, 1979; ErgÖnül, 2006), creating some uncertainty about the cause of this epidemic. This uncertainty emphasises the need for accurate and systematic diagnosis through effective point-of-care methods.

Increases in incidence can also result from changes in socioeconomic and human activities, such as expansion into risky new habitats for exploitation or dwelling, or land-cover change, such as reforestation of previously agricultural areas (Barbour and Fish, 1993; Chaves et al., 2008; Barrett and Higgs, 2007; Hay et al., 2005). Human infection with VBPs increases with the product of entomological risk (the abundance of infected vectors) and exposure of people to vectors, which can change independently and sometimes synergistically. For example, the incidence of dengue is higher on the Mexican side of the Mexico–Texas border than on the other (Reiter et al., 2003), where open windows compensate for the absence of air-conditioning but expose people to mosquito biting.

Exposure to ticks, paradoxically, might be higher in people of high and low income than in those with intermediate income (see Figure A6-3). Incidence of Lyme disease in parts of Europe has been shown to be higher in people with high income living in new homes in broad-leaf woodlands where wildlife co-occur, including rodents and birds that serve as reservoirs for spirochaetes and ticks (Linard et al., 2007). Generally, outdoor recreational opportunities associated with wealth can result in increased exposure to vectors. Conversely, hardship precipitated by population displacements due to civil conflict, loss of protective

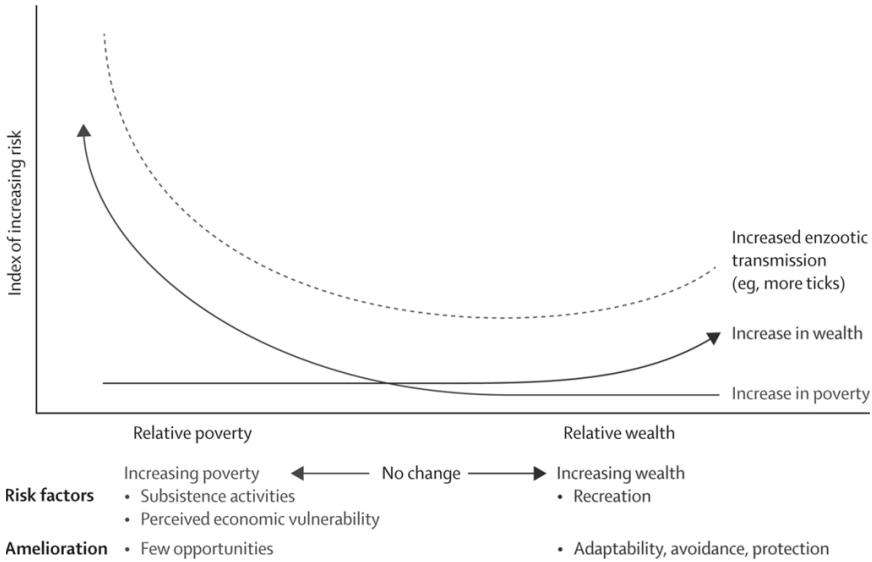

FIGURE A6-3 Interactions between economic status and disease risk. Interactions are particularly applicable where contact with infectious agents is largely due to human activities outdoors, such as tick-borne diseases. Human activities take place against a backdrop of variable inherent risk from zoonotic vector-borne pathogens, which is measured as the density of host-seeking infected vectors such that the overall risk curve can rise or fall.

housing through natural disasters, or use of natural environmental resources driven by economic transitions can lead to increased contact between people and vectors (Randolph, 2010; Beyrer et al., 2007). A clear example comes from a large upsurge of tick-borne encephalitis in 2009, immediately after the economic downturn in three eastern European countries that already had high background poverty and where foods are harvested from forests for subsistence and commerce (Godfrey and Randolph, 2011). Human activities resulting in exposure to VBPs is sometimes reflected in different seasonal patterns, such as cases of tick-borne encephalitis in different parts of Europe (see Figure A6-4). In eastern Europe, the timing of cases matches the season of forest food harvest more closely than the seasonal pattern of tick abundance, while in western Europe the earlier peak of cases coincides with summer recreational activity.

Poverty and wealth, however, probably affect final disease outcomes asymmetrically, because economic duress restricts the potential for ameliorative actions (eg, limiting of outdoor activities, protection from vector bites, or costly vaccination in the case of tick-borne encephalitis). This hypothesis could partly explain the difference between a large upsurge (two to 30 times) in reported tick-borne encephalitis cases in the early 1990s in central and eastern Europe

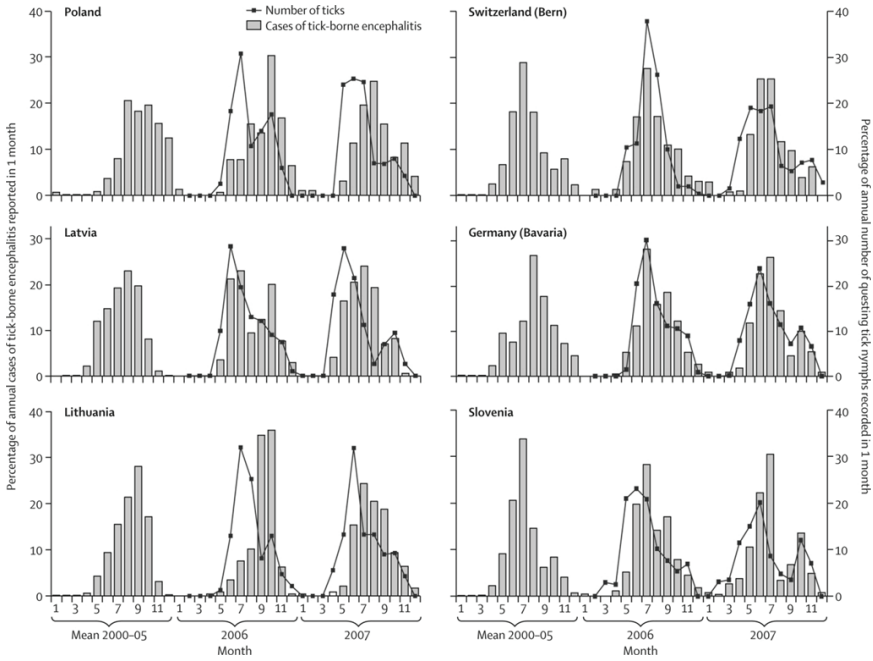

FIGURE A6-4 Seasonal patterns of tick-borne encephalitis cases and abundance of questing nymphal ticks (*Ixodes ricinus*). The data for ticks are lagged by 1 month to account for the interval between a tick bite and diagnosis of tick-borne encephalitis.

after the fall of Soviet rule and a gradual and steady increase in western Europe (Figure A6-1) (Randolph, 2010). Political and civil unrest that commonly occur with armed conflict could also account for the sudden re-emergence of plague in Vietnam in the late 1960s and in Madagascar and Mozambique at the end of the 1980s (Duplantier et al., 2005). Failure of public health services and overcrowded, unsanitary living conditions increased human contact with flea-bearing rodents and decreased routine surveillance, allowing rapid emergence with no warning. These examples of social strife enabling new epidemics of vector-borne diseases will probably recur, and awareness and investment in public health infrastructure can help to reduce their effect.

Understanding of the mechanistic processes linking land use and socioeconomic conditions with disease enables prediction of future trends and control or mitigation. Economic and public health assistance could be targeted towards populations at high disease risk because of social strife caused by conflict or natural disasters, and urban planning could be used to minimise the use of risky habitat by people for living and recreation. Unfortunately, although correlations exist between land use and disease incidence or measures of risk, rigorous and

mechanistic analyses that identify causal factors that are needed for intelligent urban planning are absent in most cases. For example, in the USA, specific types of land use (agriculture and urbanisation) are associated with a higher incidence of West Nile virus in people at the county scale, but the mechanism underlying this pattern is unknown (Kilpatrick, 2011; Bowden et al., 2011). This gap in our knowledge makes it difficult to anticipate and avoid future epidemics associated with rapid urbanisation and land-use change.

Climate Change and Vector-Borne Diseases

Although several components of vector-borne disease systems (principally the vector and the pathogen) are highly sensitive to climate, evidence shows that climate change has been less important in the recent emergence of vector-borne

BOX A6-2
Climate Change and Vector-Borne Disease

It is now well established in the scientific community that climate change has played and will play a mixed and minor part in the emergence of most vector-borne pathogens (VBPs) and diseases generally (Rogers and Randolph, 2006; Lafferty, 2009). Nonetheless, a persistent stream of reviews are published that claim that climate change is a primary driving force. These reviews stem from two semi-independent assumptions that have developed in the past decade: first, that climate change will lead to more widespread and more abundant VBPs as more of the planet starts to closely resemble the tropics where VBPs are presently most abundant; and second, that the arrival of exotic and upsurges of endemic VBPs are due to climate changes. Both these assumptions originate from plausible arguments, because the natural distribution and intensity of VBPs are indeed highly sensitive to climate (Russell et al., 2009). They were partly inspired by repeated publications of highly influential and visually arresting maps at the end of the 20th century that presented predictions of expanding malaria derived from mathematical models. Problematically, these models were not parameterised with data for key variables (eg, vector abundance) (Martens et al., 1995). The belief that warming will intensify VBPs is reinforced by speculative reports that describe the general coincidence of increased disease incidence with warming in recent decades (Gould and Higgs et al., 2009; Gray et al., 2009). Spatiotemporal analyses of variation in long trends suggest that in many cases climate has not consistently changed in the right way, at the right time, and in the right places to account for the recorded epidemiology of emergent VBPs (Šumilo et al., 2007).

The effects of climate on transmission are several, non-linear, and act in opposing directions. Thus, prediction of the overall effect of climate and climate change on vector-borne disease systems needs a complete understanding and parameterisation of VBP models (Reiter, 2008; Rogers and Randolph, 2006). Specifically, higher temperatures increase three aspects of transmission for vector-borne pathogens: vector biting rate, vector development rate, and

diseases than have changes in land use, animal host communities, human living conditions, and societal factors, probably because of countering influences of climate (see Box A6-2). An exception seems to be the increased transmission of VBPs with warming along the cold latitudinal and altitudinal edges of their present distribution. The differential effect of climate at the edge and core of a pathogen's distribution stems partly from the non-linear relation between the fraction of the population exposed in an epidemic and transmission potential (which can be quantified as R_0). Specifically, initial increases in R_0 to more than one (ie, allowing pathogen spread to create an epidemic) lead to large rises in case burden, but further increases in R_0 have diminishing effects, especially for pathogens with sterilising immunity. Expansions in the distribution of a disease might have disproportionate effects on public health if the newly exposed populations have little immunity. Examples of VBP range expansions along cold edges are dengue virus in

pathogen replication (thereby reducing the extrinsic incubation period or the time between a vector feeding on an infected host and being able to transmit the pathogen). However, they frequently decrease a fourth, vector survival, especially when associated with moisture stress. As a result, increased temperatures might lead to increases or decreases in transmission depending on the relative effects of these factors (Rogers and Randolph, 2006). A key challenge is that biological models frequently have difficulty accurately predicting changes in vector abundance, which is the most variable factor in the transmission potential of VBPs.

The best science clearly suggests that effects of climate change on VBPs will be variable, as would be expected from all such complex systems (Rohr et al., 2011). Thus, although continuing climate change could increase transmission or distributions of some VBPs in the future, for most diseases other factors will be more important and, crucially, be manageable with public health initiatives (e.g., drug treatment, vaccines, and bed nets). These factors include changes in the biotic elements of the environment (e.g., wildlife hosts), drug resistance, reduced health service provision, and political and socioeconomic factors that change human exposure and susceptibility to infections.

Governments and public health agencies want predictions of the disease burden and risk in the future. To obtain such predictions, a robust understanding of how all aspects of climate affect rates of the processes involved in transmission needs to be developed (Rogers and Randolph, 2006), and the breadth of analyses should be expanded to include all potential factors affecting incidence of infection and prevalence of disease, both biological and non-biological. Predictions will necessitate truly cross-disciplinary collaborations, marrying biologists' pursuit of improved models of vector abundance, infection prevalence, and pathogen evolution (e.g., drug resistance) with understanding from medical and social scientists about developments in treatment and interventions, land-use change, and human societal factors. Such cooperation would further our knowledge, which is presently based on assumptions about what global warming will do, to a more evidence-based set of predictions.

Texas, USA (Brunkard et al., 2007), Lyme disease in Canada (Ogden et al., 2009), and tick-borne encephalitis at increasing altitude in Slovakia (Lukan et al., 2010).

In core transmission areas, not only are the effects of climate change less important than other factors, but warming might even decrease transmission if decreases in vector survival overwhelm other factors (Randolph and Rogers, 2000) (see Box A6-2). An analysis of several decades of severe malaria incidence (the best studied disease with respect to climate change) at five locations spanning a range of elevations in western Kenya identified initial rises in incidence followed by two decades of decreases at two locations and increases with high variability in three others (Chaves et al., 2012). These mixed patterns challenge expectations that continuing climate change will lead to increased malaria and suggest that changes in transmission potential of malaria and other VBPs are primarily driven instead by a mix of factors such as demographic shifts, land-use change, interventions (eg, bed nets), drug resistance, and climate. The relative contributions of each factor can be rigorously assessed only by careful comparisons of the same pathogen over time and with valid accurate baseline data, which were lacking in a previous study (Gething et al., 2010).

Evolution of Vector-Borne Pathogens

One underappreciated aspect of growing human populations, global land-use change, and the introduction of human commensal vectors is the selective pressure exerted on pathogens, causing them to evolve to take advantage of new environments, including hosts and vectors. Both West Nile virus and chikungunya evolved rapidly (a feature typical of viruses and especially RNA viruses) (Holmes, 2003) after being introduced to new locations and encountering new anthropophilic vectors. The original genotype of West Nile virus (NY99) was replaced by another (WN02) (Davis et al., 2005) that differs by three consensus nucleotide changes and exhibits increased transmission efficiency in *C pipiens* and *Culex tarsalis* mosquitoes (Moudy et al., 2007; Kilpatrick et al., 2008). Similarly, on Réunion between 2005 and 2006, one nucleotide change occurred in chikungunya virus that increased infection in the recently introduced mosquito species *Aedes albopictus* (Tsetsarkin and Weaver, 2011). The same genetic change appeared independently in viruses isolated from Réunion, west Africa, and Italy, but was not identified in mosquitoes from India at the start of the continuing epidemics there in 2006 (de Lambellerie et al., 2008). When *A albopictus* rather than *A aegypti* became the main vector in India from 2007, however, the same genetic substitution spread rapidly and subsequent substitutions seem to be enabling even more efficient virus circulation and persistence, which could presage further expansion of the chikungunya virus (de Lambellerie et al., 2008).

More generally, the transmission of many VBPs is less efficient when the vector feeds on several hosts, only some of which can be infected by the pathogen (Kilpatrick et al., 2007). It is no coincidence that the dominant human VBPs

malaria and dengue are transmitted most intensely where they are vectored by mosquitoes that feed almost entirely on people. What has been less appreciated is the selective pressure imposed on zoonotic pathogens (especially those for which people are still a dead-end host) to adapt to be efficiently transmitted by human specialist vectors like *Anopheles gambiae, A aegypti*, and, to a slightly lesser extent, *A albopictus* (which sometimes feeds on non-human mammals and birds) where people are highly abundant. As the abundance of human commensal vectors increases with urbanisation and deforestation, so do the opportunities for strictly human transmission of pathogens.

Control of VBPs

Novel introductions and increases in incidence of endemic VBPs draw attention to the need for effective control and treatment of individuals with associated diseases. A key challenge in the attempt to control many VBPs is that they are zoonotic and transmission intensity in vectors is driven primarily by wildlife reservoirs. As a result, the dominant method used to control directly transmitted pathogens—vaccines—protects only individuals with financial and logistical access and has no effect on underlying transmission intensity. Thus, natural or vaccine-acquired herd immunity has no protective effect in people, and exposure is governed primarily by contact with vectors.

Control of zoonoses in wildlife is difficult at best, and eradication is often impossible (Barrett and Higgs, 2007). Vaccines for wildlife hosts—in development for West Nile virus (Kilpatrick et al., 2010) and field tested at a small scale for Lyme borreliosis (Tsao et al., 2004)—offer some reasons for optimism, but substantial work remains before they can be deployed as effective instruments on a large scale. Additionally, for vector-borne pathogens, transmission is thought to be frequency dependent, such that culling of livestock or wildlife that decreases host abundance (short of eradication) might increase transmission. Vectors are likely to seek out, feed on, and infect the hosts that remain after culling efforts, and the remaining hosts will subsequently be fed on by a greater number of susceptible vectors per host than they were before culling (Wonham et al., 2004). Control of frequency-dependent pathogens by culling would thus be expected to result in short but intensified epizootics that could lead to additional human infections, with the exact public burden depending in part on patterns of vector feeding on people and other hosts (Kilpatrick et al., 2006c, 2007).

Another control strategy used for VBPs, active or passive use of animals to divert vector feeding away from people to protect them against infection (so-called zooprophylaxis) (Hess and Hayes, 1970), has had mixed effects. Feeding on additional alternative hosts sometimes results in increased vector densities, which could result in higher transmission even if a smaller proportion feed on people (Yamamoto et al., 2009; Cohen and Gurtler, 2001). A more recent incarnation of this basic idea—termed the dilution effect—postulates that naturally

occurring biodiversity could, in some instances, also divert vectors from infectious hosts (Ostfeld and Keesing, 2000). As with empirical attempts of zooprophylaxis, the effects of biodiversity, or, more accurately, variable host community assemblages, are not uniform with respect to risk of infection, because of the complexity of interactions between hosts, vectors, and pathogens (Randolph and Dobson, 2012; Kilpatrick et al., 2006b). The more direct strategy of vector control targeted at larval mosquitoes (including elimination of larval habitat) has been more effective than has zooprophylaxis and has even resulted in local eradication of a disease (Killeen, 2003). Additionally, new techniques to develop vectors resistant to pathogens by infecting them with naturally occurring intracellular insect parasites (eg, *Wolbachia*) offer some promise (Hoffman et al., 2011).

In many cases, the most effective long-term public health strategies will combine efforts by clinicians and public health officials to treat and alter the behaviour of patients to avoid infection with actions by others to reverse the ecological drivers of transmission. Behavioural change is especially important at the leading edge of invading endemic or exotic pathogens where personal protective behaviours are often absent. Reversal of ecological drivers of disease emergence necessitates identification of the causes of increases in incidence and subsequent targeting with appropriate control measures, which needs integration between researchers, public health agencies, the government, and the public. For example, risk related to specific types of land use could be ameliorated by urban planning and management of host and vector communities through landscaping, hunting, or restoration of ecological communities.

Similarly, increases in incidence related to socioeconomic changes could be reduced with prudent development and assistance after disasters and social upheaval (Bogich et al., 2012). The vaccination campaign against tick-borne encephalitis, for example, targeted children in Latvia in response to the massive upsurge in incidence in the early 1990s. This campaign, together with a reduction in high-risk activities in tick-infested forests (presumably as a result of enhanced awareness), effectively reduced the mean national incidence by 74 percent by 1999, with the greatest reductions in counties where incidence was previously highest (Šumilo et al., 2008). Even modest changes in societal structure and socioeconomic development can increase exposure to zoonoses; an awareness of changing risk would allow communication of appropriate warnings to alert unsuspecting members of the public. Prevention of the introduction of foreign pathogens is far more difficult than is control of endemic VBPs because it is an inevitable result of the globalisation of trade and travel. History suggests that successful control needs prompt identification, swift action, and occasionally draconian social measures.

Conclusions

VBPs impose an important global burden on public health, including widespread human diseases that were formerly zoonotic, such as malaria and dengue, as well as zoonotic diseases for which people are dead-end hosts, such as Lyme disease, West Nile virus, and Crimean-Congo haemorrhagic fever. Widespread land-use change, globalisation of trade and travel, and social upheaval are driving the emergence of zoonotic VBPs, including along local invasion fronts. Recognition that a large fraction of the public health burden of both endemic and exotic VBPs comes from infection at the invading front would enable prospective action to address the ecological and sociological drivers of transmission. Financial and technological hurdles persist in developing countries, making diagnosis and control difficult where the diseases are stubbornly most prevalent. Inadequate knowledge prevents populations in developed countries from taking actions that would minimise the diseases' effects. Development projects that address disease can help to overcome these challenges, and clinicians and public health professionals can play important parts in the reduction of the burden of vector-borne disease.

Search Strategy and Selection Criteria

We searched PubMed and ISI Web of Knowledge with the terms "emerging infection,*" "vector-borne diseas.*" "zoonos*" or names of specific vector-borne infections, in combination with "control," "exotic," "climate change," "socioeconom,*" "land use," or "evolution" for reports published in any language before July, 2012. Searches were done at all stages, from the initial drafting of the paper to submission of the revised and final version. We also relied on our own familiarity with the scientific literature. We largely selected reports from the past 6 years, but did not exclude older publications that were informative and useful. We also searched the reference lists of reports identified by our searches and selected those that we judged to be relevant. Reviews and book chapters are cited to provide readers with comprehensive sources of references, but primary research is also included where possible within the space allowed. Our reference list was modified on the basis of comments from peer reviewers.

Contributors

AMK and SER conceived the ideas and wrote the report.

Conflicts of Interest

We declare that we have no conflicts of interest.

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References

Barbour, A.G., and D. Fish. 1993. The biological and social phenomenon of Lyme disease. *Science* 260: 1610–16.

Barrett, A.D.T., and S. Higgs. 2007. Yellow fever: a disease that has yet to be conquered. *Annual Review of Entomology* 52: 209–29.

Beyrer, C., J.C. Villar, V. Suwanvanichkij, S. Singh, S.D. Baral, and E.J. Mills. 2007. Neglected diseases, civil conflicts, and the right to health. *Lancet* 370: 619–27.

Bogich, T.L., R. Chunara, D. Scales, et al. 2012. Preventing pandemics via international development: a systems approach. *PLoS Medicine* 9: e1001354.

Bowden, S.E., K. Magori, and J.M. Drake. 2011. Regional differences in the association between land cover and West Nile virus disease incidence in humans in the United States. *Am J Trop Med Hyg* 84: 234–38.

Brownstein, J.S., C.C. Freifeld, and L.C. Madoff. 2009. Digital disease detection: harnessing the web for public health surveillance. *New England Journal of Medicine* 360: 2153–57.

Brunkard, J.M., J.L.R. Lopez, J. Ramirez, et al. 2007. Dengue fever seroprevalence and risk factors, Texas–Mexico border, 2004. *Emerg Infect Dis* 13: 1477–83.

Bryant, J.E., E.C. Holmes, and A.D.T. Barrett. 2007. Out of Africa: a molecular perspective on the introduction of yellow fever virus into the Americas. *PLoS Pathogens* 3: 668–73.

Centers for Disease Control and Prevention. 2012a. *West Nile virus*. http://www.cdc.gov/ncidod/dvbid/westnile/index.htm (accessed Oct 2, 2012).

Centers for Disease Control and Prevention. 2012b. *Reported Lyme disease cases by state, 2002–2011*. http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html (accessed Sept 10, 2012).

Chaves, L.F., J.M. Cohen, M. Pascual, and M.L. Wilson. 2008. Social exclusion modifies climate and deforestation impacts on a vector-borne disease. *PLoS Neglected Tropical Diseases* 2: e176.

Chaves, L.F., M. Hashizume, A. Satake, and N. Minakawa. 2012. Regime shifts and heterogeneous trends in malaria time series from Western Kenya Highlands. *Parasitology* 139: 14–25.

Cohen, J.E. and R.E. Gurtler. 2001. Modeling household transmission of American trypanosomiasis. *Science* 293: 694–98.

D'Ortenzio, E., M. Grandadam, E. Balleydier, et al. 2011. A226V Strains of chikungunya virus, Reunion Island, 2010. *Emerging Infectious Diseases* 17: 309–11.

Davis, C.T., G.D. Ebel, R.S. Lanciotti, et al. 2005. Phylogenetic analysis of North American West Nile virus isolates, 2001–2004: evidence for the emergence of a dominant genotype. *Virology* 342: 252–65.

de Lambellerie, X., E. Leroy, R.N. Charrel, K.A. Tsetsarkin, S. Higgs, and E.A. Gould. 2008. Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? *Virol J* 3:33.

Duffy, M.R., T.H. Chen, W.T. Hancock, et al. 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 360: 2536–43.

Duplantier, J.M., J.B. Duchemin, S. Chanteau, and E. Carniel. 2005. From the recent lessons of the Malagasy foci towards a global understanding of the factors involved in plague reemergence. *Veterinary Research* 36: 437–53.

Effler, P.V., L. Pang, P. Kitsutani, et al. 2005. Dengue fever, Hawaii, 2001–2002. *Emerging Infectious Diseases*; 11: 742–49.

Ergönül, Ö. 2006. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis* 6: 203–14.

Farajollahi, A., D.M. Fonseca, L.D. Kramer, and A.M. Kilpatrick. 2011. Bird biting mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infection, Genetics and Evolution* 11: 1577–85.

Ferguson, N.M., D.A.T. Cummings, C. Fraser, J.C. Cajka, P.C. Cooley, and D.S. Burke. 2006. Strategies for mitigating an influenza pandemic. *Nature* 442: 448–52.

Fonseca, D.M., J.L. Smith, R.C. Wilkerson, and R.C. Fleischer. 2006. Pathways of expansion and multiple introductions illustrated by large genetic differentiation among worldwide populations of the southern house mosquito. *American Journal of Tropical Medicine and Hygiene* 74: 284–89.

Gaynor, A.M., M.D. Nissen, D.M. Whiley, et al. 2007. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathogens* 3: e64.

Gerstl, S., S. Dunkley, A. Mukhtar, M. De Smet, S. Baker, and J. Maikere. 2010. Assessment of two malaria rapid diagnostic tests in children under five years of age, with follow-up of false-positive pLDH test results, in a hyperendemic falciparum malaria area, Sierra Leone. *Malaria Journal* 9: 28.

Gething, P.W., D.L. Smith, A.P. Patil, A.J. Tatem, R.W. Snow, and S.I. Hay. 2010. Climate change and the global malaria recession. *Nature* 465: 342–45.

Godfrey, E.R., and S.E. Randolph. 2011. Economic downturn results in tick-borne disease upsurge. *Parasites and Vectors* 4: e35.

Gould, E.A., and S. Higgs. 2009. Impact of climate change and other factors on emerging arbovirus diseases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103: 109–21.

Gray, J.S., H. Dautel, A. Estrada-Pena, O. Kahl, and E. Lindgren. 2009. Effects of climate change on ticks and tick-borne diseases in Europe. *Interdisciplinary Perspectives in Infectious Diseases* 2009: 593232, 12 pages.

Hay, S.I., C.A. Guerra, A.J. Tatem, P.M. Atkinson, and R.W. Snow. 2005. Urbanization, malaria transmission and disease burden in Africa. *Nat Rev Mic* 3: 81–90.

Hess, A.D., and R.O. Hayes. 1970. Relative potentials of domestic animals for zooprophylaxis against mosquito vectors of encephalitis. *American Journal of Tropical Medicine and Hygiene* 19: 327–34.

Hoffmann, A.A., B.L. Montgomery, J. Popovici, et al. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 476: 454–57.

Holmes, E.C. 2003. Error thresholds and the constraints to RNA virus evolution. *Trends in Microbiology* 11: 543–46.

Hoogstraal, H. 1979. Epidemiology of tick-borne Crimean Congo hemorrhagic fever in Asia, Europe, and Africa. *J Med Entomol* 15: 307–417.

Hotez, P.J., M.E. Bottazzi, C. Franco-Paredes, S.K. Ault, and M.R. Periago. 2008. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. *PLoS Neglected Tropical Diseases* 2: e300.

Hufnagel, L., D. Brockmann, and T. Geisel. 2004. Forecast and control of epidemics in a globalized world. *Proc Natl Acad Sci USA* 101: 15124–29.

Killeen, G.F. 2003. Following in Soper's footsteps: northeast Brazil 63 years after eradication of *Anopheles gambiae*. *Lancet Infectious Diseases* 3: 663–66.

Kilpatrick, A.M. 2011. Globalization, land use, and the invasion of West Nile virus. *Science* 334: 323–27.

Kilpatrick, A.M., P. Daszak, S.J. Goodman, H. Rogg, L.D. Kramer, C. Cedeño, and A.A. Cunningham. 2006a. Predicting pathogen introduction: West Nile virus spread to Galapagos. *Conservation Biology* 20: 1224–31.

Kilpatrick, A.M., P. Daszak, M.J. Jones, P.P. Marra, and L.D. Kramer. 2006b. Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society B: Biological Sciences* 273: 2327–33.

Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, and P. Daszak. 2006c. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biology* 4: 606–10.

Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, P. Daszak, and D.M. Fonseca. 2007. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. *American Journal of Tropical Medicine and Hygiene* 77: 667–71.

Kilpatrick, A.M., M.A. Meola, R.M. Moudy, L.D. Kramer. 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathogens* 4: e1000092.

Kilpatrick, A.M., A.P. Dupuis, G.J.J. Chang, and L.D. Kramer. 2010. DNA vaccination of American robins (*Turdus migratorius*) against West Nile virus. *Vector Borne Zoonotic Disease* 10: 377–80.

Lafferty, K.D. 2009. The ecology of climate change and infectious diseases. *Ecology* 90: 888–900.

Lambin, E.F., A. Tran, S.O. Vanwambeke, C. Linard, and V. Soti. 2010. Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *International Journal of Health Geographics* 9: 54.

Levi, T., A.M. Kilpatrick, M. Mangel, and C.C. Wilmers. 2012. Deer, predators, and the emergence of Lyme disease. *Proceedings of the National Academy of Sciences* 109: 10942–47.

Linard, C., P. Lamarque, P. Heyman, et al. 2007. Determinants of the geographic distribution of Puumala virus and Lyme borreliosis infections in Belgium. *International Journal of Health Geographics* 6: 15.

Lipkin, W.I. 2010. Microbe hunting. *Microbiology and Molecular Biology Reviews* 74: 363–77.

Lloyd-Smith, J.O., A.P. Galvani, and W.M. Getz. 2003. Curtailing transmission of severe acute respiratory syndrome within a community and its hospital. *Proceedings of the Royal Society of London B: Biological Sciences* 270: 1979–89.

Logiudice, K., S.T.K. Duerr, M.J. Newhouse, K.A. Schmidt, M.E. Killilea, and R.S. Ostfeld. 2008. Impact of host community composition on Lyme disease risk. *Ecology* 89: 2841–49.

Lukan, M., E. Bullova, and B. Petko. 2010. Climate warming and tick-borne encephalitis, Slovakia. *Emerging Infectious Diseases* 16: 524–26.

Martens, W.J.M., L.W. Niessen, J. Rotmans, T.H. Jetten, and A.J. McMichael. 1995. Potential impact of global climate change on malaria risk. *Environmental Health Perspectives* 103: 458–64.

Mendis, K., B.J. Sina, P. Marchesini, and R. Carter. 2001. The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 64: 97–106.

Moudy, R.M., M.A. Meola, L.L. Morin, G.D. Ebel, and L.D. Kramer. 2007. A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by *Culex* mosquitoes. *American Journal of Tropical Medicine and Hygiene* 77: 365–70.

Nash, D., F. Mostashari, A. Fine, et al. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. *New England Journal of Medicine* 344: 1807–14.

Ogden, N.H., L.R. Lindsay, M. Morshed, P.N. Sockett, and H. Artsob. 2009. The emergence of Lyme disease in Canada. *Canadian Medical Association Journal* 180: 1221–24.

Ostfeld, R., and F. Keesing. 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Canadian Journal of Zoology* 78: 2061–78.

Pialoux, G., B.A. Gaüzière, S. Jaureguiverry, and M. Strobel. 2007. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 7: 319–27.

Randolph, S.E., and A.D.M. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-infection paradigm. *Parasitology* 139: 847–63.

Randolph, S.E., on behalf of the EDEN-TBD team. 2010. Human activities predominate in determining changing incidence of tick-borne zoonoses in Europe. *European Surveillance* 15: 24–31.

Randolph, S.E., and D.J. Rogers. 2000. Fragile transmission cycles of tick-borne encephalitis virus may be disrupted by predicted climate change. *Proceedings of the Royal Society B: Biological Sciences* 267: 1741–44.

Reiter, P., S. Lathrop, M. Bunning, et al. 2003. Texas lifestyle limits transmission of dengue virus. *Emerging Infectious Disease* 9: 86–89.

Reiter, P. 2008. Climate change and mosquito-borne disease: knowing the horse before hitching the cart. *Scientific and Technical Review OIE* 27: 383–98.

Reiter, P. 2010. The standardised freight container: vector of vectors and vector-borne diseases. *Scientific and Technical Review OIE* 29: 57–64.

Rezza, G., L. Nicoletti, R. Angelini, et al. 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* 370: 1840–46.

Rogers, D.J., and S.E. Randolph. 2006. Climate change and vector-borne diseases. *Advances in Parasitology* 62: 345–81.

Rohr, J.R., A.P. Dobson, P.T.J. Johnson, et al. 2011. Frontiers in climate change-disease research. *Trends in Ecological Evolution* 26: 270–77.

Russell, R.C., B.J. Currie, M.D. Lindsay, J.S. Mackenzie, S.A. Ritchie, and P.I. Whelan. 2009. Dengue and climate change in Australia: predictions for the future should incorporate knowledge from the past. *Medical Journal of Australia* 190: 265–68.

San Martin, J.L., O. Brathwaite, B. Zambrano, et al. 2010. The epidemiology of dengue in the Americas over the last three decades: a worrisome reality. *American Journal of Tropical Medicine and Hygiene* 82: 128–35.

Schuffenecker, I., I. Iteman, A. Michault, et al. 2006. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. *PLoS Medicine* 3: 1058–70.

Stenseth, N.C., B.B. Atshabar, M. Begon, et al. 2008. Plague: past, present, and future. *PLoS Medicine* 5: 9–13.

Šumilo, D., L. Asokliene, T. Avsic-Zupanc, et al. 2008. Behavioural responses to perceived risk of tick-borne encephalitis: vaccination and avoidance in the Baltics and Slovenia. *Vaccine* 26: 2580–88.

Šumilo, D., L. Asokliene, A. Bormane, V. Vasilenko, I. Golovljova, and S.E. Randolph. 2007. Climate change cannot explain the upsurge of tick-borne encephalitis in the Baltics. *PLoS One* 2: e500.

Tatem, A.J., S.I. Hay, and D.J. Rogers. 2006. Global traffic and disease vector dispersal. *Proceedings of the National Academy of Sciences* 103: 6242–47.

Tsao, J.I., J.T. Wootton, J. Bunikis, M.G. Luna, D. Fish, and A.G. Barbour. 2004. An ecological approach to preventing human infection: vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *Proceedings of the National Academy of Sciences* 101: 18159–64.

Tsetsarkin, K.A., and S.C. Weaver. 2011. Sequential adaptive mutations enhance efficient vector switching by Chikungunya virus and its epidemic emergence. *PLoS Pathogens* 7: e1002412.

Weaver, S.C., and W.K. Reisen. 2010. Present and future arboviral threats. *Antiviral Res* 85: 328–45.

Wonham, M.J., T. de-Camino-Beck, and M.A. Lewis. 2004. An epidemiological model for West Nile virus: invasion analysis and control applications. *Proceedings of the Royal Society B: Biological Sciences* 271: 501 07.

Yamamoto, S.S., V.R. Louis, A. Sie, and R. Sauerborn. 2009. The effects of zooprophylaxis and other mosquito control measures against malaria in Nouna, Burkina Faso. *Malaria Journal* 8: 5.

Yasuoka, J., and R. Levins. 2007. Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *American Journal of Tropical Medicine and Hygiene* 76: 450–60.

Yergolkar, P.N., B.V. Tandale, V.A. Arankalle et al. 2006. Chikungunya outbreaks caused by African genotype, India. *Emerging Infectectious Diseases* 12: 1580–83.

Yozwiak, N.L., P. Skewes-Cox, M.D. Stenglein, A. Balmaseda, E. Harris, J.L. DeRisi. 2012. Virus identification in unknown tropical febrile illness cases using deep sequencing. *PLoS Neglected Tropical Diseases* 6: e1485.

A7

CLIMATE TELECONNECTIONS, WEATHER EXTREMES, AND VECTOR-BORNE DISEASE OUTBREAKS[1]

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Introduction: Vectors, Ecology, and Climate

Fluctuations in climate lead to extremes in temperature, rainfall, flooding, and droughts. These climate extremes create ideal ecological conditions that promote mosquito-borne disease transmission that impacts global human and animal health (see Figure A7-1). For example, abnormally high temperatures can affect mosquito populations by reducing mosquito survival, altering susceptibility of mosquitoes to pathogens, increasing mosquito development rates, changing their seasonal activity, increasing pathogen replication and shortening the extrinsic incubation period in the mosquito, and changing disease transmission patterns and seasonality (Gubler et al., 2001; Epstein, 2005; Linthicum et al., 2014). Elevated rainfall may increase immature habitats for mosquitoes, and elevated humidity can increase mosquito survival (Turell et al., 2001; Glass et al., 2000; Reisen et al., 1993). Drought conditions can change immature mosquito habitats and enhance container breeding mosquito habitats (Chretien et al., 2007). One well-known driver of such global-scale climate fluctuations is the El Niño/Southern Oscillation (ENSO) phenomenon that is exemplified by periodic extreme warming and cooling of the eastern equatorial Pacific Ocean with attendant consequences on precipitation and temperature worldwide especially across the global tropics. Such extremes include flooding as a result of persistent and above-normal rainfall and drought resulting from extended periods of below-normal rainfall and above-normal temperatures (see Figure A7-2). Such extremes in regional climate can create ecological conditions that influence the emergence of mosquito vectors, their distribution and abundance, population dynamics, and transmission of mosquito-borne disease (Anyamba et al., 2012). In this paper we show that outbreaks of Rift Valley fever and chikungunya, two important emerging mosquito-borne diseases, are coupled to specific climate anomaly patterns.

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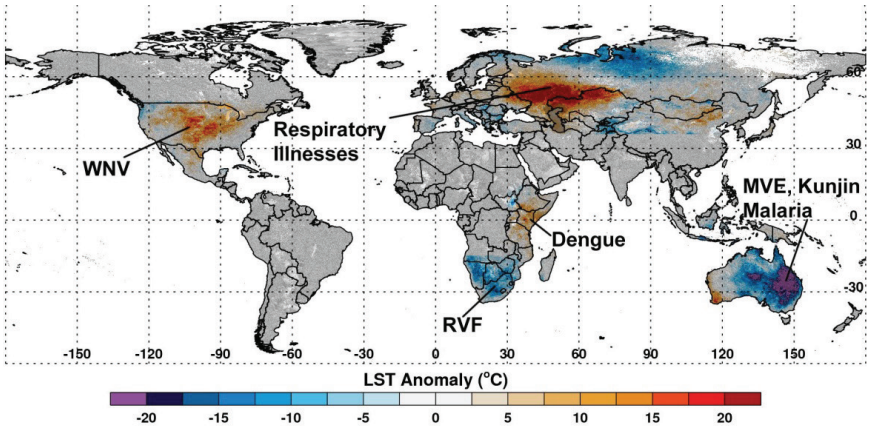


FIGURE A7-1 Land surface temperature (LST) anomaly extremes composites for June, July, and August 2010–2012 for various regions associated with vector-borne diseases including West Nile virus disease [WNV] (USA), Rift Valley fever [RVF] (Southern Africa), dengue (East Africa), Murray Valley encephalitis [MVE], Kunjin, malaria (Australia), and environment-linked respiratory illnesses (Russia).
SOURCE: Assaf Anyamba (USRA at NASA Goddard Space Flight Center) and Kenneth Linthicum.
Data: LST data from the MODIS instrument on-board NASA's Earth Observing System Terra satellite.

Next we describe significant worldwide weather anomalies that impacted vector-borne disease outbreaks during the 2010–2012 period. Using 2000–2012 normalized difference vegetation index (NDVI) and land surface temperature (LST) data from NASA's satellite-based Moderate Resolution Imaging Spectroradiometer (MODIS) we map the magnitude and extent of these weather anomalies for diverse regions including the continental United States, Russia, East Africa, Southern Africa, and Australia, and we demonstrate that shifts in temperature and/or precipitation have significant impacts on ecology patterns with attendant consequences for public health. Weather extremes resulted in excessive rainfall and flooding as well as severe drought which created exceptional conditions for extensive mosquito-borne disease outbreaks of Rift Valley fever, Murray Valley encephalitis, dengue, West Nile virus disease, and air pollution associated with extensive fires and high temperatures. Finally we describe climate teleconnections between several vector-borne, rodent-borne, and environmentally linked diseases, and describe how risks may develop if El Niño conditions develop in the winter of 2014 and spring of 2015 (Chretien et al., 2015).

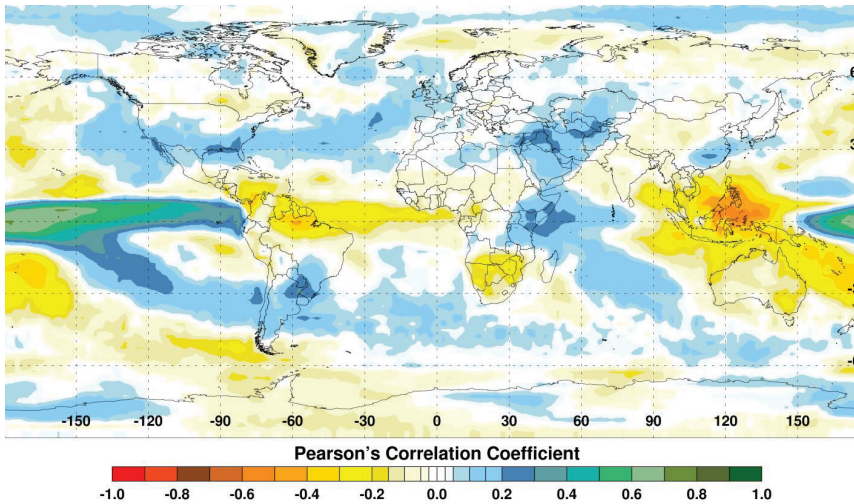


FIGURE A7-2 Summary map showing the correlation between monthly NINO3.4 sea surface temperatures (SSTs) and rainfall anomalies (1979 to 2008). El Niño events are associated with extremes of elevated or depressed rainfall (blue/green or yellow/red colors, respectively).
SOURCE: Anyamba et al., 2012. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

Climate Teleconnections and Vector-Borne Disease Patterns

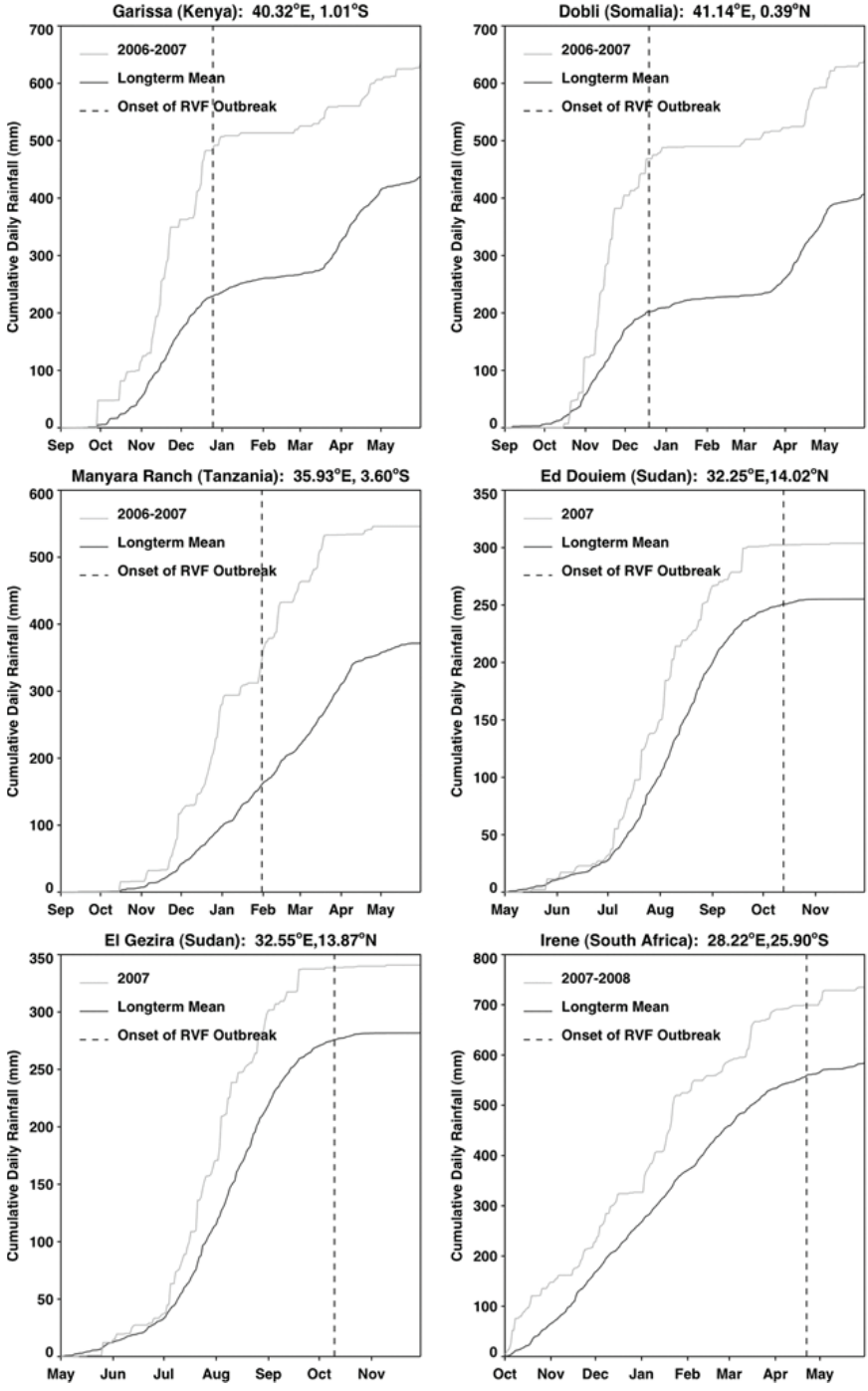
ENSO is a climate phenomenon that is associated with extremes in global climate on interannual time scales through its dislocation of major centers of and redistribution of precipitation across the global tropics and extra-tropics. It has been shown to be associated with the occurrence of several human and animal diseases including Rift Valley fever, Murray Valley encephalitis, chikungunya, malaria, hantavirus pulmonary syndrome, and cholera precipitated by extremes in rainfall and temperature among other factors (Anyamba et al., 2009; Linthicum et al., 1999; Nicholls, 1986; Bouma and Dye, 1997; Kovats et al., 2003; Engelthaler et al., 1999; Pascual et al., 2000). The impact of ENSO on earth's climate system, especially over the tropics, through interannual variations in temperature, atmospheric circulation, and rainfall at various distant locations, is termed *teleconnection*. These teleconnections produce differential anomaly patterns in major climate variables with near-cyclical transitions through time from the warm El Niño phase to the cold La Niña phase with a periodicity of 5–7 years (Diaz and Markgraf, 2000). There is likelihood for outbreaks of mosquito-borne diseases to occur at or near the same time during an ENSO cycle, demonstrating how extremes in rainfall resulting in either persistent flood or severe drought can

influence the regional dynamics of mosquito vector populations at distant locations around the world, especially in the tropics (Nicholls, 1986; Glantz, 1991; Engelthaler et al., 1999; Linthicum et al., 1999).

Globally the El Niño phase of ENSO causes predictable patterns of flooding and drought. Figure A7-2 depicts the close correlation between sea surface temperatures (SST) and rainfall anomalies. There is a strong tendency for above (below) normal rainfall during El Niño (La Niña) events over East Africa (Southern Africa, Southeast Asia). Elevated rainfall conditions and floods generally occur over Eastern Africa, the southern half of the United States, Southern Brazil/Northern Argentina, eastern and central Pacific Islands, Ecuador, and Peru. Reduced rainfall conditions leading to drought occur over a large area of Southeast Asia, Australia, northern and north-eastern Brazil, and Southern Africa. The opposite conditions may prevail during the La Niña phase of ENSO (Glantz, 1991).

ENSO-produced extremes in regional climate can create ecological conditions that influence the transmission of mosquito-borne diseases of global public health relevance (Gubler et al., 2001; Gage et al., 2008). Teleconnection studies examining the link between climate and disease have been limited by poor reporting and a lack of georeferenced disease data. We analyzed and illustrated how recent outbreaks of two mosquito-borne diseases, chikungunya and Rift Valley fever (Chretien et al., 2007; Gage et al., 2008; Panning et al., 2008; Anyamba et al., 2009, 2010, 2012), over Africa, the western Indian Ocean basin islands, and Asia were linked to specific climate anomaly patterns. We georeferenced disease occurrence records, and examined the spatial and temporal associations of these disease outbreaks and how they were impacted by variable climate and ecological patterns.

The relationship between rainfall and Rift Valley fever was determined through a logistic regression of presence or absence of disease reports on cumulative rainfall anomalies for the 4 months immediately preceding each Rift Valley fever outbreak (Anyamba, 2009; data not shown). There was a significant relationship found between cumulative rainfall anomalies and Rift Valley fever presence with at least 99.9 percent confidence. This relationship for East Africa, Sudan, and South Africa was strongly positive. Figure A7-3 shows that for each of the selected outbreak locations in each region, persistent above-normal rainfall for 3–4 months preceded the first case of Rift Valley fever. Rainfall anomalies for each of these regions at the times of disease activity are depicted in Figure A7-4 and listed in Table A7-1. The magnitude of such rainfall anomalies creates ideal ecological conditions for an increase in Rift Valley fever mosquito vector emergence and survival. These findings confirmed experimental (Linthicum et al., 1984) and field (Linthicum et al., 1985) observations that persistent, widespread, and above-normal rainfall is required to flood mosquito habitats to produce ecological conditions supporting the emergence of virus-infected ground pool *Aedes* mosquito populations and ultimately lead to large populations of vector competent mosquitoes on a large geographic scale that would result in a Rift



Valley fever epizootic. However, in Madagascar a negative relationship was found, with the model predicting higher odds of Rift Valley fever outbreaks when rainfall was less than normal (see Figure A7-4). Although the 2008 Madagascar Rift Valley fever outbreak was initially triggered by elevated rainfall (Anyamba et al., 2010), the subsequent spread of the outbreak may have been caused by the introduction of infected livestock from the southern part of the country to areas in the north where competent *Culex* mosquito vectors are associated with human habitations. Large outbreaks of chikungunya have historically been transmitted by *Aedes aegypti* mosquitoes in large highly populated urban areas of Asia and highly populated areas of Africa with smaller outbreaks in rural areas. The location of chikungunya outbreaks between 2004 and 2010 in relation to human population density is illustrated in Figure A7-5. Outbreaks occurred in coastal urban centers with large population densities (Chretien et al., 2007; Anyamba et al., 2010). Chikungunya cases between January 1979 and February 2010 were analyzed by Anyamba et al. (2012) and examined for relationships with surface air temperature anomalies and precipitation anomalies as shown in Figures A7-6 and A7-7, respectively. These figures show the frequency distribution of the number of reported chikungunya outbreak events against rainfall and temperature anomalies. Rainfall and temperature anomalies were calculated by subtracting the long-term 1979–2010 monthly rainfall or temperature means from the rainfall or temperature values in each month in each year of the study period, such that the rainfall or temperature anomaly for each month could be plotted as greater than or less than the zero long-term baseline (shown as a vertical dotted line in Figures A7-5 and A7-6). Temperature anomalies which persisted over a 4-month period were classified as hot if anomalies were > 0 or cool if < 0. Precipitation anomalies which persisted over a 4-month period were classified as drought if < 0 and wet if > 0. In East Africa, Central Africa, and South Asia, 94 percent, 68 percent, and 80 percent of the outbreaks, respectively, occurred during warmer-than-normal temperatures, and these differences were significant at $P < 0.05$ (see Figures A7-6A–C). In Southeast Asia, however, 52 percent of the outbreaks occurred during cooler than normal temperatures (see Figure A7-6D). In East Africa chikungunya reports were significantly positively correlated with drought conditions at $P < 0.05$ (see Figure A7-7A), and were not significantly correlated in South Asia (see Figure A7-7C).

FIGURE A7-3 Cumulative daily rainfall profiles for periods of Rift Valley fever activity for selected outbreak sites in Africa. Cumulative daily rainfall (green lines) profiles for periods of Rift Valley fever activity and mean long-term cumulative daily rainfall (red lines) for sites with reported Rift Valley fever activity. Dotted line represents when the first case of Rift Valley fever was identified at each location. Each of the outbreak locations was preceded by above-normal rainfall for 3-4 months.
SOURCE: Anyamba et al., 2012b, reproduced with permission from IEEE.

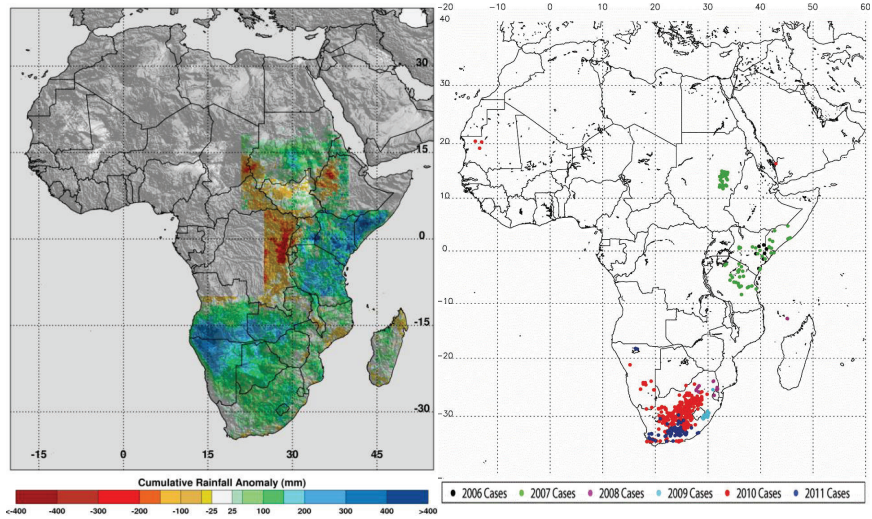


FIGURE A7-4 **A**-Left: Cumulative rainfall anomalies associated with Rift Valley fever outbreaks for East Africa (September 2006–December 2006), Sudan (June 2007–September 2007), Southern Africa (October 2007–April 2008, October 2008–April 2009, October 2009–April 2010, October 2010–April 2011), and Madagascar (October 2007–November 2008); **B**-Right: Corresponding map depicting location of RVF case reports from 2006 to 2011.
SOURCES: (A-Left) Anyamba et al., 2012. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license; (B-Right) Anyamba et al., 2012b, reproduced with permission from IEEE.

In Central Africa (see Figure A7-7B) and in Southeast Asia (see Figure A7-7D) outbreaks were significantly negatively correlated with drought at $P < 0.05$. The positive correlation between chikungunya outbreaks and warmer than normal temperatures in Africa and South Asia was consistent with nonsylvatic transmission by *Ae. aegypti* and *Ae. albopictus* in highly populated domestic settings where domestic and peridomestic stored water supplies were the likely source of the mosquitoes (Chretien et al., 2007; Anyamba et al., 2012). Our analyses suggest that in a changing and variable climate, mosquito-transmitted viruses and their mosquito vectors are going to adapt to the existing climatic and ecological conditions in new regions, and disease transmission will vary accordingly and may not be the same manifestation as observed in the original endemic regions. Combining satellite-derived measurements and analyses of climate and ecology with an understanding of mosquito vector biology and human and animal population immunity status can contribute substantially towards reducing the global burden of vector-borne diseases. Better understanding of climate teleconnection

TABLE A7-1 Total Season Rainfall, Long-Term Means, and Anomalies for Selected Periods from 2006 to 2011 Extracted from the Global Precipitation Climatology Project (Adler et al., 2003) for Regions Presented in Figures A7-4 and A7-8

Region	Season	Total (mm)	Mean (mm)	Anomaly (%)
East Africa (Somalia/Kenya)[a]	September -December 2006	495.51	247.36	102.54
Sudan	June-September 2007	563.95	354.23	63.77
US (Texas)	June-August 2011	59.40	174.11	–65.88
East Africa (Somalia/Kenya)[b]	December 2010-February 2011	40.65	80.94	–52.26
South Africa (Free State/North West)	December 2010-February 2011	363.92	253.69	43.45
SE Australia (New South Wales)	September-November 2010	255.28	95.59	174.01

[a] Refers to the specific location of Rift Valley outbreak in 2006–2007.
[b] Location of cluster of dengue outbreaks in East Africa in 2010–2011.
SOURCE: Data from NASA Goddard Space Flight Center (http://precip.gsfc.nasa.gov/), as described by Adler et al., 2003.

events and their link to mosquito-borne diseases will likely allow parts of Africa, the Indian Ocean basin islands, and elsewhere within the greater tropics to have from several months to more than a year warning prior to Rift Valley fever outbreaks, permitting more efficacious targeting of vaccine, virus surveillance, mosquito control, animal quarantine, and public education strategies.

Extreme Weather and Disease Outbreaks

The anomalous conditions observed during 2010–2012 were the most extreme weather events in the 12-year record of Terra MODIS data, and they present a good opportunity to quantify these weather impacts on mosquito-transmitted diseases using various satellite-based parameters of surface conditions. The timing and unique intensity of these events is corroborated by analyses using longer-term climate data sets (Trenberth and Fasullo, 2012; Blunden and Arndt, 2013; Hoerling et al., 2013). Anyamba et al. (2012, 2014) postulated that because both severe drought and flooding may create ecological conditions for enhancing vector-borne disease emergence, then under such extreme weather events as have been documented for 2010–2012 significant increases

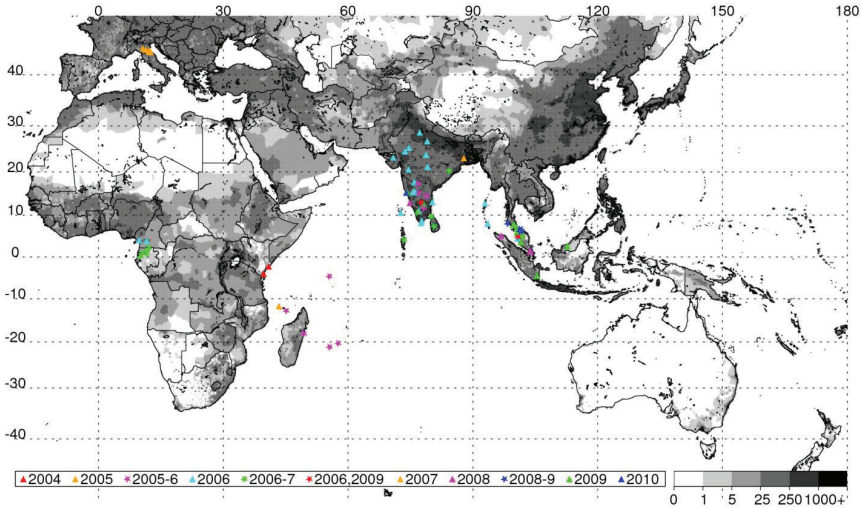


FIGURE A7-5 Distribution of chikungunya outbreaks (2004–2010) in relation to human population density. Each symbol represents the year(s) when an outbreak was reported at a specific geographic location. Most chikungunya activity has occurred in locations with high population densities (> 500 people per square kilometer).
SOURCE: Anyamba et al., 2012. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

in outbreaks of vector-borne diseases under conditions of both extreme drought and above-normal rainfall should be observed. They mapped locations of documented 2010–2012 extremes at selected locations around the globe using various satellite datasets, and described and illustrated the impacts of these extremes on epidemics/epizootics of West Nile virus disease (USA 2012), dengue (East Africa 2010–2011), Murray Valley encephalitis (SE Australia 2010), and Rift Valley fever (East Africa 2006, Sudan 2007, South Africa 2010–2011) for the selected regions shown in Figures A7-4 and A7-8. Rainfall statistics for regions where and times when these outbreaks occurred are shown in Table A7-1. Rainfall deficits were associated with elevated West Nile virus and dengue transmission, and surpluses associated with Murray Valley encephalitis and Rift Valley fever outbreaks.

Record high temperatures and persistent drought (rainfall shortfalls of 65 percent) (Table A7-1) were linked to the highest period of West Nile virus activity on record in Texas and the rest of the continental United States (see Figure A7-9A, B). The 2012 epidemic of West Nile virus disease across the continental United States (see Figure A7-8) was the largest such outbreak since the introduction of West Nile virus into the United States in 1999, and the spike in human

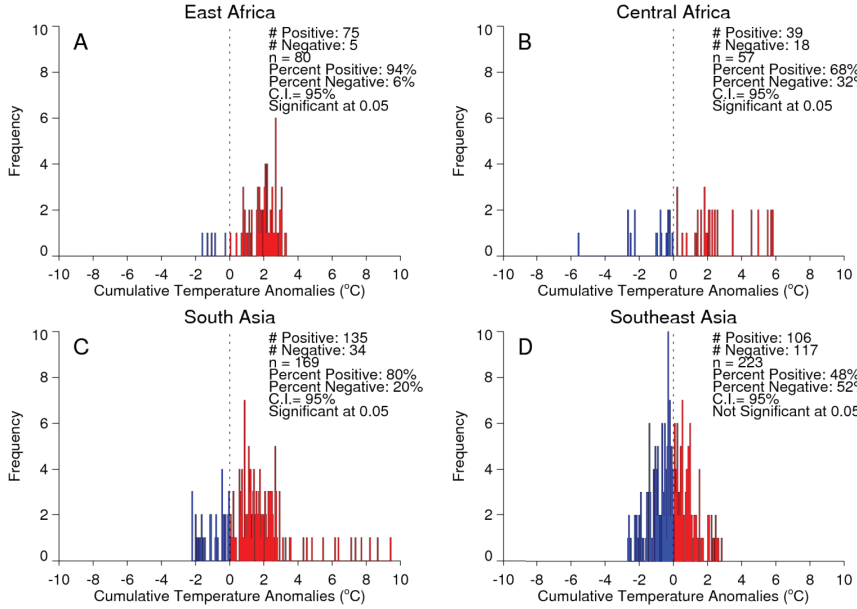


FIGURE A7-6 Frequency distributions of chikungunya outbreak events and 4-month cumulative *temperature* anomalies for East Africa (A), Central Africa (B), South Asia (C), and Southeast Asia (D). The 4-month anomaly threshold is used to represent periods of cool temperatures or drought and extreme high temperatures. The dashed line at zero depicts the long-term mean temperature (1979–2009 base mean period) with warmer than normal temperatures shown to the right (red) and cooler than normal temperatures shown to the left (blue) of the line. Cases shown to the right of the dashed line occurred during a period of elevated temperatures, with a persistence of 4 months.
SOURCE: Anyamba et al., 2012. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

West Nile virus disease cases in 2012 can in part be associated with extreme drought (see Figure A7-9B; Epstein and Defilippo, 2001). Mean summer temperatures in June to August 2012 ranged from 30°C to 33°C, exceeding long-term means (see Figure A7-9A). Elevated temperatures may have increased the efficiency of transmission of West Nile virus by both *Culex pipiens* and *Cx. tarsalis* mosquitoes, the likely vectors, by elevating population development and survival, biting rates, and viral replication within these mosquito species (Kilpatrick et al., 2008; Johnson and Sukhdeo, 2013; Moudy et al., 2007).

Across Eurasia, the summer drought of June–August 2010 was centered in western Russia (see Figure A7-1) with the drought area extending to Belarus, Poland, Germany, Ukraine, and Kazakhstan (Munich, 2010; Trenberth and Fasullo, 2012). Cumulative seasonal land surface temperatures reached as high

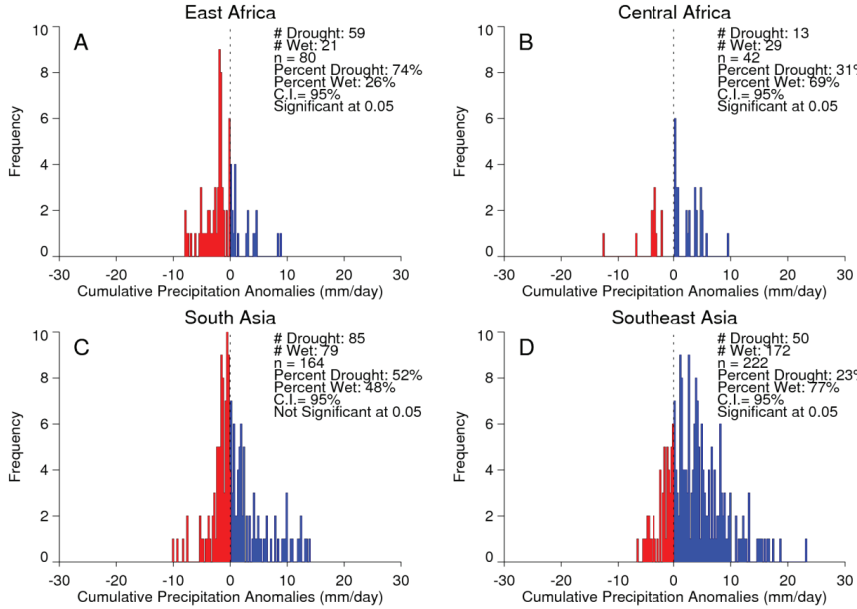


FIGURE A7-7 Frequency distributions of chikungunya outbreak events and 4-month cumulative *precipitation* anomalies in East Africa (A), Central Africa (B), South Asia (C), and Southeast Asia (D). The 4-month anomaly threshold is used to represent periods of either persistent above-normal rainfall/wetness or persistent drought conditions. The dashed line at zero depicts the long-term mean rainfall with greater than normal precipitation shown to the right (blue) and lower than normal precipitation shown to the left (red) of the line. Cases shown to the left of the dashed line occurred during a drought period, with a persistence of 4 months.
SOURCE: Anyamba et al., 2012. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

as 20°C above normal with declines in NDVI of up to 40 percent below normal (see Figure A7-1). As in the southern United States, the drought caused extreme fire conditions. The fires led to smoke pollution and an increase in the number of upper respiratory illnesses, and resulted in more than 15,000 deaths in Russia during the 2010 summer.

In 2011 an unprecedented extensive dengue outbreak occurred in East Africa and was associated with anomalously elevated land surface temperatures and drought (52 percent below-normal rainfall) (see Table A7-1, Figure A7-8). Dengue virus, transmitted by *Ae. aegypti* mosquitoes, has been linked to increased storage of water around households during hot, dry climatic conditions in densely populated areas (Epstein, 2005; Chretien et al., 2007; Padmanabha et al., 2010). These conditions are thought to increase populations of that mosquito species

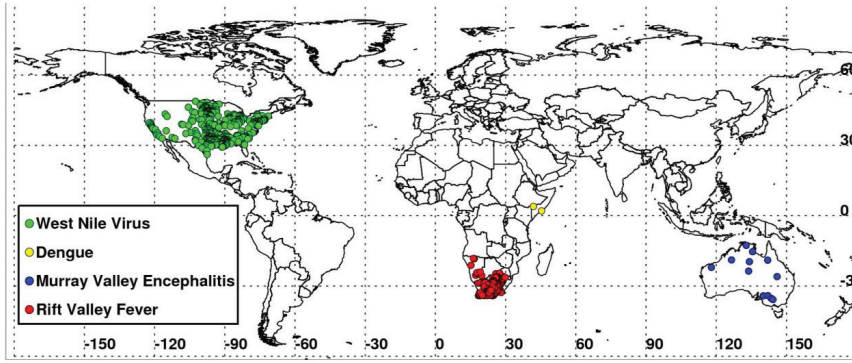


FIGURE A7-8 Global distribution of epidemics/epizootics of mosquito-borne disease outbreaks during 2010–2012 associated with weather extremes, showing the outbreak locations of West Nile virus disease (United States, 2012), dengue (East Africa, 2011), Rift Valley fever (Southern Africa, 2011), and Murray Valley encephalitis (Australia, 2011). SOURCE: Anyamba et al., 2014. Available from *PLoS ONE* under Creative Commons license.

(Subra, 1983). Extensive drought and elevated higher temperatures likely increased the abundance of container-breeding dengue virus vector mosquitoes in urban settings leading to the dengue outbreaks focused in Mogadishu, Somalia, and Mandera, Kenya (IRIN, 2011) (see Table A7-1, Figure A7-8).

During the extended La Niña event of 2010–2011 more than 40 percent higher than normal rainfall fell in much of South Africa. These extremely wet conditions led to the flooding of low-lying areas, or dambos/pans, and created ideal ecological conditions to hatch ground pool *Aedes* species mosquito eggs infected with Rift Valley fever virus. Additionally, there was a downward shift in mean seasonal (December–February) temperatures from about 40°C to 30°C in South Africa as shown in Figures A7-9C and A7-9D. These cool and wet conditions, which persisted through December 2010 to February 2011, permitted increased mosquito vector populations and increased virus infection rates in mosquitoes (Turell, 1993; Grobbelaar et al., 2011), and subsequent Rift Valley fever virus transmission. The outbreak was the most extensive and widespread epizootic/epidemic of Rift Valley fever observed in the region since the 1970s (see Figure A7-8), severely impacting domestic animal production and human health in southern Africa (Grobbelaar et al., 2011; Metras et al., 2012). MODIS time series NDVI and LST anomalies during the 2010/11 La Niña were the most persistent and extreme anomalies ever observed for the southern Africa region during the 12-year history record of consistent measurements.

Accordingly, the peak of the La Niña event from November 2010 to January 2011 produced persistent and heavy rainfall, cooler temperatures, vegetation

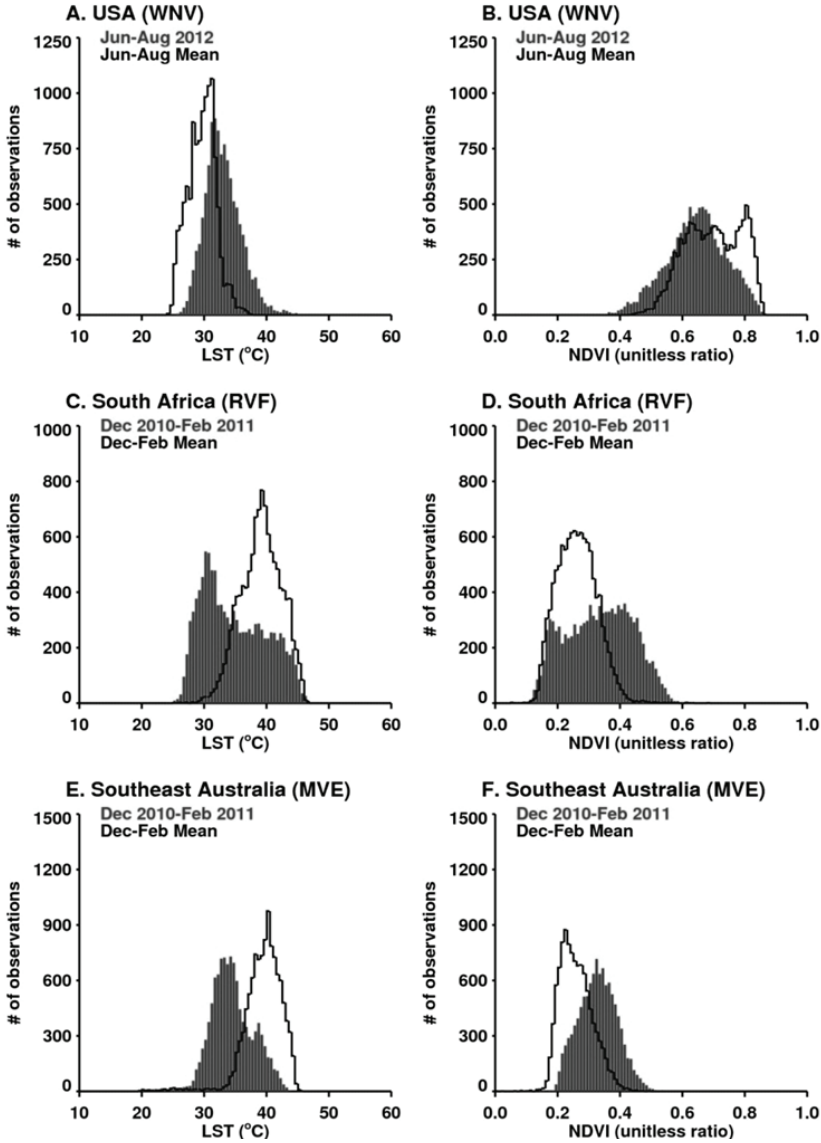


FIGURE A7-9 Distribution of land surface temperature (LST) and normalized difference vegetation index (NDVI) during periods of disease outbreaks in selected regions: (A, B) Lincoln, Nebraska, USA: West Nile virus disease, 40.8069N, 96.6817W, (C, D) Bloemfontein, South Africa: Rift Valley fever, 29.1183S, 26.2249E, and (E, F) Peterborough, Australia: Murray Valley encephalitis, 32.9733S, 138.8376E.
SOURCE: Anyamba et al., 2014. Available from *PLoS ONE* under Creative Commons license.

growth, and created the ideal conditions for increasing Murray Valley encephalitis virus mosquito vector populations. These conditions (174 percent above-normal rainfall) led to outbreaks of the virus over northern and eastern Australia (Knox et al., 2012) (see Table A7-1, Figure A7-8). *Culex annulirostris*, the primary Murray Valley encephalitis mosquito vectors, propagate well during cooler temperatures associated with heavy rainfall periods in the tropics and subtropics (Van Den Hurk et al., 2010). During this epidemic period there was a reduction in mean seasonal temperatures from 40°C to 30°C in the period from December 2010 to January 2011 compared to the long-term mean distribution for eastern Australia (see Figures A7-9E and A7-9F).

These regional examples described above illustrate how extreme weather events can impact mosquito-borne disease outbreaks. These environmentally enhanced outbreaks can vary globally depending on the virus and its transmission ecology and the geographic location. The status of a particular disease, its seasonality, and other factors may enhance the potential for globalization of such pathogens (Anyamba et al., 2012). The analysis of temperature and vegetation conditions presented here and in Anyamba et al. (2014) provides a method for consistently quantifying weather extremes from region to region, and demonstrates the unique capability of satellite data in monitoring and mapping the magnitude and extent of such events. It is likely that as extreme weather events become more common under a changing and more variable climate (Cai et al., 2015), countries will face challenging and costly adaptation strategies.

Summary: Extremes and the Near Future

The National Oceanic and Atmospheric Administration's (NOAA's) Climate Prediction Center (CPC) issued, on December 4, 2014, their most recent El Niño conditions advisory (http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/enso_advisory/index.shtml). The advisory indicated that there is a 65 percent chance that El Niño conditions will be present during the Northern Hemisphere winter and last into the Northern Hemisphere spring of 2015. Additionally, the *RVF Monitor* website (http://www.ars.usda.gov/Business/docs.htm?docid=23464) has the current suite of satellite global climate surveillance products for monitoring El Niño and vegetation conditions and implications for Rift Valley fever activity in Africa and the Arabian Peninsula region. The global products including NDVI, SST, and outgoing longwave radiation (OLR—a proxy indicator for rainfall), as well as data from terrestrial rainfall monitoring stations, are useful in illustrating the current situation of global climate anomalies with implications for public health.

The U.S. Department of Defense Armed Forces Health Surveillance Center's Global Emerging Infections Surveillance and Response System (DoD-AFHSC/GEIS); United States Department of Agriculture-Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS/

CMAVE); and the Global Inventory Modeling and Mapping Studies (GIMMS) Group at NASA Goddard Space Flight Center monitor global climate and ecological conditions of relevance to various disease outbreaks. The current NOAA El Niño watch forecasts a 65 percent chance of the development of El Niño conditions this winter 2014 and into the coming spring 2015, which may result in climate perturbations and anomalies that will affect various vector-borne and rodent-borne pathogen ecologies globally and likely result in disease outbreaks. Given current observations and forecast information, the following regions are at increased risk for disease outbreaks (see Figure A7-10):

1. Indonesia, Malaysia, Thailand, and most of the Southeast Asia Islands: High likelihood of increased dengue fever and possibly chikungunya transmission caused by drought conditions which (1) increase water storage around houses leading to elevated *Ae. aegypti* populations and (2) elevate ambient air temperatures which will reduce the extrinsic incubation period for the virus in *Ae. aegypti* and *Ae. albopictus* mosquito vectors increasing vectorial capacity; and likelihood of increased respiratory illnesses attributable to uncontrolled burning of tropical forests during extreme drought conditions.
2. Coastal Peru, Venezuela, and Colombia: Elevated risk of malaria epidemics due to elevated anopheline mosquito populations that will develop when various types of potential habitats are flooded after heavy rainfall.
3. Bangladesh, coastal India, and Sri Lanka: Elevated risk for cholera and malaria outbreaks due to increased rainfall and flooding.
4. East Africa (Kenya, Tanzania, Uganda, Somalia, and Ethiopia): Elevated risk for Rift Valley fever and malaria illnesses resulting from elevated mosquito vector populations, as well as increased risk for cholera due to heavy rainfall in dry land areas and human contamination of water supply. Note: In spite of significant protective Rift Valley fever virus antibodies in livestock older than 3 years, infected mosquitoes may be produced in the region and significant transmission may occur particularly in areas not affected during the recent 2006–2007 epizootic/epidemic. In addition, areas previously affected have now restocked after the recent severe drought from 2010–2011. There also is a risk for co-infection with other mosquito-borne diseases such as dengue, chikungunya, and o'nyong'nyong.
5. Southwest United States (New Mexico, Arizona, Colorado, Utah, Texas, and California): Hantavirus pulmonary syndrome and plague due to elevated rodent populations caused by heavy rainfall. In addition, elevated potential for transmission of arboviruses, such as West Nile virus, caused by heavy rainfall and elevated culicine mosquito populations.
6. Southern and Southeast United States, particularly along the Gulf Coast: Elevated rainfall conditions may increase *Ae. albopictus* and *Ae. aegypti* populations, potentially increasing the likelihood of local transmission of

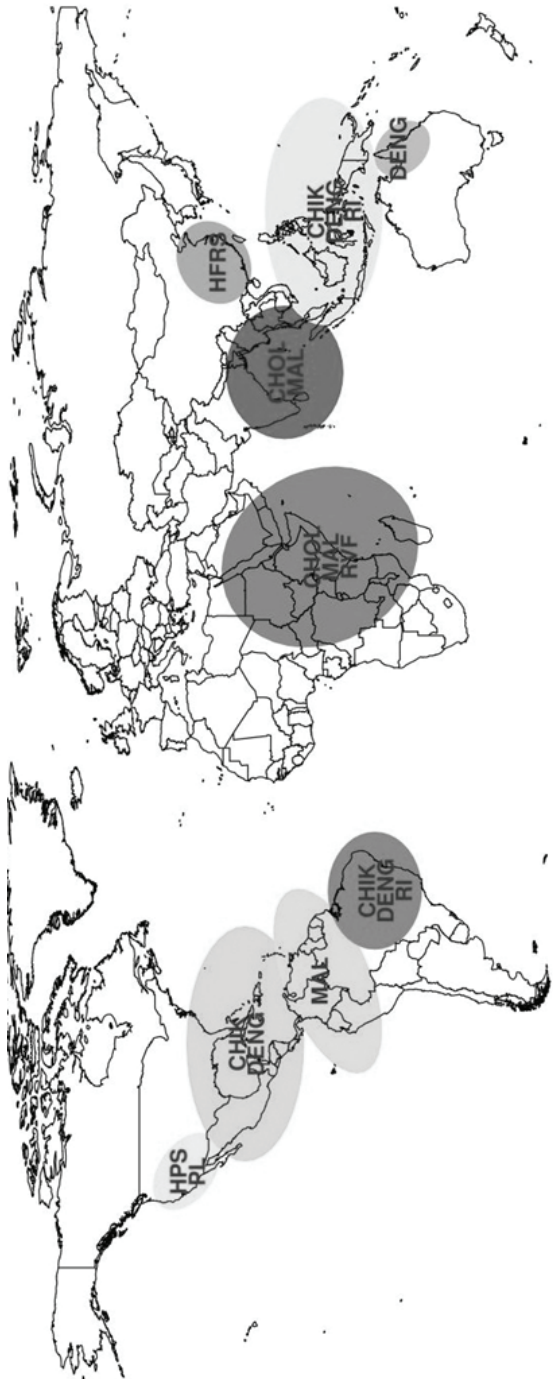


FIGURE A7-10 Potential El Niño regional teleconnections with patterns of vector-borne disease, rodent-borne disease, water-borne disease, and environment-linked respiratory illness patterns.
NOTES: CHIK = Chikungunya; CHOL = Cholera; DENG = Dengue Fever; HFRS = Hemorrhagic Fever with Renal Sundrome; HPS = Hantavirus Pulmonary Syndrome; MAL = Malaria; PL = Plague; RI = Respiratory Illness; RVF = Rift Valley Fever.
SOURCE: Chretien et al., 2015. Available from *PLoS Currents Outbreaks* under Creative Commons license.

dengue and chikungunya virus within existing or potential endemic areas or following continued pathogen introduction from current outbreaks in the Caribbean Islands and/or Central/South America.

7. Northeast Brazil: Elevated risk for dengue and respiratory illnesses due to drought conditions and potential large-scale forest fires. Additionally, increased risk for chikungunya introduction from the ongoing outbreak in the Caribbean and/or Central/South America into Brazil.

As described by Anyamba et al. (2012, 2014) outbreaks of mosquito-borne diseases on epidemic scales, such as those experienced during 2005–2011 in Africa, the western Indian Ocean islands, and in Asia, place a huge burden on public health care systems and the economy. Outbreaks such as those of chikungunya are also an impediment to tourism, a major contributor to the gross national product of countries and small island nation states everywhere. Given the potential implications from the developing El Niño in the winter of 2014 and spring of 2015 it is imperative that countries in potentially impacted regions anticipate the changing, variable, and extreme nature of the climate to prevent or minimize the emergence and reemergence of such diseases. There is an urgent need for public health authorities to take advantage of climate observations and analyses in times of extreme climate variability to enhance response and mitigation planning including: vector surveillance and control, virus surveillance, vaccination, and public education in areas that may be impacted by disease outbreaks. In addition, climate-based predictions offer opportunities for virologists, epidemiologists, entomologists, physicians, and veterinarians to understand the biological and cyclic nature of these diseases and how their episodic occurrence relates to livestock and human immunity in recently infected areas, and the potential for reemergence of the diseases in livestock and human populations.

References

Adler, R. F., G. J. Huffman, A. Chang, R. Ferraro, P.-P. Xie, J. Janowiak, B. Rudolf, U. Schneider, S. Curtis, D. Bolvin, A. Gruber, J. Susskind, P. Arkin, and E. Nelkin. 2003. The version-2 Global Precipitation Climatology Project (GPCP) monthly precipitation analysis (1979-present). *Journal of Hydrometeorology* 4:1147-1167.

Anyamba, A., J.-P. Chretien, J. Small, C. J. Tucker, P. B. Formenty, J. H. Richardson, S. C. Britch, D. C. Schnabel, R. L. Erickson, and K. J. Linthicum. 2009. Prediction of a Rift Valley fever outbreak. *Proceedings of the National Academy of Sciences of the United States of America* 106:955-959.

Anyamba, A., K. J. Linthicum, J. Small, S. C. Britch, E. Pak, S. de La Rocque, P. Formenty, A. W. Hightower, R. F. Breiman. J.-P. Chretien, C. J. Tucker, D. Schnabel, R. Sang, K. Haagsma, M. Latham, H. B. Lewandowski, S. Osman Magdi, M. Ally Mohamed, P. M. Nguku, J.-M. Reynes, and R. Swanepoel. 2010. Prediction, assessment of the Rift Valley fever activity in East and Southern Africa 2006–2008 and possible vector control strategies. *American Journal of Tropical Medicine and Hygiene* 83(S2):43-51.

Anyamba, A., K. J. Linthicum, J. L. Small, K. M. Collins, C. J. Tucker, E. W. Pak, S. C. Britch, J. R. Eastman, J. E. Pinzon, and K. L. Russell. 2012. Climate teleconnections and recent patterns of human and animal disease outbreaks. *Public Library of Science Neglected Tropical Diseases* 6(1):e1465. doi:10.1371/journal.pntd.0001465.

Anyamba, A., J. L. Small, S. C. Britch, C. J. Tucker, E. W. Pak, C. A. Reynolds, J. Crutchfield, and K. J. Linthicum. 2014. Recent weather extremes and impacts on agricultural production and vector-borne disease outbreak patterns. *Public Library of Science ONE* 9(3): e92538. doi:10.1371/journal.pone.0092538.

Blunden, J., and D. S. Arndt. 2013. State of the climate in 2012. *Bulletin of the American Meteorological Society* 94:S1-S258.

Bouma, M. J., and C. Dye. 1997. Cycles of malaria associated with El Niño in Venezuela. *Journal of the American Medical Association* 278:1772-1774.

Cai, W., G. Wang, A. Santoso, M. J. McPhaden, L. Wu, F.-F. Jin, A. Timmermann, M. Collins, G. Vecchi, M. Lengaigne, M. H. England, D. Dommenget, K. Takahashi, and E. Guilyardi. 2015. Increased frequency of extreme La Niña events under greenhouse warming. *Nature Climate Change* 5:132-137.

Chretien, J.-P., A. Anyamba, S. A. Bedno, R. F. Breiman, R. Sang, K. Sergon, A.M. Powers, C. O. Onyango, J. Small, C. J. Tucker, and K. J. Linthicum. 2007. Drought-associated chikungunya emergence along coastal East Africa. *American Journal of Tropical Medicine and Hygiene* 76:405-407.

Chretien, J.-P., A. Anyamba, J. L. Small, S. C. Britch, J. L. Sanchez, A. C. Halbach, C. J. Tucker, and K. J. Linthicum. 2015. Global climate anomalies and potential infectious disease risks: 2014-2015. *PLoS Currents Outbreaks* Jan 26. Edition 1. doi: 10.1371/currents.outbreaks.95fbc4a8f b4695e049baabfc2fc8289f.

Diaz, H. F., and V. Markgraf. 2000. *El Niño: Historical and paleoclimatic aspects of the Southern Oscillation*. New York: Cambridge University Press. Pp. 323-348.

Engelthaler, D. M., D. G. Mosley, J. E. Cheek, C. E. Levy, K. K. Komatsu, P. Ettestad, T. Davies, D. T. Tanda, L. Miller, J. W. Frampton, R. Porter, and R. T. Bryan. 1999. Climatic and environmental patterns associated with hantavirus pulmonary syndrome, Four Corners region, United States. *Emerging Infectious Diseases* 5:87-94.

Epstein, P. R. 2005. Climate change and human health. *New England Journal of Medicine* 353:1433-1436.

Epstein, P. R., and C. Defilippo. 2001. West Nile virus and drought. *Global Change and Human Health* 2(2):105-107. doi:10.1023/A:1015089901425

Gage, K. L., T. R. Burkot, R. J. Eisen, and E. B. Hayes. 2008. Climate and vectorborne diseases. *American Journal of Preventive Medicine* 35:436-450.

Glantz, M. H. 1991 Introduction. In *Teleconnections linking worldwide climate anomalies*, edited by M. H. Glantz, R. W. Katz, and N. Nicholls. New York: Cambridge University Press. Pp. 1-12.

Glass, G. E., J. E. Cheek, J. A. Patz, T. M. Shields, T. J. Doyle, D. A. Thoroughman, D. K. Hunt, R. E. Enscore, K. L. Gage, C. Irland, C. J. Peters, and R. Bryan. 2000. Using remotely sensed data to identify areas of risk for hantavirus pulmonary syndrome. *Emerging Infectious Diseases* 63:238-247.

Grobbelaar, A. A., J. Weyer, P. A. Leman, A. Kemp, J. T. Paweska, and R. Swanepoel**.** 2011. Molecular epidemiology of Rift Valley fever virus. *Emerging Infectious Diseases* 17:2270-2276.

Gubler, D. J., P. Reiter, K. L. Ebi, W. Yap, R. Nasci, and J. A. Patz. 2001. Climate variability and change in the United States: Potential impacts on vector- and rodent-borne diseases. *Environmental Health Perspectives* 109(S2):223-233.

Hoerling, M., A. Kumar, R. Dole, J. W. Nielse-Gammon, J. Eischeid, A. Kumar, J. W. Nielsen-Gammon, P. Pegion, J. Perlwitz, X.-W. Quan, T. Zhang, P. Pegion, and M. Chen. 2013. Anatomy of an extreme event. *Journal of Climate* 26: 2811-2832. doi: http://dx.doi.org/10.1175/JCLI-D-12-00270.1

IRIN (Integrated Regional Information Networks). 2011. Kenya: Medics overwhelmed as dengue fever spreads. *IRIN Humanitarian News and Analysis*. http://www.irinnews.org/Report/93848/KENYA-Medics-overwhelmed-as-dengue-fever-spreads (accessed April 10, 2013).

Johnson, B. J., and M. V. K. Sukhdeo. 2013. Drought-induced amplification of local and regional West Nile virus infection rates in New Jersey. *Journal of Medical Entomology* 50:195-204.

Kilpatrick, A. M., M. A. Meola, R. M. Moudy, and L. D. Kramer. 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *Public Library of Science Pathogens* 4: e1000092. doi: 10.1371/journal.ppat.1000092.

Knox, J., R. U. Cowan, J. S. Doyle, M. K. Ligtermoet, J. S. Archer, J. N. C. Burrow, S. Y. C. Tong, B. J. Currie, J. S. Mackenzie, D. W. Smith, M. Catton, R. J. Moran, C. A. Aboltins, and J. S. Richards. 2012. Murray Valley encephalitis: A review of clinical features, diagnosis and treatment. *Medical Journal of Australia* 196:322-326.

Kovats, R., M. J. Bouma, S. Hajat, E. Worrall, and A. Haines. 2003. El Niño and health. *Lancet* 362:1481-1489.

Linthicum, K. J., F. G. Davies, C. L. Bailey, and A. Kairo. 1984. Mosquito species encountered in a flooded grassland dambo in Kenya. *Mosquito News* 44:228-232.

Linthicum, K. J., F. G. Davies, A. Kairo, and C. L. Bailey. 1985. Rift Valley fever virus (family Bunyaviridae, genus *Phlebovirus*). Isolations from Diptera collected during an interepizootic period in Kenya. *Journal of Hygiene-Cambridge* 95:197-209.

Linthicum, K. J., A. Anyamba, C. J. Tucker, P. W. Kelley, M. F. Myers, and C. J. Peters. 1999. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science* 285:397-400.

Linthicum, K. J., A. Anyamba, B. Killenbeck, W.-J. Lee, H. C. S. Lee, T. A. Klein, J.-C. Kim, J. A. Pavlin, S. C. Britch, S. Small, C. J. Tucker, and J. C. Gaydos. 2014. Association of temperature and historical dynamics of malaria in the Republic of Korea, including reemergence in 1993. *Military Medicine* 179:806-814.

Métras, R., T. Porphyre, D. U. Pfeiffer, A. Kemp, P. N. Thompson, L. M. Collins, and R. G. White. 2012. Exploratory space-time analyses of Rift Valley fever in South Africa in 2008–2011. *Public Library of Science Neglected Tropical Diseases* 6(8):e1808. doi:10.1371/journal.pntd.0001808.

Moudy, R. M., M. A. Meola, L. L. Morin, G. D. Ebel, and L. D. Kramer. 2007. A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by *Culex* mosquitoes. *American Journal of Tropical Medicine and Hygiene* 77:365-370.

Munich, R. E. 2010. Geo risks research: Heat wave, drought, wildfires in Russia (Summer 2010). https://www.munichre.com/site/touchnaturalhazards/get/documents_E439647210/mr/assetpool.shared/Documents/5_Touch/_NatCatService/Catastrophe_portraits/event_report_hw_dr_wf_russia_touch_en.pdf (accessed January 29, 2015).

Nicholls, N. A. 1986. A method for predicting Murray Valley encephalitis in southeast Australia using the Southern Oscillation. *Australian Journal of Experimental Biology & Medical Science* 64:587-594.

Padmanabha, H., E. Soto, M. Mosquera, C. C. Lord, and L. P. Lounibos. 2010. Ecological links between water storage behaviors and *Aedes aegypti* production: Implications for dengue vector control in variable climates. *Ecohealth* 7:78-90.

Panning, M., K. Grywna, M. van Esbroeck, P. Emmerich, and C. Drosten. 2008. Chikungunya fever in travellers returning to Europe from the Indian Ocean region, 2006. *Emerging Infectious Diseases* 14:416-422.

Pascual, M., X. Rodó, S. P. Ellner, R. Colwell, and M. J. Bouma. 2000. Cholera dynamics and El Niño-Southern Oscillation. *Science* 289:1766-1769.

Reisen, W. K., R. P. Meyer, S. B. Presser, and J. L. Hardy. 1993. Effect of temperature on the transmission of Western equine encephalomyelitis and St. Louis encephalitis viruses by *Culex tarsalis* (Diptera, Culicidae). *Journal of Medical Entomology* 30:151-160.

Subra, R. 1983. The regulation of preimaginal populations of *Aedes aegypti* L. (Diptera:Culicidae) on the Kenya coast. I. Preimaginal population dynamics and the role of human behavior. *Annals of Tropical Medicine and Parasitology* 77:195-201.

Trenberth, K. E., and J. T. Fasullo. 2013. Climate extremes and climate change: The Russian heat wave and other climate extremes of 2010. *Journal of Geophysical Research: Atmospheres* 117:D17103.

Turell, M. J. 1993. Effects of environmental temperature on the vector competence of *Aedes taeniorhynchus* for Rift Valley fever and Venezuelan equine encephalitis viruses. *American Journal of Tropical Medicine and Hygiene* 49:672-670.

Turell, M. J., M. O'Guinn, D. J. Dohm, and J. W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology* 38:130-134.

Van Den Hurk, A. F., S. B. Craig, S. M. Tulsiani, and C. C. Jansen. 2010. Emerging tropical diseases in Australia. Part 4. Mosquito-borne diseases. *Annals of Tropical Medicine and Parasitology* 8:623-640.

A8

CHANGING PARADIGMS FOR TICK-BORNE DISEASES IN THE AMERICAS[1]

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Introduction

Ticks transmit a greater diversity of viral, bacterial, and protozoan diseases than any other arthropod vector on earth (Jongejan and Uilenberg, 2004; IOM, 2011). Through 2014, at least 27 ecologically and epidemiologically distinct tick-borne diseases were identified in the Western Hemisphere; remarkably, nearly half of these were discovered during the last 20 years (see Table A8-1). Against this background of expanding pathogen recognition are also unprecedented surges in the incidence of several tick-borne infections throughout the Americas.

During 2013, 48,821 cases of autochthonous, nationally notifiable, vector-borne disease were reported to the United States Centers for Disease Control and Prevention (CDC) (CDC, 2014). Overall, approximately 95 percent of reported

[1] The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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TABLE A8-1 Tick-Borne Pathogens Affecting Humans in the Western Hemisphere

Pathogen	Year Identified as a Cause of Tick-Borne Disease	Principal Tick Vector(s)	Country or Countries with Cases of Disease
Rickettsia rickettsii	1909	*Dermacentor andersoni* *Dermacentor variabilis* *Rhipicephalus sanguineus* *Amblyomma sculptum* *Amblyomma aureolatum*	Canada, United States, Mexico, Costa Rica, Panama, Colombia, Brazil, Argentina
Borrelia mazzottii	1921	*Carios talaje*	Mexico, Panama
Francisella tularensis	1924	*D. variabilis* *D. andersoni* *Amblyomma americanum*	Canada, United States, Mexico
Borrelia venezuelensis	1927	*Carios rudis*	Colombia, Venezuela
Borrelia turicatae	1930	*Ornithodoros turicata*	United States, Mexico
Borrelia hermsii	1935	*Ornithodoros hermsi*	Canada, United States
Borrelia parkeri	1941	*Ornithodoros parkeri*	United States
Colorado tick fever virus	1946	*D. andersoni*	Canada, United States
Lineage I Powassan virus	1963	*Ixodes marxi* *Ixodes cookei* *Ixodes spinipalpis*	Canada, United States
Babesia microti	1970	*Ixodes scapularis*	United States
Borrelia burgdorferi	1982	*I. scapularis* *Ixodes pacificus*	Canada, United States
Ehrlichia chaffeensis	1987	*A. americanum*	United States
Babesia duncani	1993	Unknown	United States
Anaplasma phagocytophilum	1994	*I. scapularis* *I. pacificus*	Canada, United States
Babesia divergens-like organism	1996	Unknown	United States
Rickettsia africae	1998	*Amblyomma variegatum*	Guadeloupe
Ehrlichia ewingii	1999	*A. americanum*	United States
Lineage II Powassan virus[a]	2001	*I. scapularis* *D. andersoni*	Canada, United States
Rickettsia parkeri	2004	*Amblyomma maculatum* *Amblyomma triste* *Amblyomma tigrinum*	United States, Uruguay, Argentina
Rickettsia sp. 364D	2010	*Dermacentor occidentalis*	United States

TABLE A8-1 Continued

Pathogen	Year Identified as a Cause of Tick-Borne Disease	Principal Tick Vector(s)	Country or Countries with Cases of Disease
Rickettsia sp. Atlantic rainforest	2010	*Amblyomma ovale* *A. aureolatum*	Brazil
Ehrlichia muris-like agent	2011	*I. scapularis*	United States
Heartland virus	2012	*A. americanum*	United States
Borrelia americana	2013	Unknown	United States
Borrelia andersonii	2013	Unknown	United States
Borrelia miyamotoi	2013	*I. scapularis* *I. pacificus*	United States
Borrelia mayonii	2014	*I. scapularis*	United States

NOTES: In some circumstances the distribution of the pathogen in ticks extends to other countries from which no documented cases of human disease have been reported. Dates approximate the recognition of a specific agent and its direct association with ticks and disease in humans. In some instances the named disease preceded discovery of the causative agent by many years. In other situations, the discovery of the agent in ticks preceded its association with human disease, or the agent was discovered simultaneously with the disease but remained without a formal name or was misidentified as another species before its correct designation.

[a] Also known as deer tick virus, this agent was first detected in *Dermacentor andersoni* ticks in Colorado in 1952 (Thomas et al., 1960; Kuno et al., 2001).

SOURCES: Ricketts, 1909; Bates et al., 1921; Parker et al., 1924; Dunn, 1927; Weller and Graham, 1930; Wheeler et al., 1935; Davis et al., 1941; Florio et al., 1946; McLean et al., 1963; Western et al., 1970; Steere et al., 1983; Maeda et al., 1987; Quick et al., 1993; Bakken et al., 1994; Herwaldt et al., 1996; Parola et al., 1998; Buller et al., 1999; Kuno et al., 2001; Paddock et al., 2004; Shapiro et al., 2010; Spolidorio et al., 2010; Pritt et al., 2011; McMullan et al., 2012; Gugliotta et al., 2013; Clark et al., 2013, Clark et al., 2014; Pritt et al., 2014.

cases of vector-borne disease were associated with ticks, making these the most medically important group of arthropods in the United States. Lyme disease alone accounted for almost 75 percent of all reported cases of indigenously acquired vector-borne disease. This compilation does not include many other regionally important and occasionally life-threatening tick-borne infections such as Colorado tick fever (CTF), tick-borne relapsing fever, and Heartland virus infection that are not nationally notifiable (Forrester et al.; 2015, Yendell et al., 2015; Pastula et al., 2014). In comparison, indigenously acquired mosquito- and flea-borne diseases comprised only approximately 5 percent of the nationally reported cases of vector-borne disease for 2013.

Since 2000, the numbers of reported cases of notifiable tick-borne diseases in the United States have followed consistent upward trends (see Figures A8-1, A8-2, and A8-3). During 2000–2008, the annual reported incidence of Rocky Mountain spotted fever (RMSF) in the United States increased from 1.7 to 9.4 cases per million persons, representing the steepest rise to the highest rate ever recorded (Openshaw et al., 2010). Likewise, from 2000 to 2007, the incidence of infections caused by *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* increased linearly, from 0.80 to 3.0 and 1.4 to 3.0 cases per million population, respectively (Dahlgren et a1., 2011). Nonetheless, these figures underestimate the true burden of tick-borne infections (IOM, 2011). Recent analyses of Lyme disease statistics provide a salient example. Using data acquired from a survey of 7 large commercial laboratories in the United States that performed tests for Lyme disease during 2008, investigators identified an estimated 240,000 to 440,000 source patients for that year (Hinckley et al., 2014). Although Lyme disease is the most commonly reported arthropod-borne infection in the United States, fewer than 30,000 cases were reported to the CDC in 2008, suggesting that national surveillance underestimates the annual magnitude of Lyme disease by about a factor of 10.

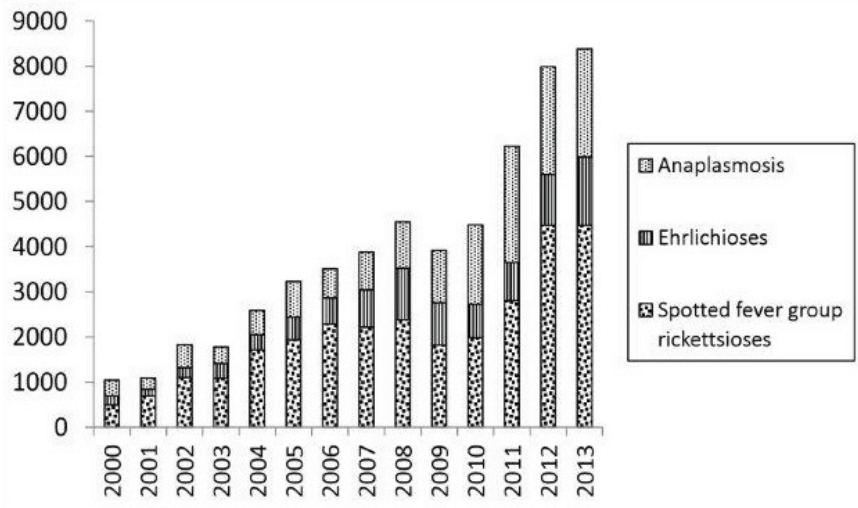

FIGURE A8-1 Reported cases of spotted fever group rickettsioses (including Rocky Mountain spotted fever, *Rickettsia parkeri* rickettsiosis, and 364D rickettsiosis), ehrlichioses (including *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and *Ehrlichia muris*-like ehrlichioses), and anaplasmosis in the United States, 2000–2013.

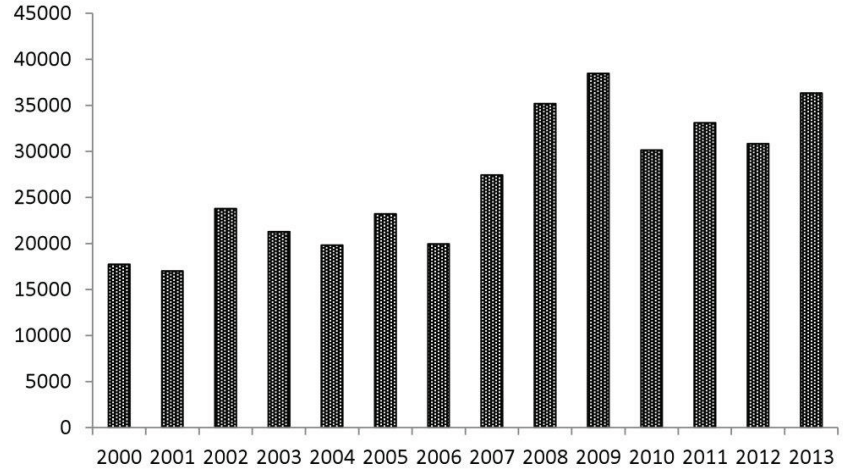

FIGURE A8-2 Reported cases of Lyme disease in the United States, 2000–2013. SOURCE: Adams et al., 2014 (CDC) (http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html).

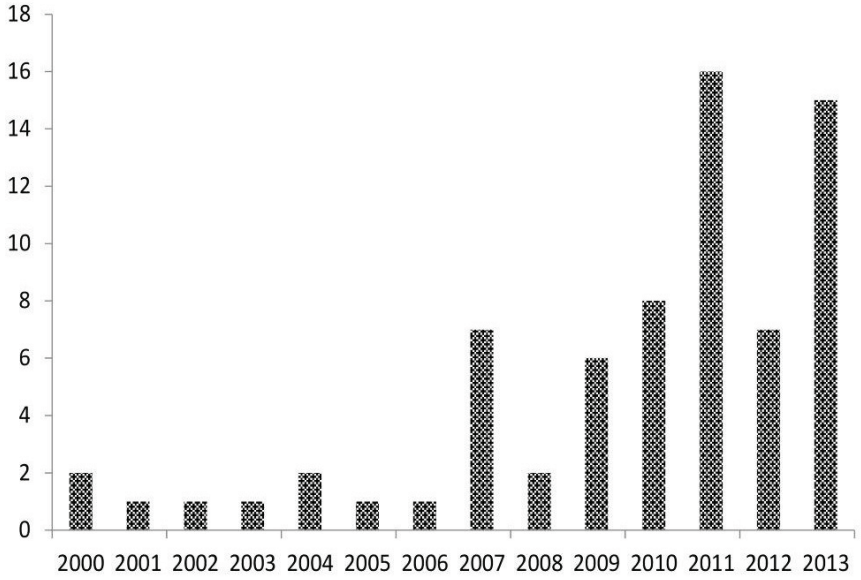

FIGURE A8-3 Reported cases of Powassan virus disease in the United States, 2000–2013. SOURCE: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention (http://www.cdc.gov/powassan/statistics.html).

Similar trends in rising case counts of tick-borne diseases have been identified in other countries of the Western Hemisphere. In several states of Brazil, the number of reported cases of Brazilian spotted fever (BSF) has risen steadily during the last decade (see Figure A8-4; Amâncio et al., 2011; Barros e Silva et al., 2014). Since 2004, RMSF has reemerged in many regions of Mexico, particularly in the states of Baja California, Sonora, and Yucatan (Zavala-Castro et al., 2008; Bustamente Moreno and Pon Méndeza, 2010a; Álvarez Hernández and Contreras Soto, 2013). Similar trends have been recognized in Colombia (Hidalgo et al., 2007, 2011) and Panama (Estripeaut et al., 2007; Tribaldos et al., 2011), where RMSF reemerged more than 50 years after the sentinel outbreaks were identified in these countries during the first half of the 20th century (Patino et al., 1937; Rodaniche and Rodaniche, 1950).

Collectively, these observations highlight several recurring themes: (1) the scope and magnitude of tick-borne diseases are continuously evolving and expanding; (2) changes in the distribution and determinants of these diseases may occur over relatively brief intervals of time and space; and (3) the epidemiology of historically recognized tick-borne infections may evolve alongside the discovery of newly characterized pathogens. The following discussion examines the growing list of tick-borne pathogens, explores new perspectives on the pathogenesis of these infections in humans, and briefly considers certain aspects of the dynamic and multifaceted natural histories of these diseases in the Western Hemisphere.

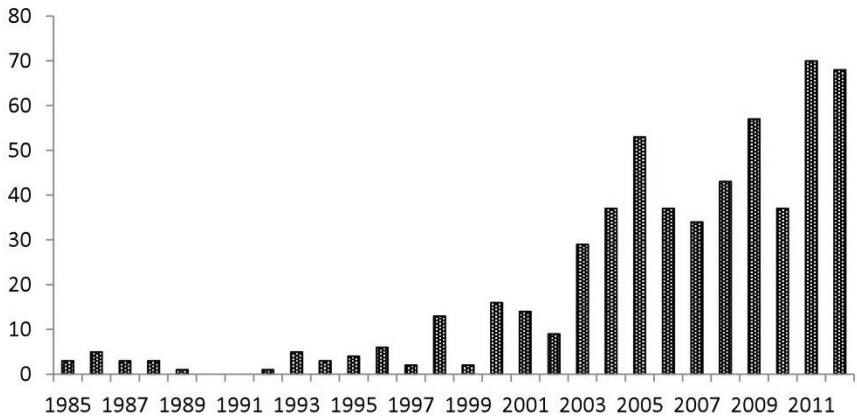

FIGURE A8-4 Reported cases of laboratory-confirmed Brazilian spotted fever in São Paulo State, Brazil, 1985–2012.
SOURCE: Data from São Paulo State government's *Centro de Vigilância Epidemiológica "Alexandre Vranjac" (CVE)*.

The Expanding Diversity of Tick-Borne Pathogens

Thirteen newly recognized tick-borne pathogens have been identified and characterized in the Western Hemisphere during the last 20 years (see Table A8-1). Perhaps more remarkable is the diversity of organisms represented by these newly identified pathogens, including several arboviruses and various *Borrelia, Ehrlichia*, and *Rickettsia* species, as well as the recognition that certain tick species can transmit multiple pathogens, in some cases as many as seven. It is highly probable that many tick-borne viruses and bacteria will be discovered throughout the enormously broad and ecologically diverse expanse of the Western Hemisphere. By example, a molecular survey of host-seeking western black-legged ticks (*Ixodes pacificus*) and small mammals from a single county bordering San Francisco Bay in California revealed an unprecedented seven *Borrelia* species, including two emerging human pathogens and two species novel for North America (Fedorova et al., 2014). Some recently identified tick-borne agents, such as *Rickettsia parkeri*, *Rickettsia* 364D (provisionally named *Rickettsia philipii*), and lineage II Powassan virus (also known as deer tick virus), represent bacteria or viruses that were identified in ticks many decades before they were formally recognized as human pathogens, including some that are evincing increased disease burdens (Kuno et al., 2001; Paddock et al., 2004; Shapiro et al., 2010; Nofchissey et al., 2013; El Khoury et al., 2013). Nonetheless, many of these agents are new to science and medicine, and even virulent pathogens, such as Heartland virus, continue to be identified, often by coupling classical diagnostic methods like cell culture isolation and electron microscopy with evolving molecular technologies (McMullan et al., 2012; Goldsmith et al., 2013).

The initial recognition of a tick-borne disease agent typically lags behind its detection in nature. Indeed, these agents characteristically afflict human populations as cryptic or misidentified infectious processes for decades or even centuries before these are correctly characterized. RMSF was identified in a "typhus fever" patient who died in Maryland in 1901 by testing autopsy tissues 90 years later. This retrospective diagnosis preceded the first official description of RMSF in the eastern United States by 30 years (Dumler, 1991). Historical accounts of a life-threatening, typhus-like illness, afflicting 44 persons in a small settlement in North Carolina during the summer of 1759, also document an illness clinically and epidemiologically suggestive of RMSF, 140 years before the disease was "discovered" in the western United States in 1900 (Tigertt, 1987). A febrile, highly lethal disease locally called *febre pintada* (spotted fever) has been recorded in the Brazilian state of Minas Gerais, an area where BSF is endemic (Amâncio et al., 2011), since the beginning of the 17th century (Magalhães, 1952).

The Lyme disease spirochete, *Borrelia burgdorferi* sensu stricto, hereinafter *B. burgdorferi*, is another case in point. DNA of *B. burgdorferi* was amplified from archival specimens of white-footed mice (*Peromyscus leucopus*) collected in Massachusetts during the 1890s (Marshall et al., 1994) and from black-legged ticks (*Ixodes scapularis*) collected on Long Island, New York during the 1940s

(Persing et al., 1990), which establishes the presence of the pathogen in vector and reservoir hosts in the northeastern United States decades before the formal recognition of Lyme disease. Willy Burgdorfer's epochal discovery of this spirochete in *I. scapularis* ticks collected from vegetation on Shelter Island, New York, represents one of the major biomedical breakthroughs of the 20th century (Burgdorfer et al., 1982; Burgdorfer, 1984). Lyme disease was initially attributed to a single bacterial species, but subsequently was found to be caused by several closely related species forming the ever-expanding *B. burgdorferi* sensu lato (s. l.) complex. Nineteen additional species have been confirmed or proposed since *B. burgdorferi* was characterized and named in 1984 (Margos et al., 2011; Rudenko et al., 2011; Ivanova et al., 2013), and more undescribed species await characterization (Fedorova et al., 2014). *Borrelia burgdorferi* was the sole member of the complex thought to infect humans in North America for 3 decades until *B. bissettii*-like spirochetes were detected in three residents of a rural community in north-coastal California (Girard et al., 2011), and *B. americana* and *B. andersonii* were incriminated as human pathogens in the southeastern United States (Clark et al., 2013). More recently, *B. americana*-like strains were recovered from patients residing in the northeastern, southeastern, northwestern, and southwestern United States (Clark et al., 2014).

Discovery of new agents has important ramifications that may change clinical and epidemiological perceptions of previously identified tick-borne infections. In Missouri, investigators used molecular methods to discriminate infections caused by *Ehrlichia ewingii* from those caused by *E. chaffeensis* (Buller et al., 1999). That finding revealed a clinically and ecologically similar illness previously obscured because of overlapping disease manifestations and a shared tick vector. Indeed, surveys examining the relative prevalence of *Ehrlichia* spp. in reservoir hosts and *Amblyomma americanum* ticks in the United States suggest that *E. ewingii* occurs in these species at frequencies similar to, or in some cases greater than, infection with *E. chaffeensis* (Paddock and Yabsley, 2007). However, *E. ewingii* appears to cause a milder illness, particularly in immunosuppressed patients. Without molecular methods, these infections would have remained submerged among those caused by *E. chaffeensis*, contributing to a falsely heterogeneous description of *E. chaffeensis* ehrlichiosis. Likewise, an ehrlichial species very closely related to *Ehrlichia muris* (designated the *E. muris*-like agent) identified by molecular methods from patients in Minnesota and Wisconsin, appears to cause most and perhaps all of the serologically diagnosed cases of ehrlichiosis in the upper Midwestern United States, where neither *E. chaffeensis* nor *E. ewingii* are endemic (Pritt et al., 2011; Hoang Johnson et al., 2015). Because the *E. muris*-like agent appears to cause milder disease in humans than *E. chaffeensis,* and is transmitted by *I. scapularis* ticks rather than by *A. americanum* ticks (Stromdahl et al., 2014), the clinical, epidemiological, and ecological features of these diseases are distinct (Hoang Johnson et al., 2015).

Equally telling is a recent example involving arboviruses. A retrospective evaluation of 14 patients diagnosed with Powassan encephalitis in New York State from 2004 to 2012 yielded laboratory and epidemiological evidence indicating that many of these cases were caused by lineage II (i.e., deer tick virus) rather than lineage I (i.e., classical Powassan) virus (El Khoury et al., 2013). The ecologies of the two lineages of Powassan virus are markedly different: lineage I is maintained principally between *Ixodes cookei* ticks and groundhogs (*Marmota momax*) or *Ixodes marxi* and striped skunks (*Mephitis mephitis*), whereas lineage II is maintained predominantly between *I. scapularis* and deer mice (*Peromyscus maniculatus*). Because *I. cookei* and *I. marxi* ticks rarely attach to humans, the number of Powassan cases caused by lineage I is almost certainly lower than those caused by lineage II Powassan virus which is more readily transmitted to humans by *I. scapularis* ticks. Furthermore, *I. scapularis* ticks may be coinfected with lineage II Powassan virus, *B. burgdorferi*, *A. phagocytophilum*, or other zoonotic agents that can confound the clinical and epidemiological features of Powassan encephalitis caused by lineage II virus. Close examination of three other newly recognized tick-borne diseases further illustrates the foregoing general trends.

Rickettsia parkeri

Disease caused by *R. parkeri* was first described in 2004 (Paddock et al., 2004). Unrecognized infections undoubtedly have occurred in humans for many years before the index case, as suggested by descriptions of non-fatal cases of RMSF associated with attachment-site ulcers from coastal areas of Virginia and Maryland during the 1920s and 1930s. Although *R. parkeri* rickettsiosis and RMSF are clinically, epidemiologically, and ecologically distinct diseases (Paddock and Goddard, 2015; Romer et al., 2011), cases of *R. parkeri* rickettsiosis have been embedded among national surveillance data for RMSF for decades. Accordingly, the reporting category for RMSF in the United States was modified in 2010 to include diseases caused by *R. parkeri* and other spotted fever group rickettsioses (Openshaw et al., 2010). Through 2014, cases of *R. parkeri* rickettsiosis have been identified from Alabama, Florida, Georgia, Kentucky, Maryland, Mississippi, North Carolina, Texas, and Virginia. Moreover, the magnitude of *R. parkeri* rickettsiosis is likely greater than currently appreciated because 8–56 percent of *Amblyomma maculatum* ticks, the principal vector species, are infected with *R. parkeri* (Paddock and Goddard, 2015). By comparison, *Rickettsia rickettsii*, the etiologic agent of RMSF, was detected in only 1 (0.02 percent) of 5,286 *Dermacentor variabilis* ticks removed from humans during one U.S. study from 1997–2009 (Stromdahl et al., 2011).

During the last decade, *R. parkeri* was detected in at least three human-biting *Amblyomma* tick species in Argentina, Brazil, Peru, and Uruguay, and more than 15 cases of *R. parkeri* rickettsiosis were identified in South America though 2014

(Romer et al., 2011, 2014). In Brazil, the discovery in 2010 of a second pathogen, closely related to *R. parkeri* and designated *Rickettsia* sp. Atlantic rainforest (Spolidorio et al., 2010), helped solve an epidemiological conundrum created by vastly different clinical features described for the same tick-borne disease. During 2007–2012, 734 laboratory confirmed cases of BSF were reported to the National Disease Surveillance System in Brazil, including 180 (24.5 percent) and 324 (44 percent) from the states of Santa Catarina and São Paulo, respectively. Surprisingly, no BSF-associated deaths were reported from Santa Catarina during this period, whereas the case-fatality rate of BSF in São Paulo was approximately 41 percent (Barros e Silva et al., 2014). A careful comparison of clinical characteristics of BSF patients in Santa Catarina versus São Paulo revealed marked differences in severity, which suggests that cases designated as "BSF" in these two states were in fact two distinct diseases (Angerami et al., 2009). Although no significant differences were identified between the frequency of fever, rash, or malaise, the rates of hemorrhage, severe neurological manifestations, and death differed notably (see Table A8-2). Acarological surveys from different regions of Santa Catarina subsequently identified *Rickettsia* sp. Atlantic rainforest infecting approximately 3–9 percent of human-biting *Amblyomma* spp. ticks in these areas with no evidence of *R. rickettsii* (Medeiros et al., 2011; Barbieri et al., 2014).

TABLE A8-2 Comparison of Selected Signs and Symptoms Reported for Patients with Laboratory-Confirmed Brazilian Spotted Fever in the States of São Paulo and Santa Catarina, Brazil, During 2003–2006. No Significant Differences in the Frequency of Fever, Rash, or Malaise Were Identified; Nonetheless, Significant Differences in Severe Manifestations and Death Were Apparent

Sign or Symptom	São Paulo (n = 126)	Santa Catarina (n = 61)	*P* value
Fever	112 (89 percent)	58 (95 percent)	0.16
Rash	44 (35 percent)	30 (49 percent)	0.06
Malaise	73 (58 percent)	35 (57 percent)	0.9
Adenopathy	5 (4 percent)	30 (49 percent)	< 0.01
Petechiae	46 (36 percent)	5 (8 percent)	< 0.01
Hemorrhage	33 (26 percent)	1 (2 percent)	
Hypotension	30 (24 percent)	2 (3 percent)	< 0.01
Coma	24 (19 percent)	0	< 0.01
Convulsion	18 (14 percent)	0	< 0.01
Death	46 (37 percent)	0	< 0.01

SOURCE: Adapted from Angerami et al., 2009.

These findings strongly suggest that reported cases of tick-borne spotted fever in Santa Catarina are caused by a *Rickettsia* species different than the pathogen associated with classical BSF in São Paulo.

Borrelia miyamotoi

Until PCR and sequencing techniques came into routine use during the early 1990s, *B. miyamotoi* had been categorized unknowingly with *B. burgdorferi* s. l. for more than a decade. First described in Japan in 1995 and named in honor of tick researcher Kenji Miyamoto, this relapsing-fever group spirochete was isolated initially from *Ixodes persulcatus* ticks and the blood of a rodent (*Apodemus argenteus*) (Fukunaga et al., 1995). *Borrelia miyamotoi* was subsequently detected in North America in *I. scapularis* ticks, and 1.9–2.5 percent of host-seeking nymphs collected in Connecticut, Maryland, New Jersey, New York, or Rhode Island were found to contain this *Borrelia* species (Scoles et al., 2001). In Canada, *B. miyamotoi* was detected in 23 (0.5 percent) of 4,938 *I. scapularis* ticks collected by passive surveillance in eight provinces during 2012 (Dibernardo et al., 2014). *Borrelia miyamotoi* is passed transstadially and transovarially within *I. scapularis* ticks, and the white-footed mouse has been incriminated as a reservoir host. Approximately 12 percent of all *Borrelia*-positive ticks detected in the areas surveyed by Scoles et al., (2001) were infected with *B. miyamotoi* versus the Lyme disease spirochete *B. burgdorferi* (88 percent), which indicates that a sizable proportion of spirochete-positive ticks previously thought to contain *B. burgdorferi* by microscopy were instead infected with this novel *Borrelia*. The authors also posited, correctly as it turned out, that at least some of the *B. burgdorferi* infections reported earlier in wild-caught *I. scapularis* larvae were probably *B. miyamotoi*, not *B. burgdorferi*. The foregoing assumptions are supported convincingly by experimental and field and laboratory evidence for *I. scapularis* and other members of the medically relevant *I. persulcatus* group of ticks, such as *I. pacificus* (Lane and Burgdorfer, 1987; Rollend et al., 2013; Padgett et al., 2014). In the study by Lane and Burgdorfer (1987), spirochetes visualized in tissue smears of *I. pacificus* F2 larval progeny, but not those present in all three parasitic stages of the F1 generation, were reactive with a monoclonal antibody (H5332) once deemed specific for *B. burgdorferi*, but now recognized to be more broadly reactive with other borreliae.

A 13-year survey carried out in 24 of California's 58 counties revealed that about half of spirochete-infected *I. pacificus* adults assayed for borreliae were infected with *B. miyamotoi* and the other half with *B. burgdorferi* s. l. (Padgett et al., 2014). These results have important epidemiological implications, namely, that a considerable percentage of adult ticks thought to be infected with *B. burgdorferi* when assayed 20–30 years earlier using less specific serological methods are likely to have been infected with *B. miyamotoi,* and that the risk of human exposure to *B. burgdorferi* and *B. miyamotoi* following the bite of

an adult *I. pacificus* is similar (Padgett et al., 2014). Nevertheless, the risk in California of acquiring infection with either spirochete from an adult *I. pacificus* tick is very low, as less than 1 percent of 6,036 tested adult ticks were infected with either *B. burgdorferi* or *B. miyamotoi*. By comparison, 3.2 percent of 2,188 nymphal *I. pacificus* were infected with *B. burgdorferi* versus 1.4 percent with *B. miyamotoi*.

On a global scale, multiple species of *Ixodes* ticks and several small mammals and birds are known to host *B. miyamotoi* in Asia, Europe, and North America. In the United States, *B. miyamotoi* infects white-footed mice in the northeastern and north-central regions (Barbour et al., 2009), and wild turkeys (*Meleagris gallopavo*) in the south-central region (Scott et al., 2010); whereas, nothing is known about its vertebrate hosts in the western United States. As is true for so many emerging tick-borne illnesses, the full clinical spectrum produced by different genogroups of *B. miyamotoi* requires clarification. What is clear, however, is that infection with *B. miyamotoi* may cause much more than a mild relapsing fever-like illness (Krause et al., 2013, 2014; Chowdri et al., 2013), as reported recently for an elderly patient who developed meningoencephalitis following infection with *B. miyamotoi* in the northeastern United States (Gugliotta et al., 2013).

Heartland Virus and Other Potentially Tick-Borne Arboviruses

The first human cases of Heartland virus disease were discovered when the virus was cultured serendipitously from blood specimens of two Missouri patients who were suspected initially to have *E. chaffeensis* ehrlichiosis (McMullan et al., 2012). Although the cell cultures showed cytopathic effects, no ehrlichial morulae were identified; subsequently, electron microscopy identified a *Bunyavirus*, and next-generation sequencing further characterized this pathogen as a newly recognized *Phlebovirus* (see Figure A8-5A). Because Heartland virus grows slowly in Vero cells that traditionally are used to isolate arboviruses, this virus may not have been readily discovered if the clinical samples had been received by an arbovirology laboratory. Prior to this discovery, no human pathogenic *Phlebovirus* was known to occur in the Western Hemisphere (Matsuno et al., 2014). The most closely related virus, severe fever with thrombocytopenia syndrome virus described from China in 2011, demonstrates only 70 percent homology on nucleic acid sequencing (Yu et al., 2011; McMullan et al., 2012). Despite its recent identification in 2009, and given the similar clinical features of Heartland virus with other tick-borne bacterial or rickettsial diseases, it is likely that cases of Heartland virus disease may have been misdiagnosed clinically as one of these diseases in the United States for many years (Figure A8-5B). The percent nucleotide divergence of Heartland virus strains from various locations in the United States suggest that these foci had been evolving separately for quite some time (Muehlenbachs et al., 2014). Through 2014, cases of Heartland virus disease,

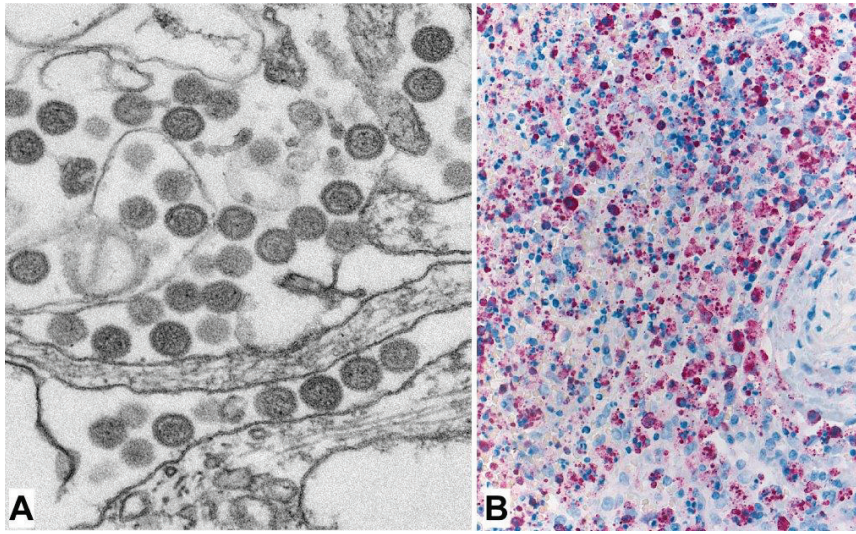

FIGURE A8-5 A. Electron photomicrograph of Heartland virus in cell culture. B. Immunohistochemical staining of Heartland virus antigens (red) in the spleen of a patient who died in 2004.
SOURCES: Images courtesy of Cynthia Goldsmith, CDC; Sherif Zaki, CDC.

including several deaths, have been identified in Georgia, Kentucky, Missouri, Tennessee, and Oklahoma (Pastula et al., 2014; CDC unpublished data), and it is likely that the distribution of Heartland virus in the United States will resemble closely that of its vector, *A. americanum* (Savage et al., 2013).

The recent discovery of Bourbon virus, a newly recognized *Thogotovirus* isolated from an ill patient in Kansas, resulted from a careful laboratory assessment of a patient suspected initially to be infected with Heartland virus (Kosoy et al., 2015). Although it is currently unknown how Bourbon virus is transmitted to humans, the initial case-patient reported tick exposure and removed an imbedded tick several days prior to the onset of illness characterized by fever, fatigue, thrombocytopenia, and leukopenia. Despite treatment with doxycycline and other antimicrobial agents, the patient failed to improve, developed multiorgan failure and died 11 days after illness onset from cardiopulmonary arrest. Testing of the patient's specimen for Heartland virus antibodies using plaque reduction neutralization revealed a unique virus that was subsequently identified by next-generation sequencing and phylogenetic analysis as a member of the genus *Thogotovirus*. Thogotoviruses primarily associated with hard or soft ticks (McCauley et al., 2012), and field studies are in progress to determine if Bourbon virus is yet another example of the expanding diversity of tick-borne pathogens.

The Expanding Clinical Spectrum of Tick-Borne Diseases

Our understanding of the pathogenesis of tick-borne diseases has become more nuanced during the last 50 years, augmented by the identification of host factors that place persons at risk for more severe illness, and by the recognition that some strains of a specific pathogen may dictate specific manifestations or severity. Increasing awareness of the astonishing complexity among the pathogen, its vector, and the human host has been leveraged by transformational advances in diagnostic techniques that accurately provide species and strain identity of the infectious agent.

Underrecognized Manifestations of Previously Recognized Pathogens

During 2012–2013, three cases of Lyme carditis associated with sudden cardiac deaths were identified by postmortem examinations of patients ranging in age from 26 to 38 years (see Figure A8-6; Ray et al., 2013). Prior to this report, only four deaths attributed to Lyme carditis had been described since the initial characterization of the disease in the early 1980s. Indeed, fatal carditis is considered an extremely rare manifestation of Lyme disease. A retrospective evaluation

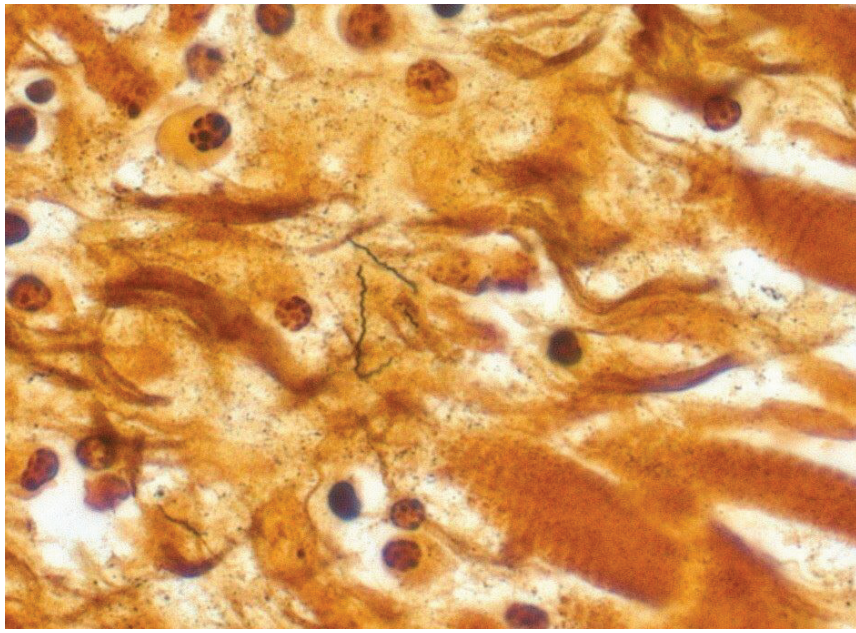

FIGURE A8-6 *Borrelia burgdorferi* spirochete identified by using Warthin-Starry silver impregnation technique in heart tissue of a patient with sudden cardiac death.
SOURCE: Image courtesy of Atis Muehlenbachs, CDC.

of 121,894 cases of Lyme disease from seven selected high-incidence states that occurred between 1995 and 2013 identified two suspected cases of fatal Lyme disease carditis, representing only 0.002 percent of the total patients and 0.1 percent of the 1,696 cases for whom carditis was documented (Forrester et al., 2014). Nonetheless, the frequency of sudden death attributable to cardiac infection with *B. burgdorferi*, albeit rare, may be greater than previously believed.

Before 2003, nearly all reported cases of Powassan virus disease experienced severe neurologic illness, usually meningoencephalitis (Artsob, 1988; Gholam et al., 1999). Since then, several cases without neurologic features have been documented (Hoang Johnson et al., 2010). In addition, an increasing number of aseptic meningitis cases due to Powassan virus infections have been reported (Minnesota Department of Health, 2014). These less severe clinical presentations were associated mainly with lineage II Powassan virus infections originating from the Midwest (Neitzel et al., 2013; Hoang Johnson et al., 2010; Ebel et al., 1999; Brackney et al., 2008). Some of these perceived differences in severity between the two lineages might be due to increased recognition and testing in certain locations of potentially less severe disease cases (Neitzel et al., 2013; Hoang Johnson et al., 2010; Hinten et al., 2008); whereas, ecological data suggest that there has been a true increase in the circulation of lineage II Powassan virus over the last 30 years (Nofchissey et al., 2013).

Strain-Specific Variations Associated with Virulence and Tissue Tropism

Advanced molecular techniques have allowed strain separation of *R. rickettsii* isolates obtained from patients in North, Central, and South America. In the United States and Mexico, two predominant genogroups have been associated with disease in humans, while cases of RMSF in Central and South America have been associated exclusively with a third distinct genogroup (Paddock et al., 2014; Labruna et al., 2014). Case-fatality rates of RMSF in Costa Rica, Panama, Colombia, Brazil, and Argentina characteristically are three to four times greater than those observed in the United States, and an unusually virulent genogroup of *R. rickettsii* may be responsible for the higher case-fatality rates in many Latin American countries (Parola et al., 2009; Labruna et al., 2014).

In north coastal California, 13 outer-surface-protein (*ospC*) allelles belonging to 12 genotypes of *B. burgdorferi* were identified among several thousand host-seeking *I. pacificus* nymphs (Girard et al., 2009). Approximately 20 *ospC* genotypes had been described from North America through 2008, including at least 4 and possibly as many as 9 that are invasive for humans. The most prevalent genotype in northern California is a novel strain, designated H3, previously not detected in *B. burgdorferi*-infected *I. scapularis* ticks in the northeastern United States, or in humans anywhere. Surprisingly, strain H3 was found in 25 percent of 222 *B. burgdorferi*-infected nymphs collected from dense woodlands in Mendocino County (Girard et al., 2009). The presence of this strain, and the

absence of a few other strains causing disseminated disease in people in the northeastern United States, especially the highly invasive *ospC* genotypes I and K, may represent one of several factors contributing to the low incidence of Lyme disease in California. Intriguingly, serum specimens from 24 percent of the subjects tested from a community at high risk for Lyme disease in the same county were PCR positive for *B. burgdorferi* infection (Girard et al., 2011). Among 20 *B. burgdorferi*-infected study subjects whose spirochete DNA could be typed, 95 percent contained the highly invasive *ospC* genotype A even though only 11 percent of infected nymphs collected countywide harbored this strain (Girard et al., 2009).

Co-Infections with Multiple Tick-Borne Agents

Increasing awareness that a single tick species may contain multiple and varied pathogenic agents has leveraged medical awareness that human hosts may be infected simultaneously by two or more pathogens following a single tick bite or from concurrent single-pathogen tick attachments. The most widely described examples represent co-infections transmitted by *I. scapularis* and include instances of Lyme disease and babesiosis, Lyme disease and human anaplasmosis, and Lyme disease, human anaplasmosis, and babesiosis (Krause et al., 1996; Swanson et al., 2006; Horowitz et al., 2013). The risk for co-infection with multiple pathogens differs by geographic location and depends to a large degree on the prevalence of the pathogens in reservoir hosts and tick species. Nonetheless, the risk of exposure to multiple pathogens following a single tick bite are not well understood, and a recent study suggests that certain co-infections may occur at frequencies other than predicted by independent assortment of the various pathogens. Specifically, evaluation of questing nymphal *I. scapularis* ticks collected in Dutchess County, New York, during 2011–2012 revealed 83 percent more co-infections with *B. microti* and *B. burgdorferi* than predicted by chance alone; whereas, fewer confections with *B. microti* and *A. phagocytophilum* were identified than predicted by chance (Hersh et al., 2014).

In the United States, the highest frequencies of co-infections in humans have been reported from New England (Swanson et al., 2006). From one study, 75 (39 percent) of 192 patients from Massachusetts and Connecticut diagnosed with Lyme disease, human anaplasmosis, or babesiosis during 1997–2000 were co-infected with two or more pathogens (Krause et al., 2002). Co-infected patients are significantly more likely to present with a greater diversity of signs and symptoms, as well as longer durations of illness, caused in part by a delay in diagnosis of the secondary or tertiary co-infection (Krause et al., 2002; Horowitz et al., 2013). Co-infections also occur with Powassan virus and *A. phagocytophilum* (Hoang Johnson et al., 2010), and with Heartland virus and *Ehrlichia chaffeensis* (CDC, unpublished data).

Host Factors and Clinical Expression of Disease

African-American male patients in the United States with glucose-6-phosphate dehydrogenase (G6PD) deficiency have a greater likelihood of experiencing severe or fatal RMSF than patients with normal G6PD activity (Walker et al., 1983a,b). Specifically, the genotype represented by G6PD A- was identified four times more often than the expected frequency in that cohort of RMSF patients. The overall frequency of G6PD A- also is generally higher among the at-risk population of several Latin America countries versus the United States and may contribute to the much greater case-fatality rates associated with *R. rickettsii* infections in South America. For example, the overall case-fatality rate of BSF in Minas Gerais State during 2000 to 2008 was 40 percent (Amâncio et al., 2011), and in São Paulo State during 2007–2012 it was 41 percent (Barros e Silva et al., 2014). By contrast, contemporary RMSF case-fatality rates in the United States have been less than 5 percent (Openshaw et al., 2010).

Advanced age is a risk factor for disease severity for many tick-borne infections. By example, the age-specific incidence for ehrlichiosis and anaplasmosis show a striking age-related increase in frequency among older persons (Demma et al., 2005b). Cholesterol dependence by the pathogenic bacteria *E. chaffeensis* and *A. phagocytophilum* may correlate with greater disease severity in older patients, because cholesterol levels typically rise with increasing age, and these bacteria lack the genes necessary for the biosynthesis of lipid A (Lin and Rikihisa, 2003). Furthermore, symptoms of babesiosis are more diverse, longer lasting, and more frequently require hospitalization in elderly patients than in younger individuals (Krause et al., 2003). Despite children being tested for Heartland virus, none has tested positive, and all known cases of Heartland virus disease have occurred in adults (Pastula et al., 2014), and the few deaths attributed to Heartland virus occurred in persons older than 60 years of age, many of whom had co-morbid conditions (Muehlenbachs et al., 2014; CDC, unpublished data). Although it is unclear why older adults are more likely to have more clinically apparent or severe disease when infected with Heartland virus, this finding mirrors what occurs with many other viral infections, such as West Nile virus and influenza virus (Lindsey et al., 2010; Quandelacy et al., 2014).

Recent Epidemiological and Ecological Shifts in Ticks and Tick-Borne Diseases

Tick-borne zoonoses are highly sensitive to manifold factors, often anthropogenic, that include microclimate, climate, host availability, habitat fragmentation, invasive forest pathogens and land use (Levia et al., 2012; Swei et al., 2012; Pfäffle et al., 2013; Léger et al., 2013). Changes in one or more of these variables often create ecological ripples across landscapes that culminate in modified environments favorable for the propagation and perpetuation of certain tick vectors. These dynamic and cumulative processes, associated intimately with concurrent

movements of pathogens, reservoir hosts, and host species, result in the emergence of tick-borne infections in human populations that reside or intrude into regions newly colonized by the particular tick species (Ogden et al., 2013). On a microscopic level, the perception of ticks as "crawling pins" has evolved into a far more complex host–pathogen association, as microbiome analyses reveal a remarkable diversity of bacteria and viruses that coexist within these arthropods and likely affect pathogen transmission.

Epidemiological Changes Over Time and Space

During the 1970s, most cases of CTF occurred among males aged 20-39 years (Goodpasture et al., 1978; Spruance and Bailey, 1973). More recently CTF cases in Montana, Utah, and Wyoming from 1995–2003 occurred in a higher proportion of females and people older than 50 years (Brackney et al., 2010). Changes in care-seeking behavior, testing, or surveillance practices, or true differences in exposures during recreational activities among persons of all ages and both sexes, may underlie these demographic changes. Overall, the number of CTF cases has decreased dramatically from more than 200 cases diagnosed per year in the United States from 1970–1984 to a median of 55 cases per year from 1987–2001 and only 5 cases per year from 2002—2012 (Bowen, 1988; Marfin and Campbell, 2005; Yendell et al., 2015). This decline may be an artifact of changes in testing and reporting practices. For example, Colorado historically reported the largest number of CTF cases (Bowen, 1988; Tsai, 1991; Marfin and Campbell, 2005), and when CTF was removed from the list of notifiable conditions in Colorado in 1997, the number of nationally reported cases plummeted (Yendell et al., 2015).

The ecology and epidemiology of Lyme disease on the West Coast differs markedly from that in the northeastern United States. Californians are exposed to infected *I. pacificus* ticks predominantly in rural or semirural settings year-round in less populated northern counties while they recreate or work outdoors versus mainly peridomestic exposure in suburban areas in the Northeast (Lane et al., 1992; Salkeld et al., 2014). Risk factors for exposure to *I. pacificus* nymphs in California include spending time in forested areas having an annual growing degree-day range of 2,600 to 3,000 (Eisen et al., 2006), and having contact with wood by sitting atop logs, gathering firewood, or woodcutting (Lane et al., 1992, 2004). Moreover, Lyme disease is a highly focal disease in California, with more than four-fifths of cases reported from northern counties, especially the sparsely populated northwestern coastal region (Eisen et al., 2006). Acarological, demographic, and climatic factors contribute to the low statewide incidence (about 0.2 cases per 100,000 population) of Lyme disease. Most residents live in suburban or urban areas in more arid southern counties where both the projected (Eisen et al., 2006) and known acarologic risks are low (Lane et al., 2013). Although more than 37 million residents reside in the state's 58 counties, half of the entire population is concentrated in Los Angeles, San Diego, Orange, Riverside, and

San Bernardino counties, where *B. burgdorferi*-infected ticks are rarely encountered and diurnal questing by *I. pacificus* nymphs is minimal (Lane et al., 2013).

Range Expansions of Medically Important Ticks

Many shifts in the distribution and abundance of tick species in North America occurred during the last 50 years. Some of these observations reflect the ebb and flow of species movement within an ancestral range that is modulated by constant human intervention (Spielman et al., 1993; Paddock and Yabsley, 2007; Paddock and Goddard, 2015). The rise of *I. scapularis* populations throughout much of eastern North America reflects a series of anthropogenically driven events during the mid-19th century to the present, whereby reversal of post-Columbian deforestation, increased deer abundance, and increased development and use of forested sites by humans resulted in a proliferation of black-legged ticks and recognition of at least 7 *I. scapularis*-borne pathogens (Spielman et al., 1985; Spielman et al., 1993; IOM, 2011; Pritt et al., 2011; Hoang Johnson et al., 2015). Current data indicate that the expansion of geographic range of black-legged ticks has proceeded largely through progressive and local migration events from southern populations to proximate northern locations (Khatchikian et al., 2015). In the mid-1980s, Ipswich, Massachusetts, represented the northernmost distribution of *I. scapularis* in the northeastern United States. Within a decade, however, this tick had spread northward to the Bar Harbor region in Maine.

Similar changes in the distribution and abundance of black-legged tick populations took place across the central and upper Midwestern United States during the past 30 years. A significant increase in the prevalence of *I. scapularis* on white-tailed deer occurred along the Wisconsin River Valley during 1981–1994 (Riehle and Paskewitz, 1996). No specimens of *I. scapularis* were found during an acarological survey in the early 1990s around Chicago; whereas, established populations were detected within 20 years throughout several northeastern Illinois counties located adjacent to and inclusive of this large metropolitan area (Rydzewski et al., 2012). Recent incursions of black-legged ticks also were identified across the lower peninsula of Michigan (Hamer et al., 2010). Finally, *B. burgdorferi*-infected *I. scapularis* ticks have spread throughout southern Canada, with recent invasion events in southwestern Quebec and southern Ontario being ascribed to long-distance dispersal by migratory birds (Ogden et al., 2013).

Acarological surveys conducted at the end of the 20th century identified established populations of *A. americanum* throughout many regions of New York State where none were noted approximately 50 years earlier (Means and White, 1997; Paddock and Yabsley, 2007). Similarly, retrospective assessment of various state and national tick collections revealed only isolated and sporadic records of *A. americanum* in Nebraska during 1944–1973. Collection records of this species increased markedly during 1987–2011, and it now represents the second most frequently reported tick in the state (Cortinas and Spomer, 2013). Indeed, various

data sources suggest a general northward shift in the distribution of *A. americanum* throughout much of the Midwest and northeastern United States during the last 50 years (Springer et al., 2014).

The distribution of *A. maculatum* was described 70 years ago as occupying a narrow band extending 100–150 miles inland from the Gulf Coast of Texas, across the southern states, to the Atlantic Coast of South Carolina. Since then, collection data suggest qualitative and quantitative changes in the historically accepted range of *A. maculatum*, including established populations more than 250 miles inland in several states bordering the Gulf of Mexico as well as northern expansions in many mid-Atlantic states (Nadolny et al., 2015). Established populations of *A. maculatum* now occur in several states where few or no records of this species existed during the first half of the 20th century, including Arkansas, Delaware, Kansas, Kentucky, North Carolina, Oklahoma, and Virginia, and confirmed cases of *R. parkeri* rickettsiosis have been documented in several of these states (Paddock and Goddard, 2015).

Amblyomma variegatum, the tropical bont tick, was introduced into the Caribbean on cattle imported from Senegal, Gambia, and Guinea to Guadeloupe during the early 1800s, and is now established on more than 15 islands in this region (Parola et al., 2009; Léger et al., 2013). *A. variegatum* is a primary vector of *Rickettsia africae*, the etiologic agent of African tick-bite fever and *R. africae*-infected populations of *A. variegatum* have been identified throughout much of the Caribbean, including Guadeloupe, Martinique, St. Lucia, Nevis, St. Kitts, Antigua, Dominica, Montserrat, and the U.S. Virgin Islands (Kelly et al., 2010). In 1998, the first case of African tick-bite fever acquired in the Western Hemisphere was documented in a traveler from Guadeloupe, and it is likely that many other undocumented cases occur annually in the Caribbean (Parola et al., 1998).

Capybara and the Reemergence of Brazilian Spotted Fever

The state of São Paulo in southeastern Brazil has accounted for nearly half of all laboratory-confirmed cases of BSF during the past 30 years (Barros e Silva et al., 2014). Notably, the number of BSF cases has gradually increased from 3 in 1985 to 68 in 2013, with annual fatality rates always around 40 percent. Most of these cases occurred in rural areas where capybaras (*Hydrochoerus hydrochaeris*) sustain large populations of the tick vector *Amblyomma sculptum,* a member of the *Amblyomma cajennense* species complex (Nava et al., 2014). Besides its role as a major host for *A. sculptum*, capybaras also serve as reservoir hosts of *R. rickettsii* (Labruna, 2013). Capybaras infected with *R. rickettsii* can maintain rickettsiae in their bloodstream for several days to weeks at levels sufficient to infect noninfected ticks, thereby amplifying rickettsial infection among the tick population (Souza et al., 2009). Because *R. rickettsii* is only partially maintained through vertical transmission in *A. sculptum* (Soares et al., 2012), capybaras play a major role in the ecology of *R. rickettsii* in BSF-endemic areas in São Paulo.

Indeed, the increasing number of BSF cases has been directly attributed to the increasing expansion of capybara populations in the state of São Paulo during the same period (Labruna, 2013).

During the last 5 decades, the state of São Paulo has gone through substantial landscape transformation, in which three factors have played a major role in the expansion of capybaras: (1) the tremendous agricultural expansion of sugar cane, a preferred food source of capybara, that has developed over recent years throughout Brazil as ethanol has emerged as a biofuel; (2) the creation of strict laws prohibiting the hunting of wildlife, which protect capybaras even in urban and semiurban areas; and (3) the elimination of natural predators of capybara such as jaguars from these same areas (Ferraz et al., 2007; Moreira et al., 2013). Capybara are remarkably prolific breeders, and females can birth 6 pups each year; indeed, the density of capybara in some BSF-endemic areas of the state of São Paulo are estimated to be 40 to 60-fold higher than the densities observed in natural environments, such as Pantanal and Amazon (Ferraz et al., 2010). Collectively these changes have modified capybara behavior such that large peridomestic populations exist that enhance the likelihood of human exposure to tick vectors of *R. rickettsii*.

Rhipicephalus sanguineus and the Ecology of RMSF in Western North America

For almost a century, *D. variabilis* and *Dermacentor andersoni* were considered to be the most important vectors of RMSF in the United States. During 2002–2004, 16 cases of Rocky Mountain spotted fever were identified in a 6700-km^2 region of rural eastern Arizona (Demma et al., 2005a). From an epidemiological perspective, this was a highly unusual event, as only 8 cases of RMSF had been reported from the entire state during the preceding 15 years. An ecological assessment revealed large numbers of free-roaming dogs and *Rhipicephalus sanguineus* ticks in all life stages distributed abundantly at the case-patient households and surrounding environment (Nicholson et al., 2006). *R. rickettsii* was detected in approximately 5 percent of the nonengorged ticks and 10 percent of the engorged ticks (Eremeeva, 2012). Neither *D. variabilis* nor *D. andersoni* were found at any of the case-acquisition sites despite repeated acarological evaluations. Although investigators in the 1930s determined that *Rh. sanguineus* was a competent experimental vector of *R. rickettsii* (Parker et al., 1933), a role for this tick in the natural history of RMSF in the United States had not been demonstrated before this outbreak. It is now recognized that the specific ecological circumstances that perpetuated epidemic RMSF in these small communities also exist in other areas of Arizona. During 2003–2012, more than 250 authochthonous cases of RMSF, including 19 deaths, were reported from this state alone. During 2009–2012, the average annual incidence of RMSF in Arizona was approximately 136 cases per 100,000 persons, more than 150 times the U.S. national average (Drexler et al., 2014).

In-depth studies in Mexico during the 1940s identified *Rh. sanguineus* as a vector for *R. rickettsii* during outbreaks of RMSF in several northern states including Sonora and Sinaloa (Mariotte et al., 1944; Bustamente and Varela, 1947). In 2003, investigators in Mexicali, Mexico, determined that 60 percent of stray and privately owned dogs in the city were infested with *Rh. sanguineus* (Tinoco-Gracia et al., 2009). These findings heralded an epidemic of RMSF that occurred in Mexicali and other areas of Baja California during 2009, resulting in more than 1,000 confirmed and probable infections (Bustamente Moreno and Pon Méndez, 2010a). Surveys for *Rh. sanguineus* identified these ticks in all 14 districts of Mexicali, where 96 percent of the cases occurred (Sanchez et al., 2009; Bustamente Moreno and Pon Méndez, 2010b). These outbreaks are not necessarily generalizable to other regions of the United States or to other countries in the Americas. However, it appears that *Rh. sanguineus* is a far more important vector of RMSF than previously believed. While *R. rickettsii* has been detected or isolated from *Rh. sanguineus* ticks at different BSF-endemic areas of southeastern Brazil (Cunha et al., 2009; Gehrke et al., 2009; Moraes-Filho et al., 2009; Pacheco et al., 2011), cases have not been associated with this tick species in Brazil. Thus far, *R. rickettsii*-infected *Rh. sanguineus* ticks have been collected only from areas where the classical vectors of *R. rickettsii—A. sculptum* and *Amblyomma aureolatum*—were also present (Labruna, 2009).

Conclusions and Future Perspectives

The contemporary pace of tick-borne pathogen discovery has produced a litany of newly recognized agents and may well escalate with the advent of metagenomics. Many candidate tick-borne pathogens already have been suggested based on data from animal experimentation, serologic reactivity to particular antigens, or anecdotal reports of non-characterized illnesses following tick bites (see Table A8-3). Focused endeavors to determine viral etiologies of tick-borne disease in the New World will undoubtedly reveal novel pathogens. In North America, there are currently four tick-associated viral agents of disease—Colorado tick fever virus, Heartland virus, and Powassan lineage I and II viruses. This likely represents only a fraction of the pathogenic tick-borne arboviruses in the Western Hemisphere. Several viruses in the *Bunyaviridae* and *Arenaviridae* families have been detected with increasing frequency in human-biting ticks, and it would be surprising if some of these occasionally did not infect people (Briese et al., 2014; Pinto da Silva et al., 2005; McElroy Horne and Vanlandingham, 2014; Sayler et al., 2014). Recently, virome analyses of *I. scapularis* and *D. variabilis* have uncovered multiple previously undescribed viruses, including novel nairoviruses and phleboviruses of potential relevance for public health (Tokarz et al., 2014b; Matsuno et al., 2014). Similar studies examining the viromes of *I. pacificus, D. occidentalis*, *Rh. sanguineus*, *A. maculatum*, *O. hermsi*, and other human-biting ticks in the United States will likely yield other tick-borne viruses.

TABLE A8-3 Candidate Tick-Borne Pathogens in the Western Hemisphere

Agent	Tick associate	Data to suggest pathogenicity	Reference(s)
Rickettsia canadensis	*Haemaphysalis leporispalustris*	Tick transmission of agent to guinea pigs; fever in infected guinea pigs; seroconversion to *R. canadensis* antigens in febrile patients.	McKiel et al., 1967 Burgdorfer, 1968 Bozeman et al., 1970 Wenzel et al., 1986
Rickettsia sp. Tillamook	*Ixodes pacificus*	Death of infected mice.	Hughes et al., 1976
Rickettsia sp. *parumapertus*	*Dermacentor parumapertus*	Fever in infected guinea pigs.	Philip and Hughes, 1953
Rickettsia rhipicephali	*Rhipicephalus sanguineus*	Fever and death in infected meadow voles.	Burgdorfer et al., 1975
Punta Salinas virus	*Ornithodoros amblus*	Undifferentiated febrile illness in persons bitten by *O. amblus*	Clifford et al., 1980
Sixgun City virus	*Argas cooleyi*	Illness in suckling mice; related viruses cause disease in humans.	Yunker et al., 1972
Tacaribe virus	*Amblyomma americanum*	Associated with a non-fatal infection in a laboratory worker.	Sayler et al., 2014
Borrelia bissettii	*Ixodes pacificus*	DNA detected in human serum; cause of illness in Europe; induces pathology in mice.	Girard et al., 2011 Schneider et al., 2008

Virus isolations and serological surveys from multiple species of wildlife collected in California suggest that CTF virus, or a very similar virus, is widespread across several west-central counties, far beyond the historically recognized and relatively limited distribution of CTF in the far northeastern corner of that state (Lane et al., 1982). Remarkably, no pathogenic viruses of humans have been catalogued from among the 28 species of human-biting ixodid ticks in South America (Guglielmone et al., 2006; Nava et al., 2014), where the potential for similar discoveries is enormous.

Prospecting archival specimen banks containing blood, serum, or tissue samples from patients for whom a recognized tick-borne disease was suspected but unconfirmed with existing assays is expected to yield novel agents as well. A retrospective survey of 29 patients for whom RMSF was suspected clinically and epidemiologically identified 3 individuals who seroconverted to *Ehrlichia* antigens and subsequent prospective evaluation at a hospital in eastern Georgia where one of these patients had been treated identified 3 more individuals with acute ehrlichiosis (Fishbein et al., 1987). In a similar manner, fatal cases of Heartland virus infection have been identified retrospectively from autopsy specimens from at least 4 case-patients initially suspected to have died from ehrlichiosis (Muehlenbachs et al., 2014; CDC, unpublished data).

Investigators throughout the 20th century commented on the diversity of prokaryotic species that co-infected medically important ticks (Cowdry, 1925; Steinhaus, 1942; Martin and Schmidtmann, 1998), but contemporary molecular techniques now reveal a far greater assemblage of bacterial residents than ever imagined. The growing recognition that complex and interacting microbial communities exist within medically important ticks, and that these interactions may influence pathogen prevalence, heralds an important and evolving area of research in tick-borne diseases (Clay et al., 2006). *Amblyomma americanum*, a vector of *E. chaffeensis* and *E. ewingii*, contains many other bacterial taxa, including Acidobacteriales, Bacilliales, Burkholderiales, Caulobacterales, Enterobacteriales, Flavobacteriales, Legionellales, Pseudomonadales, Rhizobiales, Rickettsiales, and Sphingomonadales (Clay et al., 2008; Ponnusamy et al., 2014; Williams-Newkirk et al., 2014). Additional data indicate that the composition of these bacterial communities are remarkably dynamic and change in response to environmental stimuli, during acquisition of blood meals, and between various life stages (Menchaca et al., 2013; Williams-Newkirk et al., 2014). The complexity of tick-associated microbial communities extends beyond bacteria: *A. americanum* is also a vector of Heartland virus, as well as host to another bunyavirus (Lone Star virus) and a newly identified rhabdovirus (Long Island tick rhabdovirus) (Swei et al., 2013; Tokarz et al., 2014a).

In certain situations, microbial communities appear to modulate the frequency of pathogen transmission. By using an antibiotic to perturb the composition of the indigenous gut microbiota of larval *I. scapularis* ticks, investigators detected qualitative alterations in the tick peritrophic membrane that significantly reduced colonization by *B. burgdorferi* spirochetes (Narasimhan et al., 2014). During a multistate survey of field-collected *I. scapularis* ticks, male ticks harboring an uncharacterized and presumably nonpathogenic *Rickettsia* species had significantly lower rates of infection with *B. burgdorferi* than *Rickettsia*-free males (Steiner et al., 2008). Also, ticks cannot simultaneously maintain more than one *Rickettsia* species by vertical transmission, as demonstrated by the exclusion of transovarial transmission of *R. rickettsii* by *Rickettsia peacockii* in *D. andersoni* (Burgdorfer et al., 1981), *Rickettsia rhipicephali* by *Rickettsia montanensis*

in *D. variabilis* (Macaluso et al., 2002), and *R. rickettsii* by *Rickettsia bellii* in *Amblyomma dubitatum* (Sakai et al., 2014). This rickettsial interference phenomenon presumably explains the uneven distribution of RMSF cases in the Bitterroot Valley of western Montana. Almost all patients acquire the infection from exposures to *D. andersoni* ticks on the western side of the valley. It is believed that *R. peacockii*, a nonpathogenic *Rickettsia* species infecting up to 80 percent of *D. andersoni* ticks collected from the eastern side of the valley, prevents transovarial transmission of *R. rickettsii*. In contrast, almost all tick isolates of *R. rickettsii* from the Bitterroot Valley have originated from its western slopes, where only 8–16 percent of *D. andersoni* are infected with *R. peacockii* (Burgdorfer et al., 1981). These findings could stimulate new areas of research and exploration regarding control and prevention of certain tick-borne diseases by manipulating the microbiome of medically important ticks using strategies similar to those proposed for control of certain insect pests (Douglas, 2007).

Investigators who consider the complete ecological framework in which a particular tick-borne pathogen resides are poised to make remarkable discoveries. Unprecedented advances have been made in genetics, biochemistry, and molecular biology, but in many cases, these advances are applied to pursuits that are independent of or entirely unconnected to the natural history of the disease. In this context, many endeavors in this discipline result in highly compartmentalized studies that rely on elegant and sophisticated techniques, but are divorced from the ecology of the pathogen, its vectors, and its hosts (IOM, 2008). Future explorations in tick-borne disease research hold tremendous promise, but erosion of expertise in many core disciplines could seriously undermine the foundation upon which many past discoveries were based. During the last 50 years, transformational advances in molecular technology have fueled the discovery and characterization of multiple tick-borne pathogens. Ironically, the number of scientists who pursue fundamental studies in tick taxonomy, vector–pathogen–host interactions, and basic transmission dynamics, has diminished considerably during this same period. The identification of ticks should be founded predominantly on morphology, and it is axiomatic that any subsequent ecologic or epidemiologic conclusions based on an incorrect identification of the vector species are erroneous and misleading. Unfortunately, diminishing numbers of contemporary investigators have a solid foundation in tick taxonomy (Estrada-Peña et al., 2013). Identification methods based on molecular data or proteome analysis such as mass spectrometry are under development, but are not considered reference standards for tick identification, and cannot be developed or validated without convincing morphological correlation. Emphasis in these areas of expertise clearly needs to be maintained and fortified. Despite an increasingly diverse catalogue of tick-borne diseases in the United States and other countries of the Western Hemisphere, many of the resources that are necessary to properly explore the transmission dynamics, reservoir hosts, and human epidemiologic

and clinical features for tick-borne pathogens are declining, particularly in state health departments (Hadler et al., 2014).

A more complete understanding of the ecological and biological factors responsible for expanding distributions of tick vectors and reservoir hosts, as well as the microbiological dynamics within ticks that modulate pathogen emergence, is needed to develop effective strategies to mitigate the rising incidence of tick-borne diseases in the Americas. To achieve this goal, more vector biology training centers and programs that offer balanced curricula fostering ecological as well as molecular and quantitative approaches are essential, as are more academic and governmental-funded field-related job opportunities (Glaser, 2010). Also, a more concerted effort must be made by national funding agencies to promote and support field studies because these form the bedrock upon which successful epidemiological interventions are based. Lastly, vector-borne disease scientists need to become better advocates for their work, and more clearly articulate the benefits of this research to public health and welfare (Porter, 2014).

Dedication

We are honored to dedicate this article to the memory of Dr. Willy Burgdorfer (1925–2014), a world-class medical entomologist who not only discovered the Lyme disease spirochete, but made many other significant discoveries about various tick-borne agents that have a bearing on public health. He also was a very generous and kind individual, and a highly effective mentor who willingly shared his broad expertise about ticks and tick-borne diseases while training numerous neophytes in the field. Several decades ago, Willy informed one of us presciently: "There is no such thing as a clean tick." How accurately he foretold the future with his characteristic acumen.

References

Adams, D. A., R. A. Jajosky, U. Ajani, J. Kriesman, P. Sharp, D. H. Onwen, A. W. Schley, W. J. Anderson, A. Grigoryan, A. E. Aranas, M. S. Wodajo, J. P. Abellera, and Centers for Disease Control and Prevention. 2014. Summary of notifiable diseases – United States, 2012. *Morbidity and Mortality Weekly Report* 61:1-121.

Álvarez Herández, G., and J. J. Contreras Soto. 2013. Letalidad por fiebre manchada po *Rickettsia rickettsii* en pacientes de un hospital pediátrico del estado de Sonora, 2004-2012. *Salud Pública de México* 55:151-152.

Amâncio, F. F., V. D. Amorim, T. L. Chamone, M. G. de Brito, S. B. Calic, A. C. Leite, G. L. Fraga, and M. L. Ferraz. 2011. Epidemiological characteristics of Brazilian spotted fever in Minas Gerais State, Brazil, 2000-2008. *Cad Saúde Pública, Rio de Janeiro* 27:1969-1976.

Angerami, R. N., A. M. R. da Silva, E. M. M. Nascimento, et al. 2009. Brazilain spotted fever: Two faces of the same disease? A comparative study of clinical aspects between an old and a new endemic area in Brazil. *Clinical Microbiology and Infectious Disease* 15:207-208.

Artsob, H. 1988. Powassan encephalitis. In *The arboviruses: Epidemiology and ecology*, edited by T. P. Monath. Vol. 4. Boca Raton, FL: CRC Press. Pp. 29-49.

Bakken, J. S., J. S. Dumler, S. M. Chen, M. R. Eckman, L. L. Van Etta, and D. H. Walker. 1994. Human granulocytic ehrlichiosis in the upper Midwest United States. A new species emerging? *Journal of the American Medical Association* 272:212-218.

Barbieri, A. R. M., J. M. Filho, F. A. Nieri-Bastos, J. C. Souza Jr., M. P. Szabo, and M. B. Labruna. 2014. Epidemiology of *Rickettsia* ssp. strain Atlantic rainforest in a spotted fever-endemic area of southern Brazil. *Ticks and Tick-Borne Diseases* 5:848-853.

Barbour, A. G., J. Bunikis, B. Travinsky, A. G. Hoen, M. A. Diuk-Wasser, D. Fish, and J. I. Tsao. 2009. Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. *American Journal of Tropical Medicine and Hygiene* 81:1120-1131.

Barros e Silva, P. M. R., S. C. Pereira, L. X. Fonseca, F. V. P. Maniglia, S. V. Oliveira, and E. P. de Caldas. 2014. Febre maculosa: Uma análise epidemiológica dos registros do sistema de vigilância do Brasil. *Scientia Plena* 10:1-9.

Bates, L. B., L. H. Dunn, and J. H. St. John. 1921. Relapsing fever in Panama. The human tick, *Ornithodoros talaje*, demonstrated to be the transmitting agent of relapsing fever in Panama by human experimentation. *American Journal of Tropical Medicine* 1:183-210.

Bowen, G. S. 1980. Colorado tick fever. In: *The arboviruses: Epidemiology and ecology*, edited by T. P. Monath. Vol. 4. Boca Raton, FL: CRC Press. Pp. 159-176.

Bozeman, F. M., B. L. Elisberg, J. W. Humphries, K. Runcik, and D. R. Palmer, Jr. 1970. Serologic evidence of *Rickettsia canada* infection in man. *Journal of Infectious Diseases* 121:367-371.

Brackney, D. E., R. A. Nofchissey, K. A. Fitzpatrick, et al., 2008. Stable prevalence of Powassan virus in *Ixodes scapularis* in a northern Wisconsin focus. *American Journal of Tropical Medicine and Hygiene* 79:971-973.

Brackney, M. M., A. A. Marfin, J. E. Staples, L. Stallones, T. Keefe, W. C. Black, and G. L. Campbell. 2010. Epidemiology of Colorado tick fever in Montana, Utah, and Wyoming, 1995-2003. *Vector Borne and Zoonotic Diseases* 10:381-385.

Breise, T., R. Chowdry, A. Travassos de Rosa, S. K. Hutchinson, V. Popov, C. Street, R. B. Test, and W. I. Lipkin. 2014. Upolu virus and Aransas Bay viruses, two presumptive bunyaviruses, are novel members of the family Orthomyxoviridae. *Journal of Virology* 88:5298-5309.

Buller, R. S., M. Arens, S. P. Himmel, C. D. Paddock, J.W. Sumner, Y. Rikihisa, A. Unver, M. Gaudreault-Keener, F. A. Manian, A. M. Liddell, N. Schmulewitz, and G. A. Storch. 1999. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. *New England Journal of Medicine* 341:148-155.

Burgdorfer, W. 1968. Observations of *Rickettsia canada*, a recently described member of the typhus group rickettsiae. *Journal of Hygiene, Epidemiology, and Microbiology* 12:26-31.

Burgdorfer, W. 1984. Discovery of the Lyme disease spirochete and its relation to tick vectors. *Yale Journal of Biology and Medicine* 57:515-520.

Burgdorfer, W., S. F. Hayes, and A. J. Mavros. 1981. Nonpathogenic rickettsiae in *Dermacentor andersoni*: A limiting factor for the distribution of *Rickettsia rickettsii*. In: *Rickettsiae and rickettsial diseases*, edited by W. Burgdorfer and R. L. Anacker. New York: Academic Press. Pp. 585-594.

Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis. 1982. Lyme disease–a tick-borne spirochetosis? *Science* 216:1317-1319.

Bustamente, M. E., and G. Varela. 1947. IV. Estudios de fiebre manchada en Mexico: Papel del *Rhipicephalus sanguineus* en ala transmission de la fiebre manchada en la Republica Mexicana. *Revista del Instituto de Salubridad Y Enfermedades Tropicales* 8:139-141.

Bustamente Moreno, J. G., and A. Pon Méndez. 2010a. Actualización en la vigilancia epidemiológica de "rickettsiosis". Part I. *Epidemiológia Boletin* 6:1-4.

Bustamente Moreno, J. G., and A. Pon Méndez. 2010b. Actualización en la vigilancia epidemiológica de "rickettsiosis". Part II. *Epidemiológia Boletin* 7:1-3.

CDC (Centers for Disease Control and Prevention). 2014. Notice to readers: Final 2013 reports of nationally notifiable infectious diseases. *Morbidity and Mortality Weekly Report* 63:702-715.

Chowdri, H. R., J. L. Gugliotta, H. K. Goethert, P. J. Molloy, S. L. Sterling, and S. R. Telford III. 2013. *Borrelia miyamotoi* infection presenting as human granulocytic anaplasmosis: A case report. *Annals of Internal Medicine* 159:21-27.

Clark, K. L., B. Leydet, and S. Hartman. 2013. Lyme borreliosis in human patients in Florida and Georgia, USA. *International Journal of Medical Sciences* 10:915-931.

Clark, K. L., B. F. Leydet, and C. Threlkeld. 2014. Geographical and genospecies distribution of *Borrelia burgdorferi* sensu lato DNA detected in humans in the USA. *Journal of Medical Microbiology* 63:674-684.

Clay, K., O. Klyachko, N. Grindle, et al. 2008. Microbial communities and interactions in the Lone Star tick, *Amblyomma americanum*. *Molecular Ecology* 17:4371-4381.

Clifford, C. M., H. Hoogstraal, F. J. Radovsky, D. Stiller, and J. E. Keirans. 1980. *Ornithodoros* (*Alectorobius*) *amblus* (Acarina: Ixodoidea: Argasidae): Identity, marine bird and human hosts, virus infections, and distribution in Peru. *Journal of Parasitology* 66:312-323.

Cortinas, R., and S. Spomer. 2013. Lone Star tick (Acari: Ixodidae) occurrence in Nebraska: Historical and current perspectives. *Journal of Medical Entomology* 50:244-251.

Cowdry, E. V. 1925. A group of microorganisms transmitted hereditarily in ticks and apparently unassociated with disease. *Journal of Experimental Medicine* 41:817-830.

Cunha, N. C., A. H. Fonseca, J. Rezende, T. Rozental, A. R. M. Favacho, J. D. Barreira, C. L. Massard, and E. R. S. Lemos. 2009. First identification of natural infection of *Rickettsia rickettsii* in the *Rhipicephalus sanguineus* tick, in the State of Rio de Janeiro. *Pesquisa Veterinaria Brasileira* 29:105-108.

Dahlgren, F., E. Mandel, J. Krebs, R. Massung, and J. H. McQuiston. 2011. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocyophilum* in the United States, 2000-2007. *American Journal of Tropical Medicine and Hygiene* 85:124-131.

Davis, G. E., H. L. Wynns, and D. L. Beck. 1941. Relapsing fever: *Ornithodoros parkeri*, a vector in California. *Public Health Reports* 56:2426-2428.

Demma, L. J., M. S. Traeger, W. L. Nicholson, C. D. Paddock, D. M. Blau, M. E. Eremeeva, G. A. Dasch, M .L. Levin, J. Singleton Jr., S. R. Zaki, J. E. Cheek, D. L. Swerdlow, and J. H. McQuiston. 2005a. Rocky Mountain spotted fever from an unexpected vector in Arizona. *New England Journal of Medicine* 353:587-594.

Demma, L. J., R. C. Holman, J. H. McQuiston, J. W. Krebs, and D. L. Swerdlow. 2005b. Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001-2002. *American Journal of Tropical Medicine and Hygiene* 73:400-409.

Dibernardo, A., T. Cote, N. H. Ogden, and L. R. Lindsay. 2014. The prevalence of *Borrelia miyamotoi* infection, and co-infections with other *Borrelia* spp. in *Ixodes scapularis* ticks collected in Canada. *Parasites and Vectors* 7:183.

Douglas, A. E. 2007. Symbiotic microorganisms: Untapped resources for insect pest control. *Trends in Biotechnology* 25:338-342.

Drexler, N., M. Miller, J. Gerding, S. Todd, L. Adams, F. S. Dahlgren, N. Bryant, E. Weis, K. Herrick, J. Francies, K. Komatsu, S. Piontkowski, J. Velascosoltero, T. Shelhamer, B. Hamilton, C. Eribes, A. Brock, P. Sneezy, C. Goseyun, H. Bendle, R. Hovet, V. Williams, R. Massung, and J. H. McQuiston. 2014. Community-based control of the brown dog tick in a region with high rates of Rocky Mountain spotted fever, 2012-2013. *PLoS ONE* 9: e112368.

Dumler, J. S. 1991. Fatal Rocky Mountain spotted fever in Maryland—1901. *JAMA* 265:718.

Dunn, L. H. 1927. Studies on the South American tick, *Ornithodoros venezuelensis* Brumpt, in Colombia. Its prevalence, distribution, and importance as an intermediate host of relapsing fever. *Journal of Parasitology* 13:249-255.

Ebel, G. D., I. Foppa, A. Spielman, and S. R. Telford, III. 1999. A focus of deer tick virus transmission in the north central United States. *Emerging Infectious Diseases* 5:570-574.

Eisen, R. J., R. S. Lane, C. L. Fritz, and L. Eisen. 2006. Spatial patterns of Lyme disease risk in California based on disease incidence data and modeling of vector-tick exposure. *American Journal of Tropical Medicine and Hygiene* 75:669-676.

El Khoury, M. Y., J. F. Camargo, J. L. White, et al., 2013. Potential role of deer tick virus in Powassan encephalitis cases in Lyme disease-endemic areas of New York, USA. *Emerging Infectious Diseases* 19:1926-1933.

Eremeeva, M. E. 2012. Molecular epidemiology of rickettsial diseases in North America. *Ticks and Tick-Borne Diseases* 3:331-336.

Estrada-Peña, A., J. S. Gray, O. Kahl, R. S. Lane, and A. M. Nijhof. 2013. Research on the ecology of ticks and tick-borne pathogens—methodological principles and caveats. *Frontiers in Cellular and Infection Microbiology* 3:1-12.

Estripeaut, D., M. G. Aramburú, X. Sáez-Llorens, H. A. Thompson, G. A. Dasch, C. D. Paddock, S. Zaki, and M. E. Eremeeva. 2007. Rocky Mountain spotted fever, Panama. *Emerging Infectious Diseases* 13:1763-1765.

Fedorova, N., J. E. Kleinjan, D. James, L. T. Hui, H. Peeters, and R. S. Lane. 2014. Remarkable diversity of tick or mammalian-associated borreliae in the metropolitan San Francisco Bay Area, California. *Ticks and Tick-Borne Diseases* 5:951-961.

Ferraz, K. M. P. M. B., S. F. B. Ferraz, J. R. Moreira, H. T. Z. Couto, and L. M. Verdade. 2007. Capybara (*Hydrochoerus hydrochaeris*) distribution in agroecosystems: A cross-scale habitat analysis. *Journal of Biogeogeography* 34:223-230.

Ferraz, K. M. P. M. B., B. Manly, and L. M. Verdade. 2010. The influence of environmental variables on capybara (*Hydrochoerus hydrochaeris*: Rodentia, Hydrochoeridae) detectability in anthropogenic environments of southeastern Brazil. *Population Ecology* 52:263-270.

Fishbein, D. B., L. A. Sawyer, C. J. Holland, E.B. Hayes, W. Okoroanyanwu, D. Williams, K. Sikes, M. Ristic, and J. E. McDade. 1987. Unexplained febrile illnesses after exposure to ticks: infection with an *Ehrlichia*? *Journal of the American Medical Association* 257:3100-3104.

Florio, L., M. D. Stewart, and E. R. Mugrage. 1946. The etiology of Colorado tick fever. *Journal of Experimental Medicine* 83:1-10.

Forrester, J. D., J. Meiman, J. Mullins, R. Nelson, S.H. Ertel, M. Cartter, C.M. Brown, V. Lijewski, E. Schiffman, D. Neitzel, E. R. Daly, A. A. Matthewson, W. Howe, L. A. Lowe, N. R. Kratz, S. Semple, P. B. Backenson, J. L. White, P. M. Kurpiel, R. Rockwell, K. Walker, D. H. Johnson, C. Steward, B. Batten, D. Blau, M. DeLeon-Carnes, C. Drew, A. Muehlenbachs, J. Ritter, J. Sanders, S. R. Zaki, C. Molins, M. Schriefer, A. Perea, K. Kugeler, C. Nelson, A. Hinckley, P. Mead, and Centers for Disease Control and Prevention (CDC). 2014. Update on Lyme carditis, groups at high risk, and frequency of associated sudden cardiac death—United States. *Morbidity and Mortality Weekly Report* 63:982-983.

Forrester, J. D., A. M. Kjemtrup, C. L. Fritz, N. Marsden-Haug, J. B. Nichols, L. A. Tengelsen, R. Sowadsky, E. DeBess, P. R. Cieslak, J. Weiss, N. Evert, P. Ettestad, C. Smelser, J. Iralu, R. J. Nett, E. Mosher, J. S. Baker, C. van Houten, E. Thorp, A. L. Geissler, K. Kugeler, and P. Mead. 2015. Tickborne relapsing fever—United States, 1990-2011. *Morbidity and Mortality Weekly Report* 64:58-60.

Fukunaga, M., Y. Takahashi, Y. Tsuruta, O. Matsushita, D. Ralph, M. McClelland, and M. Nakao. 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *International Journal of Systematic Bacteriology* 45:804-810.

Gehrke, F. S., G. S. Gazeta, E. R. Souza, A. Ribeiro, M. T. Marrelli, and T. T. S. Schumaker. 2009. *Rickettsia rickettsii, Rickettsia felis* and *Rickettsia* sp. TwKM03 infecting *Rhipicephalus sanguineus* and *Ctenocephalides felis* collected from dogs in a Brazilian spotted fever focus in the State of Rio de Janeiro/Brazil. *Clinical Microbiology and Infection* 15:267-268.

Gholam, B. I., S. Puksa, and J. P. Provias. 1999. Powassan encephalitis: A case report with neuropathology and literature review. *Canadian Medical Association Journal* 161:1419-1422.

Girard, Y. A., B. Travinsky, A. Schotthoefer, N. Fedorova, R. J. Eisen, L. Eisen, A. G. Barbour, and R. S. Lane. 2009. Population structure of the Lyme borreliosis spirochete *Borrelia burgdorferi* in the western black-legged tick (*Ixodes pacificus*) in northern California. *Applied and Environmental Microbiology* 75:7243-7252.

Girard, Y. A., N. Fedorova, and R. S. Lane. 2011. Genetic diversity of *Borrelia burgdorferi* and detection of *B. bissettii*-like DNA in serum of north-coastal California residents. *Journal of Clinical Microbiology* 49:945-954.

Glaser, V. 2010. An interview with Robert Lane, Ph.D. *Vector Borne and Zoonotic Diseases* 10:211-215.

Goldsmith, C. S., T. G. Ksiazek, P. E. Rollin, J. A. Comer, W. L. Nicholson, T. C. T. Peret, D. D. Erdman, W. J. Bellini, B. H. Harcourt, P. A. Rota, J. Bhatnagar, M. D. Bowen, B. R. Erickson, L. K. McMullan, S. T. Nichol, W. J. Shieh, C. D. Paddock, and S. R. Zaki. 2013. Cell culture and electron microscopy for identifying viruses in diseases of unknown cause. *Emerging Infectious Diseases* 19:886-891.

Goodpasture, H. C., J. D. Poland, D. B. Francy, G. S. Bowen, and K. A. Horn. 1978. Colorado tick fever: Clinical, epidemiological, and laboratory aspects of 228 cases in Colorado in 1973-1974. *Annals of Internal Medicine* 88:303-310.

Guglielmone, A. A., L. Beati, D. M. Barros-Battesti, M. B. Labruna, S. Nava, J. M. Venzal, A. J. Mangold, M. P. Szabo, J. R. Martins, D. Gonzalez-Acuna, and A. Estrada-Pena. 2006. Ticks (Ixodidae) on humans in South America. *Experimental and Applied Acarology* 40:83-100.

Gugliotta, J. L., H. K. Goethert, V. P. Berardi, and T. S. Telford III. 2013. Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *New England Journal of Medicine* 368:240-245.

Hadler, J. L., D. Patel, K. Bradley, J.M. Hughes, C. Blackmore, P. Etkind, L. Kan, J. Getchell, J. Blumenstock, J. Engel, and Centers for Disease Control and Prevention (CDC). 2014. National capacity for surveillance, prevention, and control of West Nile virus and other arbovirus infections—United States, 2004 and 2012. *Morbidity and Mortality Weekly Report* 63:281-284.

Hamer, S. A., J. I. Tsao, E. D. Walker, and G. J. Hickling. 2010. Invasion of the Lyme disease vector *Ixodes scapularis*: Implications for *Borrelia burgdorferi* endemicty. *EcoHealth* 7:47-63.

Hersh, M. H., R. S. Ostfeld, D. J. McHenry, M. Tibbetts, J. L. Brunner, M. E. Killilea, K. LoGiudice, K. A. Schmidt, and F. Keesing. 2014. Co-infection of blacklegged ticks with *Babesia microti* and *Borrelia burgdorferi* is higher than expected and acquired from small mammal hosts. *PLoS ONE* 9:e99348.

Herwaldt, B., D. H. Persing, E. A. Précigout, W. L. Goff, D. A. Mathiesen, P. W. Taylor, M. L. Eberhard, and A. F. Gorenflot. 1996. A fatal case of babesiosis in Missouri: Identification of another piroplasm that infects humans. *Annals of Internal Medicine* 124:643-650.

Hidalgo, M., L. Orejuela, P. Fuya, P. Carrillo, J. Hernandez, E. Parra, C. Keng, M. Small, J. P. Olano, D. Bouyer, E. Castaneda, D. Walker, and G. Valbuena. 2007. Rocky Mountain spotted fever, Colombia. *Emerging Infectious Diseases* 13:1058-1060.

Hidalgo, M., J. Miranda, D. Heredia, P. Zambrano, J. F. Vesga, D. Lizarazo, S. Mattar, and G. Valbuena. 2011. Outbreak of Rocky Mountain spotted fever in Córdoba, Colombia. *Memórias do Instituto Oswaldo Cruz* 106:117-118.

Hinckley, A. F., N. P. Connally, J. I. Meek, B. J. Johnson, M. M. Kemperman, K. A. Feldman, J. L. White, and P. S. Mead. 2014. Lyme disease testing by large commercial laboratories in the United States. *Clinical Infectious Diseases* 59:676-681.

Hinten, S. R., G. A. Beckett, K. F. Gensheimer, E. Pritchard, T. M. Courtney, S. D. Sears, J. M. Woytowicz, D. G. Preston, R. P. Smith Jr., P. W. Rand, E. H. Lacombe, M. S. Holman, C. B. Lubelczyk, P. T. Kelso, A. P. Beelen, M. G. Stobierski, M. J. Sotir, S. Wong, G. Ebel, O. Kosoy, J. Piesman, G. L. Campbell, and A. A. Marfin. 2008. Increased recognition of Powassan encephalitis in the United States, 1999-2005. *Vector-Borne and Zoonotic Diseases* 8:733-740.

Hoang Johnson, D. K., J. E. Staples, M. J. Sotir, D. M. Warshauer, and J. P. Davis. 2010. Tickborne Powassan virus infections among Wisconsin residents. *Wisconsin Medical Journal* 109:91-97.

Hoang Johnson, D. K., E. K. Schiffman, J. P. Davis, D. F. Neitzel, L. M. Sloan, W. L. Nicholson, T. R. Fritsche, C. R. Steward, J. A. Ray, T. K. Miller, M. A. Feist, T. S. Uphoff, J. J. Franson, A. L. Livermore, A. K. Deedon, E. S. Theel, and B. S. Pritt. 2015. Human infection with *Ehrlichia muris*-like pathogen, United States, 2007-2013. *Emerging Infectious Diseases* 21:1785-1790.

Horowitz, H. W., M. E. Aguero-Rosenfeld, D. Holmgren, D. McKenna, I. Schwartz, M.E. Cox, and G. P. Wormser. 2013. Lyme disease and human granulocytic anaplasmosis coinfection: Impact of case definition on coinfection rates and illness severity. *Clinical Infectious Diseases* 56:93-99.

Hughes, L. E., C. M. Clifford, R. Gresrink, et al. 1976. Isolation of a spotted fever group rickettsia from the Pacific coast tick, *Ixodes pacificus*, in Oregon. *American Journal of Tropical Medicine and Hygiene* 25:513-516.

IOM (Institute of Medicine). 2008. *Vector-borne diseases: Understanding the environmental, human health and ecological connections*. Washington, DC: The National Academies Press. Pp. 65-69.

IOM. 2011. *Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: The short-term and long-term outcomes*. Washington, DC: The National Academies Press. Pp. 221-266.

Ivanova, L. B., A. Tomova, D. González-Acuña, R. Murúa, C. X. Moreno, C. Hernández, J. Cabello, C. Cabello, T. .J. Daniels, H. P Godfrey, and F. C. Cabello. 2014. *Borrelia chilensis*, a new member of the *Borrelia burgdorferi* sensu lato complex that extends the range of this genospecies in the Southern Hemisphere. *Environmental Microbiology* 16:1069-1080.

Jongejan, F., and G. Uilenberg. 2004. The global importance of ticks. *Parasitology* 129(Suppl):S3-S14.

Kelly, P., H. Lucas, L. Beati, C. Yowell, S. Mahan, and J. Dame. 2010. *Rickettsia africae* in *Amblyomma variegatum* and domestic ruminants on eight Caribbean islands. *Journal of Parasitology* 96:1086-1088.

Khatchikian, C. E., M. A. Prusinski, M. Stone, P. B. Backenson, I. N. Wang, E. Foley, S. N. Seifert, M. Z. Levy, and D. Brisson. 2015. Recent and rapid population growth and range expansion of the Lyme disease tick vector, *Ixodes scapularis*, in North America. *Evolution* 69:1678-1689.

Kosoy, O. I., A. J. Lambert, D. J. Hawkinson, et al. 2015. Novel Thogotovirus associated with a febrile illness and death, United States 2014. *Emerging Infectious Diseases*. 21:760-764.

Krause, P. J., S. R. Telford III, A. Spielman, V. Sikand, and R. Ryan. 1996. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *Journal of the American Medical Association* 275:1657-1660.

Krause, P. J., K. McKay, C. A. Thompson, V. K. Sikand, R. Lentz, T. Lepore, L. Closter, D. Christianson, S. R. Telford, D. Persing, J. D. Radolf, A. Spielman, and Deer-Associated Infection Study Group. 2002. Disease-specific diagnosis of coinfecting tickborne zoonoses: Babesiosis, human granulocytic ehrlichiosis, and Lyme disease. *Clinical Infectious Diseases* 34:1184-1191.

Krause, P. J., K. McKay, J. Gadbaw, D. Christianson, L. Closter, T. Lepore, S. R. Telford 3rd, V. Sikand, R. Ryan, D. Persing, J. D. Radolf, A. Spielman, and Tick-Borne Infection Study Group. 2003. Increasing health burden of human babesiosis at endemic sites. *American Journal of Tropical Medicine and Hygiene* 68:431-436.

Krause, P. J., S. Narasimhan, G. P. Wormser, L. Rollend, E. Fikrig, T. Lepore, A. Barbour, and D. Fish. 2013. Human *Borrelia miyamotoi* infection in the United States. *New England Journal of Medicine* 368:291-293.

Krause, P. J., S. Narasimhan, G. P. Wormser, A. G. Barbour, A. E. Platonov, J. Brancato, T. Lepore, K. Dardick, M. Mamula, L. Rollend, T. K. Steeves, M. Diuk-Wasser, S. Usmani-Brown, P. Williamson, D. S. Sarksyan, E. Fikrig, D. Fish, and Tick-Borne Diseases Group. 2014. *Borrelia miyamotoi* sensu lato seroreactivity and seroprevalence in the northeastern United States. *Emerging Infectious Diseases* 20:1183-1190.

Kuno, G., H. Artsob, N. Karabatsos, K. R. Tsuchiya, and G. J. J. Chang. 2001. Genomic sequencing of deer tick virus and phylogeny of Powassan-related viruses in North America. *American Journal of Tropical Medicine and Hygiene* 65:671-676.

Labruna, M. B. 2009. Ecology of *Rickettsia* in South America. *Annals of the New York Academy of Sciences* 1166, 156-166.

Labruna, M. B. 2013. Brazilian spotted fever: The role of capybaras. In: J. R. Moreira et al. (eds.), *Capybara: Biology, use and conservation of an exceptional neotropical species*. New York: Springer Science+Business Media. Pp. 371-383.

Labruna, M. B., F. C. P. Santos, M. Ogrzewalska, E. M. M. Nascimento, S. Colombo, A. Marcili, and R. Angerami. 2014. Genetic identification of rickettsial isolates from fatal cases of Brazilian spotted fever and comparison with *Rickettsia rickettsii* isolates from the American continents. *Journal of Clinical Microbiology* 52:3788-3791.

Lane, R. S., R. W. Emmons, V. Devlin, D. V. Dondero, and B. C. Nelson. 1982. Survey for evidence of Colorado tick fever virus outside of the known endemic area in California. *American Journal of Tropical Medicine and Hygiene* 31:837-843.

Lane, R. S., and W. Burgdorfer. 1987. Transovarial and transstadial passage of *Borrelia burgdorferi* in the western black-legged tick, *Ixodes pacificus* (Acari: Ixodidae). *American Journal of Tropical Medicine and Hygiene* 37:188-192.

Lane, R. S., S. A. Manweiler, H. A. Stubbs, E. T. Lennette, J. E. Madigan, and P. E. Lavoie. 1992. Risk factors for Lyme disease in a small rural community in northern California. *American Journal of Epidemiology* 136:1358-1368.

Lane, R. S., D. B. Steinlein, and J. Mun. 2004. Human behaviors elevating exposure to *Ixodes pacificus* (Acari: Ixodidae) nymphs and their associated bacterial zoonotic agents in a hardwood forest. *Journal of Medical Entomology* 41:239-248.

Lane, R. S., N. Fedorova, J. E. Kleinjan, and M. Maxwell. 2013. Eco-epidemiological factors contributing to the low risk of human exposure to ixodid tick-borne borreliae in southern California, USA. *Ticks and Tick-Borne Diseases* 4:377-385.

Léger, E., G. Vourc'h, L. Vial, C. Chevillon, and K. D. McCoy. 2013. Changing distributions of ticks: Causes and consequences. *Experimental and Applied Acarology* 59:219-244.

Levia, T., M. Kirkpatrick, M. Mangel, and C. Wilmers. 2012. Deer, predators, and the emergence of Lyme disease. *Proceedings of the National Academy of Sciences* 109:10942-10947.

Lin, M., and Y. Rikihisa. 2003. *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* lack genes for lipid A biosynthesis and incorporate cholesterol for their survival. *Infection and Immunity* 71:5324-5331.

Lindsey, N. P., J. E. Staples, J. A. Lehman, and M. Fischer. 2010. Surveillance for human West Nile virus disease–United States, 1999—2008. *Morbidity and Mortality Weekly Report* 59:SS-2.

Macaluso, K. R., D. E. Sonenshine, S. M. Ceraul, and A. F. Azad. 2002. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. *Journal of Medical Entomology* 39:809-813.

Maeda, K., N. Markowitz, R. C. Hawley, M. Ristic, D. Cox, and J. E. McDade. 1987. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. *New England Journal of Medicine* 316: 853-856.

Magalhães, O. 1952. Contribuição ao conhecimento das doenças do grupo tifo exantematico. *Instituto Oswaldo Cruz, Rio de Janeiro*. 968pp.

Marfin, A., and G. Campbell. 2005. Colorado tick fever and related *Coltivirus* infections. In *Tick-borne diseases of humans*, edited by J. Goodman. Washington, DC: ASM Press. Pp. 143-149.

Margos, G., S. A. Vollmer, N. H. Ogden, and D. Fish. 2011. Population genetics, taxonomy, phylogeny and evolution of *Borrelia burgdorferi* sensu lato. *Infection, Genetics and Evolution* 11:1545-1563.

Mariotte, C. O., M. E. Bustamente, and G. Varela. 1944. Hallazgo del *Rhipicephalus sanguineus* Latreille infectado naturalmente con fiebre manchada de las Montañas Rocosas, en Sonora (Mexico). *Revista del Instituto de Salubridad Y Enfermedades Tropicales* 5:297-300.

Marshall, W. F., III, S. R. Telford III, P. N. Rys, B. J. Rutledge, D. Mathiesen, S. E. Malawista, A. Spielman, and D. H. Persing. 1994. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Peromyscus leucopus*. *Journal of Infectious Diseases* 170:1027-1032.

Martin, P. A., and E. T. Schmidtmann. 1998. Isolation of aerobic microbes from *Ixodes scapularis* (Acari: Ixodidae), the vector of Lyme disease in the eastern United States. *Journal of Economic Entomology* 91:864-868.

Matsuno, K., C. Weisend, M. Kajihara, C. Matysiak, B. N. Williamson, M. Simuunza, A. S. Mweene, A. Takada, R. B. Tesh, and H. Ebihara. 2015. Comprehensive molecular detection of tick-borne phleboviruses leads to the retrospective identification of taxonomically unassigned bunyaviruses and the discovery of a novel member of the genus Phlebovirus. *Journal of Virology* 89:594-604.

McCauley, J. W., S. Hongo, N. V. Kaverin, G. Kochs, R. A. Lamb, M. N. Matrosovich, D. R. Perez, P. Palese, R. M. Presti, and E. Rimstad. 2012. Family Orthomyxoviridae. In *Virus taxonomy: Classification and nomenclature of viruses,* edited by A. M. Q. King, M. J. Adams, E. B. Carstens, and E. J. Lefkowitz. Ninth Report of the International Committee of Taxonomy of Viruses. New York: Elsevier Inc. Pp. 749-761.

McElroy Horne, K., and D. L. Vanlandingham. 2014. Bunyavirus-vector interactions. *Viruses* 6:4373-4397.

McKiel, J. A., E. J. Bell, and D. B. Lackman. 1967. *Rickettsia canada*: A new member of the typhus group of rickettsiae isolated from *Haemaphysalis leporispalustris* ticks in Canada. *Canadian Journal of Microbiology* 13:503-510.

McLean, D. M., and R. P. B. Larke. 1963. Powassan and Silverwater viruses: Ecology of two Ontario arboviruses. *Canadian Medical Association Journal* 88:182-185.

McMullan, L. K., S. M. Folk, A. J. Kelly, A. MacNeil, C. S. Goldsmith, M. G. Metcalfe, B. C. Batten, C. G. Albarino, S. R. Zaki, P. E. Rollin, W. L. Nicholson, and S. T. Nichol. 2012. A new phlebovirus associated with severe febrile illness in Missouri. *New England Journal of Medicine* 367:834-841.

Means, R. G., and D. J. White. 1997. New distribution records of *Amblyomma americanum* (L.) (Acari: Ixodidae) in New York State. *Journal of Vector Ecology* 22:133-145.

Medeiros, A. P., A. P. de Souza, A. B. de Moura, M. S. Lavina, V. Belatto, A. Sartor, F. A. Nieri-Bastos, L. J. Richtzenhain, and M. B. Labruna. 2011. Spotted fever group *Rickettsia* infecting ticks (Acari: Ixodidae) in the state of Santa Catarina, Brazil. *Memorias do Instituto Oswaldo Cruz, Rio de Janeiro* 106:926-930.

Menchaca, A. C., D. K. Visi, O. F. Strey, P. D. Teel, K. Kalinowski, M. S. Allen, and P. C. Williamson. 2013. Preliminary assessment of microbiome changes following blood-feeding and survivorship in the *Amblyomma americanum* nymph-to-adult transition using semiconductor sequencing. *PLoS ONE* 6:e67129.

Moreira, J. R., K. M. P. M. B. Ferraz, E. A. Herrera, and D. W. Macdonald. 2013. *Capybara - biology, use and conservation of an exceptional Neotropical species*. New York: Springer, 419 pp.

Minnesota Department of Health. 2014. Powassan (POW) Virus Information for Health Professionals. Available at: http://www.health.state.mn.us/divs/idepc/diseases/powassan/hcp.html. Accessed on 16 December 2014.

Moraes-Filho, J., A. Pinter, R. C. Pacheco, T. B. Gutmann, S. O. Barbosa, M. A. Gonzales, M. A. Muraro, S. R. Cecilio, and M. B. Labruna. 2009. New epidemiological data on Brazilian spotted fever in an endemic area of the state of São Paulo, Brazil. *Vector-Borne and Zoonotic Diseases* 9:73-78.

Muehlenbachs, A., C. R. Fata, A. J. Lambert, C. D. Paddock, J. O. Velez, D. M. Blau, J. E. Staples, M. B. Karlekar, J. Bhatnagar, R. S. Nasci, and S. R. Zaki. 2014. Heartland virus-associated death in Tennessee. *Clinical Infectious Diseases* 59:845-850.

Nadolny, R., H. Gaff, J. Carlsson, and D. Gauthier. 2015. Comparative population genetics of two invading ticks: Evidence of the ecological mechanisms underlying tick range expansions. *Infection, Genetics and Evolution* 35:153-162.

Narasimhan, S., N. Rajeevan, L. Liu, Y. O. Zhao, J. Heisig, J. Pan, R. Eppler-Epstein, K. DePonte, D. Fish, and E. Fikrig. 2014. Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. *Cell Host and Microbe* 15:58-71.

Nava, S., L. Beati, M. B. Labruna, A. G. Cáceres, A. J. Mangold, and A. A. Guglielmone. 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (F.) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum*, and *Amblyomma sculptum* (Ixodida: Ixodidae). *Ticks Tick-borne Diseases* 5:252-276.

Neitzel, D. F., R. Lynfield, and K. Smith. 2013. Powassan virus encephalitis, Minnesota, USA. *Emerging Infectious Diseases* 19:686.

Nicholson, W. L., C. D. Paddock, L. Demma, M. Traeger, B. Johnson, J. Dickson, J. McQuiston, and J. Swerdlow. 2006. Rocky Mountain spotted fever in Arizona: Documentation of heavy environmental infestations of *Rhipicephalus sanguineus* at an endemic site. *Annals of the New York Academy of Sciences* 1078:338-341.

Nofchissey, R. A., E. R. Deardorff, T. M. Blevins, M. Anishcenko, A. Bosco-Lauth, E. Berl, C. Lubelczyk, J. P. Mutebi, A. C. Brault, G. D. Ebel, and L. A. Magnarelli. 2013. Seroprevalence of Powassan virus in New England deer, 1979—2010. *American Journal of Tropical Medicine and Hygiene* 88:1159-1162.

Ogden, N. H., S. Mechai, and G. Margos. 2013. Changing geographic ranges of ticks and tick-borne pathogens: Drivers, mechanisms and consequences for pathogen diversity. *Frontiers in Cellular and Infection Microbiology* 3:1-11.

Openshaw, J. J., D. L. Swerdlow, J. W. Krebs, R.C. Holman, E. Mandel, A. Harvey, D. Haberling, R. F. Massung, and J. H. McQuiston. 2010. Rocky Mountain spotted fever in the United States: Interpreting contemporary increases in incidence. *American Journal of Tropical Medicine and Hygiene* 83:174-182.

Pacheco, R. C., J. Moraes-Filho, E. Guedes, I. Silveira, L. J. Richtzenhain, and R. C. Leiti. 2011. Rickettsial infections of dogs, horses and ticks in Juiz de Fora, southeastern Brazil, and isolation of *Rickettsia rickettsii* from *Rhipicephalus sanguineus* ticks. *Medical and Veterinary Entomology* 25:148-155.

Paddock, C. D., and M. J. Yabsley. 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. *Current Topics in Microbiology and Immunology* 315:289-324.

Paddock, C. D., and J. Goddard. 2015. The evolving medical and veterinary importance of the Gulf Coast tick (Acari: Ixodidae). *Journal of Medical Entomology* 52:230-252.

Paddock, C. D., J. W. Sumner, J. A. Comer, S. R. Zaki, C. S. Goldsmith, J. Goddard, S. L. McLellan, C. L. Tamminga, and C. A. Ohl. 2004. *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clinical Infectious Diseases* 38:805-811.

Paddock, C. D., A. M. Denison, R. R. Lash, L. Liu, B. C. Bollweg, F. S. Dahlgren, C. T. Kanamura, R. N. Angerami, F. C. Pereira dos Santos, R. Brasil Martines, and S. E. Karpathy. 2014. Phylogeography of *Rickettsia rickettsii* genotypes associated with fatal Rocky Mountain spotted fever. *American Journal of Tropical Medicine and Hygiene* 91:589-597.

Padgett, K., D. Bonilla, A. Kjemtrup, I. M. Vilcins, M. Hardstone Yoshimizu, L. Hui, M. Sola, M. Quintana, and V. Kramer. 2014. Large scale spatial risk and comparative prevalence of *Borrelia miyamotoi* and *Borrelia burgdorferi* sensu lato in *Ixodes pacificus*. *PLoS ONE* 9:e110853.

Parker, R. R., R. R. Spencer, and E. Francis. 1924. Tularemia XI. Tularemia infection in ticks of the species *Dermacentor andersoni* Stiles in the Bitterroot Valley, Montana. *Public Health Reports* 39:1057-1073.

Parker, R. R., C. B. Philip, and W. L. Jellison. 1933. Potentialities of tick transmission in relation to geographical occurrence in the United States. *American Journal of Tropical Medicine and Hygiene* 13:341-379.

Parola, P., J. Jourdan, and D. Raoult. 1998. Tick-borne infection caused by *Rickettsia africae* in the French West Indies. *New England Journal of Medicine* 338:1391.

Parola, P., M. B. Labruna, and D. Raoult. 2009. Tick-borne rickettsioses in America: Uunanswered questions and emerging diseases. *Current Infectious Disease Reports* 11:40-50.

Pastula, D. M., G. Turabelidze, K. F. Yates, T. F. Jones, A. J. Lambert, A. J. Panella, O. I. Kosoy, J. O. Velez, M. Fischer, and E. Staples. 2014. Heartland virus disease–United States, 2012-2013. *Morbidity and Mortality Weekly Report* 63:270-271.

Patino, L., A. Afanador, and J. H. Paul. 1937. A spotted fever in Tobia, Colombia. *American Journal of Tropical Medicine and Hygiene* 17:639-53.

Persing, D. H., S. R. Telford III, P. N. Rys, D. E. Dodge, T. J. White, S. E. Malawitsa, and A. Spielman. 1990. Detection of *Borrelia burgdorferi* DNA in museum specimens of *Ixodes dammini* ticks. *Science* 249:1420-1423.

Pfäffle, M., N. Littwin, S. V. Muders, and T. N. Petney. 2013. The ecology of tick-borne diseases. *International Journal for Parasitology* 43:1059-1077.

Philip, C. B., and L. E. Hughes. 1953. Disease agents found in the rabbit tick, *Dermcentor parumapertus*, in the southwestern United States. *Sixth International Congress of Microbiology, Rome* 5:541-548.

Pinto da Silva, E. V., A. P. A. Travassos da Rosa, M. R. T. Nunes, J. P. Diniz, R. B. Tesh, A. C. R. Cruz, C. M. A. Vieira, and P. F. C. Vasconcelos. 2005. Araguari virus, a new member of the family Orthomyxoviridae: Serologic, ultrastructural, and molecular characterization. *American Journal of Tropical Medicine and Hygiene* 73:1050-1058.

Ponnusamy, L., A. Gonzalez, W. Van Treuren, S. Weiss, C. Parobek, J. J. Juliano, R. Knight, R. M. Roe, C. S. Apperson, and S. R. Meshnick. 2014. Diversity of *Rickettsiales* in the microbiome of the lone star tick, *Amblyomma americanum*. *Applied and Experimental Microbiology* 80:354-359.

Porter, J. E. 2014. Time to speak up for research. *Science* 344:1207.

Pritt, B. S., L. M. Sloan, D. K. Johnson, et al. 2011. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *New England Journal of Medicine* 365:422-429.

Pritt, B. S., L. M. Sloan, D. K. Hoang Johnson, et al. 2014. Emergence of a novel *Borrelia* sp. agent pathogenic for humans in the upper Midwestern United States, 2012-2014. 63rd Annual Meeting of American Society for Tropical Medicine and Hygiene, New Orleans, LA. Late-breaker abstract 3238.

Quandelacy, T. M., C. Viboud, V. Charu, M. Lipsitch, and E. Goldstein. 2014. Age- and sex-related risk factors for influenza-associated mortality in the United States between 1997-2007. *American Journal of Epidemiology* 179:156-167.

Quick, R. E., B. L. Herwaldt, J. W. Thomford, M. E. Garnett, M. L. Eberhard, M. Wilson, D. H. Spach, J. W. Dickerson, S. R. Teleford 3rd, K. R. Steingart, R. Pollock, D. H. Persing, J. M. Kobayashi, D. D. Juranek, and P. A. Conrad. 1993. Babesiosis in Washington State: A new species of *Babesia*? *Annals of Internal Medicine* 119:284-290.

Ray, G., T. Schulz, W. Daniels, E. R. Daly, T. A. Andrew, C. M. Brown, P. Cummings, R. Nelson, M. L. Cartter, B. Backenson, J. L. White, P. M. Kurpiel, R. Rockwell, A. S. Rotans, C. Hertzog, L. S. Squires, J. V. Linden, M. Prial, J. House, P. Pontones, B. Batten, D. Blau, M. DeLeon-Carnes, A. Muehlenbachs, J. Ritter, J. Sanders, S. R. Zaki, P. Mead, A. Hinckley, C. Nelson, A. Perea, M. Schriefer, C. Molins, and J. Forrester. 2013. Three sudden cardiac deaths associated with Lyme carditis—United States, November 2012-July 2013. *Morbidity and Mortality Weekly Report* 62:993-996.

Ricketts, H. R. 1909. A micro-organism which apparently has a specific relationship to Rocky Mountain spotted fever. A preliminary report. *Journal of the American Medical Association* 52:379-384.

Riehle, M., and S. M. Paskewitz. 1996. *Ixodes scapularis* (Acari: Ixodidae): Status and change in prevalence and distribution in Wisconsin between 1981 and 1994 measured by deer surveillance. *Journal of Medical Entomology* 33:933-938.

Rodaniche, E. C., and A. Rodaniche. 1950. Spotted fever in Panama. Isolation of the etiologic agent from a fatal case. *American Journal of Tropical Medicine and Hygiene* 30:511-517.

Rollend, L., D. Fish, and J. E. Childs. 2013. Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: A summary of the literature and recent observations. *Ticks and Tick-Borne Diseases* 4:46-51.

Romer, Y., A. C. Seijo, F. Crudo, W. L. Nicholson, A. Varela-Stokes, R. R. Lash, and C. D. Paddock. 2011. *Rickettsia parkeri* rickettsiosis in Argentina. *Emerging Infectious Diseases*17:1169-1173.

Romer, Y., S. Nava, F. Govedic, G. Cicuttin, A. M. Denison, J. Singleton, A. J. Kelly, C. Y. Kato, and C. D. Paddock. 2014. *Rickettsia parkeri* in different ecological regions of Argentina and its association with *Amblyomma tigrinum* as a potential vector. *American Journal of Tropical Medicine and Hygiene*. 91:1156-1160.

Rudenko, N., M. Golovchenko, L. Grubhoffer, and J. H. Oliver, Jr. 2011. Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. *Ticks and Tick-Borne Diseases* 2:123-128.

Rydzewski, J., N. Mateus-Pinilla, R. E. Warner, J. A. Nelson, and T. C. Velat. 2012. *Ixodes scapularis* (Acari: Ixodidae) distribution surveys in the Chicago metropolitan region. *Journal of Medical Entomology* 49:955-959.

Sakai, R. K., F. B. Costa, T. E. H. Ueno, D. G. Ramirez, J. F. Soares, A. H. Fonseca, M. B. Labruna, and D. M. Barros-Battesti. 2014. Experimental infection with *Rickettsia rickettsii* in an *Amblyomma dubitatum* tick colony, naturally infected by *Rickettsia bellii*. *Ticks and Tick-Borne Diseases* 5:917-923.

Salkeld, D. J., M. B. Castro, D. Bonilla, A. Kjemtrup, V. L. Kramer, R. S. Lane, and K. A. Padgett. 2014. Seasonal activity patterns of the western black-legged tick, *Ixodes pacificus*, in relation to onset of human Lyme disease in northwestern California. *Ticks and Tick-Borne Diseases* 5:790-796.

Sanchez, R., C. Alpuche, H. Lopez-Gatell, C. Soria, J. Estrada, H. Olguin, M.A. Lezana, W. Nicholson, M. Eremeeva, CDC Infectious Disease Pathology Branch, G. Dasch, M. Fonseca, S. Montiel, S. Waterman, and J. McQuiston. 2009. *Rhipicephalus sanguineus*–associated Rocky Mountain spotted fever in Mexicali, Mexico: Observations from an outbreak in 2008-2009. Paper presented at the 23rd Annual meeting of the American Society for Rickettsiology, Hilton Head, South Carolina (abstract 75).

Savage, H. M., M. S. Godsey Jr., A. Lambert, N. A. Panella, K. L. Burkhalter, J. R. Harmon, R. R. Lash, D. C. Ashley, and W. L. Nicholson. 2013. First detection of Heartland virus (Bunyaviridae: Phlebovirus) from field-collected arthropods. *American Journal of Tropical Medicine and Hygiene* 89:445-452.

Sayler, K. A., A. F. Barbet, C. Chamberlain, W. L. Clapp, R. Alleman, J. C. Loeb, and J. A. Lednicky. 2014. Isolation of Tacaribe virus, a Caribbean arenavirus, from host-seeking *Amblyomma americanum* ticks in Florida. *PLoS ONE* 9:e115769.

Schneider, B. S., M. E. Schriefer, G. Dietrich, M. C. Dolan, M. G. Morshed, and N. S. Zeidner. 2008. *Borrelia bissettii* isolates induce pathology in a murine model of disease. *Vector-Borne and Zoonotic Diseases* 8:623-633.

Scoles, G. A., M. Papero, L. Beati, and D. Fish. 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. *Vector-Borne and Zoonotic Diseases* 1:21-34.

Scott, M. C., M. E. Rosen, S. A. Hamer, E. Baker, H. Edwards, C. Crowder, J. I. Tsao, and G. J. Hickling. 2010. High-prevalence *Borrelia miyamotoi* infection among wild turkeys (*Meleagris gallopavo*) in Tennessee. *Journal of Medical Entomology* 47:1238-1242.

Shapiro, M. R., C. L. Fritz, K. Tait, C. D. Paddock, W. L. Nicholson, K. F. Abramowicz, S. E. Karpathy, G. A. Dasch, J. W. Sumner, P.V . Adem, J. J. Scott, K. A. Padgett, S. R. Zaki, and M. E. Eremeeva. 2010. *Rickettsia* 364D: A newly recognized cause of eschar-associated illness in California. *Clinical Infectious Diseases* 50:541-548.

Soares, J. F., H. S. Soares, A. M. Barbieri, and M. B. Labruna. 2012. Experimental infection of the tick *Amblyomma cajennense*, Cayenne tick, with *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever. *Medical and Veterinary Entomology* 26:139-151.

Souza, C. E., J. Moraes-Filho, M. Ogrzewalska, F. C. Uchoa, M. C. Horta, S. S. Souza, R. C. Borba, and M. B. Labruna. 2009. Experimental infection of capybaras *Hydrochoerus hydrochaeris* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense*. *Veterinary Parasitology* 161:116-121.

Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annual Review of Entomology* 30:439-460.

Spielman, A., S. R. Telford III, and R. J. Pollak. 1993. The origins and course of the present outbreak of Lyme disease. In *Ecology and environmental management of Lyme disease*, edited by H. S. Ginsberg. New Brunswick, NJ: Rutgers University Press. Pp. 83-96.

Spolidorio, M. G., M. B. Labruna, E. Mantovani, P. E. Brandao, L. J. Richtzenhain, and N. H. Yoshinari. 2010. Novel spotted fever group rickettsiosis, Brazil. *Emerging Infectious Diseases* 16:521-523.

Springer, Y. P., L. Eisen, L. Beati, A. M. James, and R. J. Eisen. 2014. Spatial distribution of counties in the continental United States with records of occurrence of *Amblyomma americanum* (Ixodida:Ixodidae). *Journal of Medical Entomology* 51:342-351.

Spruance, S. L., and A. Bailey. 1973. Colorado tick fever: A review of 115 laboratory confirmed cases. *Archives of Internal Medicine* 131:288-293.

Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista. 1983. The spirochetal etiology of Lyme disease. *New England Journal of Medicine* 308:733-740.

Steiner, F. E., R. R. Pinger, C. N. Vann, N. Grindle, D. Vivitello, K. Clay, and C. Fuqua. 2008. Infection and co-infection rates of *Anaplasma phagocytophilum* variants, *Babesia* spp. *Borrelia burgdorferi*, and the rickettsial endosymbiont in *Ixodes scapularis* (Acari: Ixodidae) from sites in Indiana, Maine, Pennsylvania, and Wisconsin. *Journal of Medical Entomology* 45:289-297.

Steinhaus, E. A. 1942. The microbial flora of the Rocky Mountain wood tick, *Dermacentor andersoni* Stiles. *Journal of Bacteriology* 44:397-404.

Stromdahl, E. Y., J. Jiang, M. Vince, and A. L. Richards. 2011. Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group rickettsiae in the United States. *Vector-Borne and Zoonotic Diseases* 11:969-976.

Stromdahl, E., S. Haner, S. Jenkins, L. Sloan, P. Williamson, E. Foster, R. Nadolny, C. Elkins, M. Vince, and B. Pritt. 2014. Comparison of phenology and pathogen prevalence, including infection with the *Ehrlichia muris*-like (EML) agent, of *Ixodes scapularis* removed from soldiers in the Midwestern and the northeastern United States over a 15 year period (1997-2012). *Parasites and Vectors* 7:553.

Swanson, S. J., D. Neitzel, K. D. Reed, and E. A. Belongia. 2006. Coinfections acquired from *Ixodes* ticks. *Clinical Microbiology Reviews* 19:708-727.

Swei, A., C. J. Briggs, R. S. Lane, and R. S. Ostfeld. 2012. Impacts of an introduced forest pathogen on the risk of Lyme disease in California. *Vector-Borne and Zoonotic Diseases* 12:623-631.

Swei, A., B. J. Russell, S. N. Naccache, B. Kabre, N. Veeraraghavan, M. A. Pilgard, B. J. B. Johnson, and C. Y. Chiu. 2013. The genome sequence of lone star virus, a highly divergent bunyavirus found in the *Amblyomma americanum* tick. *PLoS ONE* 8:e62083.

Thomas, L. A., R. C. Kennedy, and C. L. Eklund. 1960. Isolation of a virus closely related to Powassan virus from *Dermacentor andersoni* collected along North Cache la Poudre River, Colo. *Proceedings of the Society of Experimental Biology and Medicine* 104:355-359.

Tigertt, W. D. 1987. A 1759 spotted fever epidemic in North Carolina. *Journal of the History of Medicine and Allied Sciences* 42:296-304.

Tinoco-Gracia, L., H. Quiroz-Romero, M. T. Quintero-Martínez, et al., 2009. Prevalence of *Rhipicephalus sanguineus* ticks on dogs in a region on the Mexico-USA border. *Veterinary Record* 164:59-61.

Tokarz, R., S. Sameroff, M. S. Leon, K. Jain, W. I. Lipkin. 2014a. Genome characterization of Long Island tick rhabdovirus, a new virus identified in *Amblyomma americanum* ticks. *Virology Journal* 11:26.

Tokarz, R., S. H. Williams, S. Sameroff, M. S. Leon, K. Jain, W. I. Lipkin. 2014b. Virome analysis of *Amblyomma americanum, Dermacentor variabilis*, and *Ixodes scapularis* ticks reveals novel highly divergent vertebrate and invertebrate viruses. *Journal of Virology* 88:11480-11492.

Tribaldos, M., Y. Zaldivar, S. Bermudez, F. Samudio, Y. Mendoza, A. A. Martinez, R. Villalobos, M. E. Eremeeva, C. D. Paddock, K. Page, R. E. Smith, and J. M. Pascale. 2011. Rocky Mountain spotted fever in Panama: A cluster description. *Journal of Infectious in Developing Countries* 5:737-741.

Tsai, T. F. 1991. Arboviral infections in the United States. *Infectious Diseases Clinics of North America* 5:73-102.

Walker, D. H., H. K. Hawkins, and P. Hudson. 1983a. Fulminant Rocky Mountain spotted fever. Its pathologic characteristics associated with glucose-6-phosphatate dehydrogenasease deficiency. *Archives of Pathology and Laboratory Medicine* 107:121-125.

Walker, D. H., D. L. Radisch, and H. N. Kirkman. 1983b. Haemolysis with rickettsiosis and glucose-6-phosphate dehydrogenase deficiency. *Lancet* 2:217.

Weller, B., and G. M. Graham. 1930. Relapsing fever in central Texas. *Journal of the American Medical Association* 95:1834-1835.

Wenzel, R. P., F. G. Hayden, D. H. M. Gröschel, et al. 1986. Acute febrile cerebrovasculitis: A syndrome of unknown, perhaps rickettsial cause. *Annals of Internal Medicine* 104:606-615.

Western, K. A., G. D. Benson, N. N. Gleason, G. R. Healy, and M. G. Schultz. 1970. Babesiosis in a Massachusetts resident. *New England Journal of Medicine* 283:854-856.

Wheeler, C. M., W. B. Herms, and K. F. Meyer. 1935. A new tick vector of relapsing fever in California. *Proceeding of the Society of Experimental and Biological Medicine* 32:1290-1292.

Williams-Newkirk, A. J., L. A. Rowe, T. R. Mixson-Hayden, and G. A. Dasch. 2014. Characterization of the bacterial communities of life stages of free living lone star ticks (*Amblyomma americanum*). *PLoS ONE* 9:e102130.

Yendell, S. J., M. Fischer, and J. E. Staples. 2015. Colorado tick fever in the United States, 2002-2012. *Vector Borne and Zoonotic Diseases* 15:311-316.

Yu, X.-J., M.-F. Liang, S.-Y. Zhang, Y. Liu, J.-D. Li, Y.-L. Sun, L. Zhang, Q. F. Zhang, V. L. Popov, C. L, J. Qu, Q. Li, Y.-P. Zhang, R. Hai, W. Wu, Q. Wang, F.-X. Zhan, X.-J. Wang, B. Kan, S.-W. Wang, K.-L. Wan, H.-Q. Jing, J.-X. Lu, W.-W. Yin, H. Zhou, X.-H. Guan, J.-F. Liu, Z.-Q. Bi, G.-H. Liu, J. Ren, H. Wang, Z. Zhao, J.-D. Song, J.-R. He, T. Wan, J.-S. Zhang, X.-P. Fu, L.-N. Sun, X.-P. Dong, Z.-J. Feng, W.-Z. Yang, Y. Hong, Y. Zhang, D. H. Walker, Y. Wang, and D.-X. Li. 2011. Fever with thrombocytopenia associated with a novel bunyavirus in China. *New England Journal of Medicine* 364:1523-1532.

Yunker, C. E., C. M. Clifford, L. A. Thomas, et al. 1972. Isolation of viruses from swallowticks, *Argas cooleyi*, in the southwestern United States. *Acta Virologica* 16:415-421.

Zavala-Castro, J. E., K. R. Dzul-Rosado, J. J. Arias León, D. H. Walker, and J. E. Zavala-Velázquez. 2008. An increase in human cases of spotted fever rickettsiosis in Yucatan, Mexico, involving children. *American Journal of Tropical Medicine and Hygiene*. 79:907-910.

A9

EMERGING VECTOR-BORNE DISEASES IN THE UNITED STATES: WHAT IS NEXT, AND ARE WE PREPARED?

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The emergence of West Nile virus in the United States in 1999 dramatically illustrated the vulnerability of the United States to exotic vector-borne diseases.

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The sociologic, environmental, and technologic drivers of vector-borne disease emergence globally and in the United States, such as expanded travel and trade, changing land use, human population growth, urbanization, and climate change, are well known and many are accelerating (Kilpatrick and Randolph, 2012; Sutherst, 2004). As such, a bewildering array of vector-borne problems has confronted the United States in recent years. New pathogens, such as the chikungunya virus, have come from abroad (Leparc-Goffart et al., 2014). Other endemic pathogens, such as Lyme disease, have markedly increased in incidence and geographic distribution (Bacon et al., 2008). Still others, such as the Heartland and Bourbon viruses, have been newly discovered, in part, by combining traditional microbiological methods with technological advances in genetic sequencing (Kosoy et al., 2015; McMullan et al., 2012). It is evident that emerging vector-borne diseases will continue to tax our public health and medical care systems for years to come. The question remains whether we will be prepared.

Current Situation in the United States

Arthropod-borne viruses (arboviruses), bacteria, and to a much lesser extent, parasites, are medically important vector-borne pathogens in the United States. Ticks and mosquitoes principally vector the arboviruses; whereas, ticks vector most vector-borne bacteria and parasites. As such, this report will focus on mosquito- and tick-borne diseases.

Arbovirus Transmission—General Aspects

The arboviruses circulate in complex transmission cycles that most often involve a vertebrate host and arthropod vector. The short mosquito generation time and time between blood meals permit rapid pathogen amplification in mosquito-borne transmission cycles and, hence, development of large human outbreaks of sudden onset that garner public attention. The mosquito-borne arbovirus amplification cycle is stochastic, and as such, may be subject to substantial random variability. Furthermore, it is influenced by factors not easily measured, such as immunity in birds, or predicted far in advance, such as weather. As a result, prediction of mosquito-borne arboviral disease outbreaks has proven notoriously difficult.

While many variations exist, arboviral transmission cycles can be simplified into two scenarios that influence many aspects of pathogen ecology, epidemiology, and strategies for control (see Figure A9-1). In the first scenario (the zoonoses), humans do not efficiently infect arthropod vectors; thus, humans do not contribute to the maintenance of the pathogen and are considered incidental hosts. In the United States, rodents and birds serve as the most important vertebrate hosts, while ticks and mosquitoes are the most important arthropod vectors.

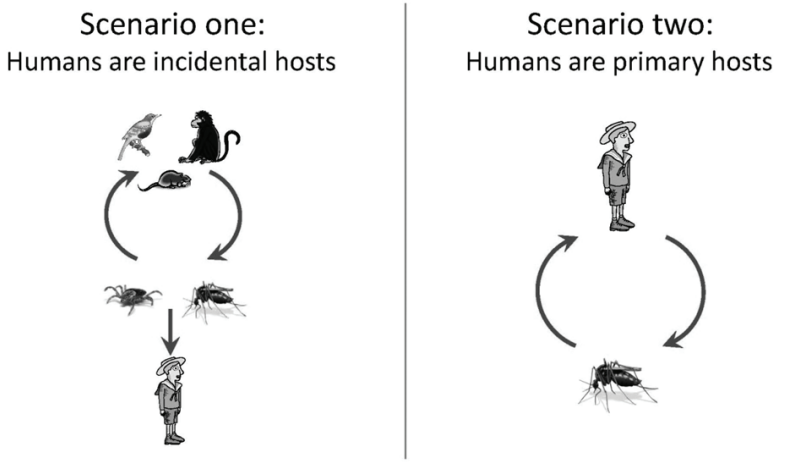

FIGURE A9-1 Two general patterns of mosquito-borne arboviral disease transmission.

High turnover rates in many animal reservoirs (e.g., small rodents, birds) limit herd immunity, facilitating the long-term maintenance of the pathogen in nature.

In the second scenario (the anthroponoses), humans develop a sufficient titer of the pathogen to efficiently infect mosquito vectors and can maintain the pathogen without other vertebrate hosts. In the United States, the only important arthropod vectors for this transmission pattern are *Aedes aegypti* and *Aedes albopictus* mosquitoes. Among the two, *Aedes aegypti* is a superior vector as it lives around human habitation and preferentially feeds on humans, often biting many persons in a single blood meal. These outbreaks occur mainly in tropical areas of the United States where *Aedes aegypti* mosquitoes are abundant (Ramos et al., 2008). Outbreaks can be explosive and may continue until sufficient human herd immunity develops.

Mosquito-Borne Arboviruses—Humans as Incidental Hosts

Mosquito-borne arboviruses have attained substantial public health importance in recent years in the United States. Among the arboviruses using humans as incidental hosts, West Nile virus produces by far the highest human infection incidence, greatest morbidity, and highest number of deaths (Petersen and Fischer, 2012). It was first recognized in the Western Hemisphere during an epizootic in birds and an outbreak of encephalitis in humans in 1999 in the New York City area (Nash et al., 2001). The presence of competent *Culex* mosquito vectors and ubiquitous avian vertebrate hosts throughout the United States permitted the virus' rapid spread to the West Coast by 2004 (Petersen and Hayes,

2008). West Nile virus is now widely endemic; human cases have occurred in all of the contiguous states, with Midwestern states in particular having recurring high incidence (see Figure A9-2) (Petersen et al., 2013). Hundreds of neuroinvasive disease cases now occur each year; regional outbreaks in 2002, 2003, and 2012 each resulted in nearly 2,000 neuroinvasive disease cases (see Figure A9-3). Outbreaks tend to occur during heat waves, likely because increased temperatures shorten the extrinsic incubation period (time from infection to infectiousness) and increase viral levels in mosquitoes, both factors conducive to amplifying transmission (Kilpatrick et al., 2008).

Although West Nile virus outbreaks are largely unpredictable, intensive surveillance in urban settings can indicate impending outbreaks with sufficient lead-time to implement safe and highly effective emergency adult mosquito control measures (Carney et al., 2008, 2011; Healy et al., 2015; Ruktanonchai et al., 2014). Unfortunately, many communities have failed to implement adequate mosquito-based surveillance, and even when data are available, concerns about cost and pesticide use often delay or prohibit application of control measures (Chung et al., 2013).

West Nile virus was shown to be a transfusion-transmitted infection in 2002 (Pealer et al., 2003). In contrast to most viral transfusion-transmitted infections

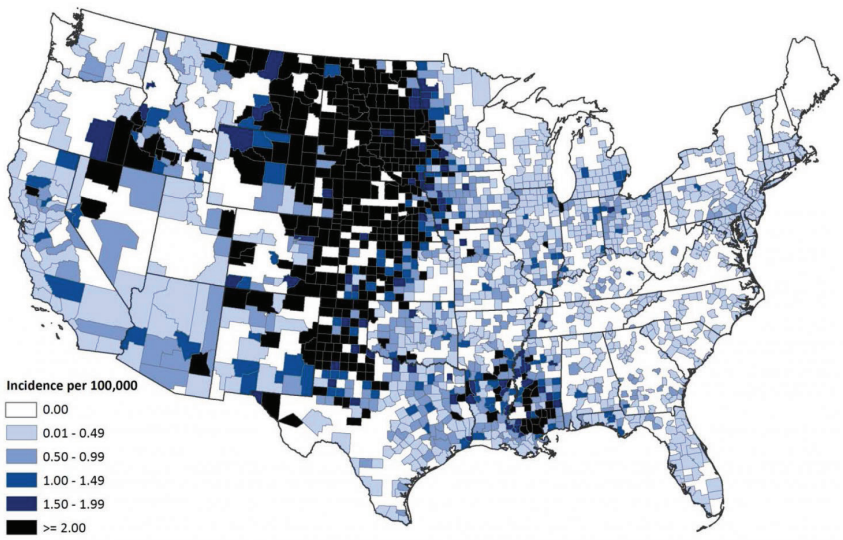

FIGURE A9-2 Average West Nile virus neuroinvasive disease incidence, by county, 1999–2014.
SOURCE: Centers for Disease Control and Prevention (http://www.cdc.gov/westnile/resources/pdfs/data/7-wnv-neuro-incidence-by-county-map_1999-2014_06042015.pdf).

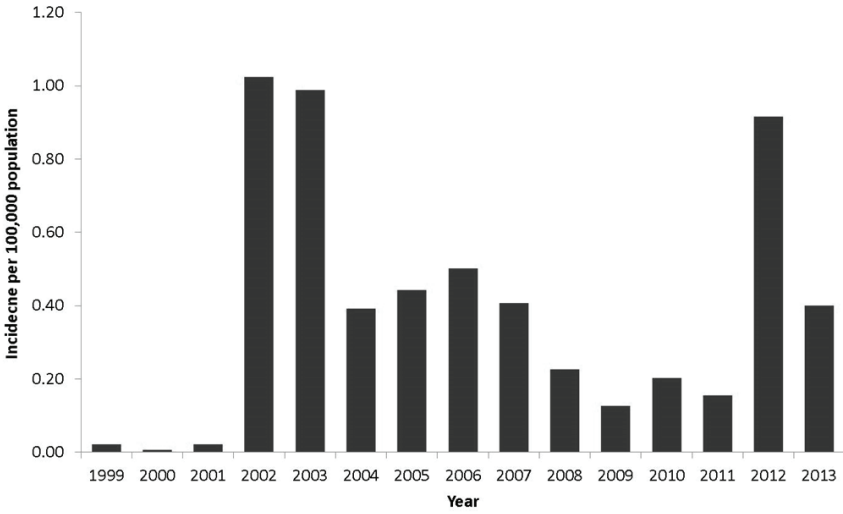

FIGURE A9-3 West Nile virus neuroinvasive disease incidence, by year, 1999–2013, United States.
SOURCE: Centers for Disease Control and Prevention (http://www.cdc.gov/westnile/resources/pdfs/cummulative/99_2013_neuroinvasivebyyear.pdf).

that cause risk by virtue of the chronicity of asymptomatic viremia in potential donor populations, the extremely high population incidence of West Nile virus infection during outbreaks produces risk despite the short duration of viremia in humans (Petersen and Busch, 2010). Since 2003, universal blood screening by nucleic acid amplification testing has nearly eliminated transfusion transmission from West Nile virus (Busch et al., 2005; Stramer et al., 2005).

Other important arboviruses involving humans as incidental hosts include the eastern equine encephalitis and La Crosse encephalitis viruses. Although their incidence has been relatively stable, their geographic distributions have changed in recent years. Evidence of increased eastern equine encephalitis virus transmission has been detected in the upper Northeast, possibly due to changes in habitat structure or climate that have influenced transmission ecology (Gibney et al., 2011). The distribution of La Crosse encephalitis virus has expanded from upper Midwestern states to those in the southeastern and mid-Atlantic regions for unclear reasons. The incidence of St. Louis encephalitis virus, which caused thousands of neuroinvasive disease cases in the mid-1970s (Creech, 1977), has been quite low in recent years, possibly because West Nile virus may have displaced St. Louis encephalitis virus in its similar ecological niche (Reisen et al., 2008).

Mosquito-Borne Arboviruses—Humans as Primary Hosts

Globally, the dengue and chikungunya viruses are now by far the most important arboviruses that use humans as primary vertebrate hosts. Thousands of dengue-infected travelers return to the contiguous United States each year from dengue endemic tropical areas (Mohammed et al., 2010), and more than 2,400 travelers returning to the contiguous United States with chikungunya virus infection were reported during the first year of its circulation in the Americas (CDC, unpublished data). Dengue and chikungunya viruses are now significant health concerns in many areas of the United States where competent mosquito vectors reside and autochthonous transmission may occur.

Dengue incidence has increased several fold in the past 15 years in endemic areas of the Western Hemisphere, which includes Puerto Rico and the U.S. Virgin Islands. Nevertheless, the spread and impact of dengue in the contiguous United States has been limited by the sporadic and limited distribution of *Aedes aegypti* and likely by other sociologic factors such as the widespread use of air conditioning (Ramos et al., 2008). While *Aedes albopictus* is a competent mosquito vector whose distribution extends throughout much of the eastern United States, recent dengue outbreaks in the contiguous states have only occurred in the southern states in areas with significant *Aedes aegypti* populations, suggesting a limited potential for *Aedes albopictus* to cause outbreaks (Bouri et al., 2012). The four dengue viruses have no known important animal reservoir.

The first autochthonous transmission of chikungunya virus in the Western Hemisphere was noted on the Caribbean island of St. Martin in late 2013 (Leparc-Goffart et al., 2014). Chikungunya virus uses the same transmission ecology as dengue (Vega-Rua et al., 2014), and the widespread distribution of the *Aedes aegypti* mosquito in the region permitted the virus' spread throughout the Caribbean, Central America, and parts of Mexico and South America within a year, causing more than one million recorded cases, including more than 30,000 suspect cases in Puerto Rico (Pan American Health Organization, 2014; Sharp et al., 2014). However, despite more than 2,400 imported cases reported in the contiguous United States in 2014, only 11 autochthonous cases were recorded, all in south Florida (Kendrick et al., 2014). These findings suggest that chikungunya will follow a pattern similar to that of dengue in the contiguous United States.

Tick-Borne Arboviruses

Colorado tick fever virus has historically been the most important tick-borne arbovirus in the United States, although reported incidence has decreased in recent years, possibly due to decreased surveillance (Yendell et al., 2015). However, the incidence of Powassan virus has increased, with 6 to 12 cases now reported each year across an expanding geographic range (Centers for Disease Control and Prevention, 2015). Two types of Powassan virus in the United States are linked to human disease. The first type, often called lineage 1 Powassan virus,

is associated with *Ixodes cookei* or *Ixodes marxi* ticks, which infrequently bite humans. Lineage 2 Powassan virus, sometimes called deer tick virus, is associated with *Ixodes scapularis* ticks (El Khoury et al., 2013). While it is not clear if a true increase or enhanced recognition account for the increasing reported Powassan virus disease incidence, other diseases associated with *Ixodes scapularis* ticks, such as Lyme disease, human anaplasmosis, and babesiosis, have greatly increased in incidence in recent years (see below).

The combination of traditional microbiologic methods, next generation sequencing, and focused surveillance efforts has resulted in identification of two novel pathogenic tick-borne arboviruses in the last 3 years in the United States. Heartland virus, the first pathogenic phlebovirus identified in North America, causes a febrile illness that can be fatal (McMullan et al., 2012; Muehlenbachs et al., 2014; Pastula et al., 2014). It is transmitted by *Amblyomma americanum* ticks, which are widely distributed in much of the Midwestern, southern, and eastern United States (Savage et al., 2013). Consequently, human cases have been identified over a wide geographic distribution, but disease and infection incidence remain unknown. Heartland virus is most closely related to the newly discovered severe fever with thrombocytopenia syndrome virus (SFTS) found in China, Korea, and Japan (Lei et al., 2015; Park et al., 2014; Saito et al., 2015).

More recently, Bourbon virus was discovered from a Kansas fatality with a history of tick bite (Kosoy et al., 2015). Bourbon virus is a type of thogotovirus, which belongs to the orthomyxovirus virus family. This is the first thogotovirus identified in the Western Hemisphere. Bourbon virus is genetically similar to tick-borne viruses found in Eastern Europe, Africa, and Asia. Human incidence and distribution as well as the arthropod vector and vertebrate hosts of Bourbon virus are unknown. The recent discoveries of Heartland and Bourbon viruses suggest that further efforts may yield additional novel tick-borne arboviruses, some possibly of public health significance.

Tick-Borne Bacterial Infections

The reported incidence of nearly all tick-borne bacterial infections has markedly increased in recent years (see Figures A9-4 and A9-5). The distributions of the major tick-borne diseases are geographically circumscribed by the distributions of their respective tick vectors (see Figure A9-6). Of particular concern is the expansion and increasing frequency of *Amblyomma americanum* (Lone Star tick), the vector of *Ehrlichia chaffeensis* and Heartland virus; and *Ixodes scapularis* (blacklegged tick), the vector of a wide array of bacterial (*Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Borrelia miyamotoi*), viral (Powassan virus), and parasitic (*Babesia microti*) pathogens.

Ixodes scapularis has a 2-year life cycle. Larvae and nymphs feed mostly on mice and other rodents, which serve as vertebrate hosts for *Borrelia burgdorferi*, the cause of Lyme disease in the United States, and *Anaplasma phagocytophylum*,

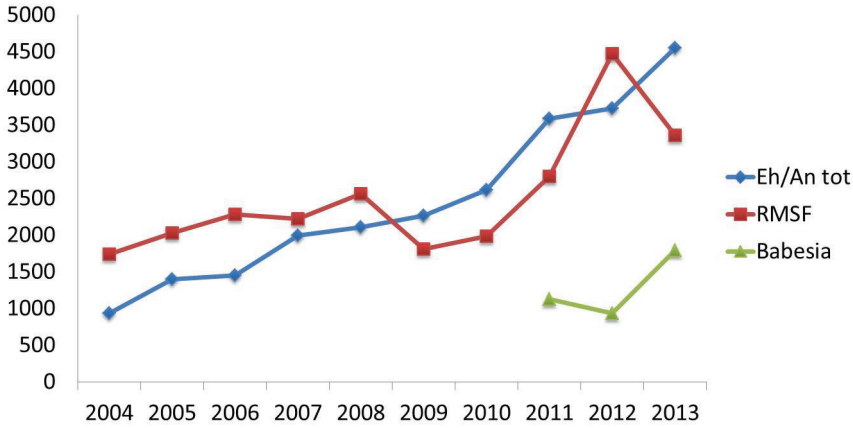

FIGURE A9-4 Incidence of ehrlichiosis and anaplasmosis (Eh/An), Rocky Mountain spotted fever (RMSF), and babesia, 2004–2013, United States. Babesiosis became nationally reportable in 2011.
SOURCE: Centers for Disease Control and Prevention.

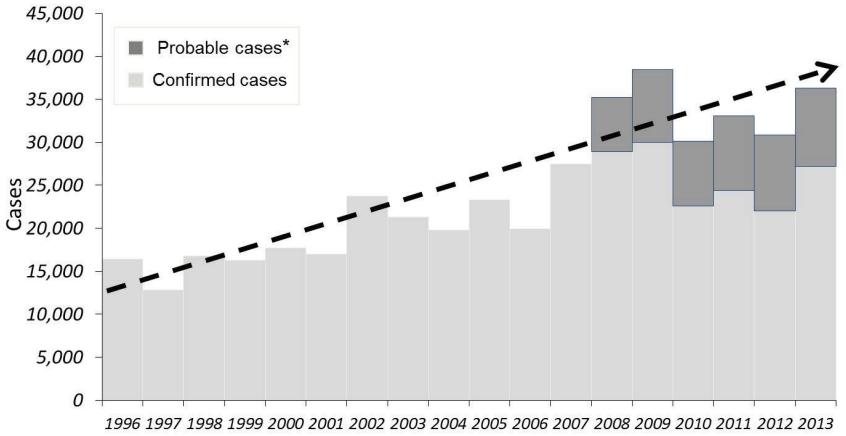

FIGURE A9-5 Reported cases of Lyme disease, 1996–2013. United States.
SOURCE: Centers for Disease Control and Prevention, 2008 (http://www.cdc.gov/lyme/stats/graphs.html).
*National Surveillance case definition revised in 2008 to include probable cases.

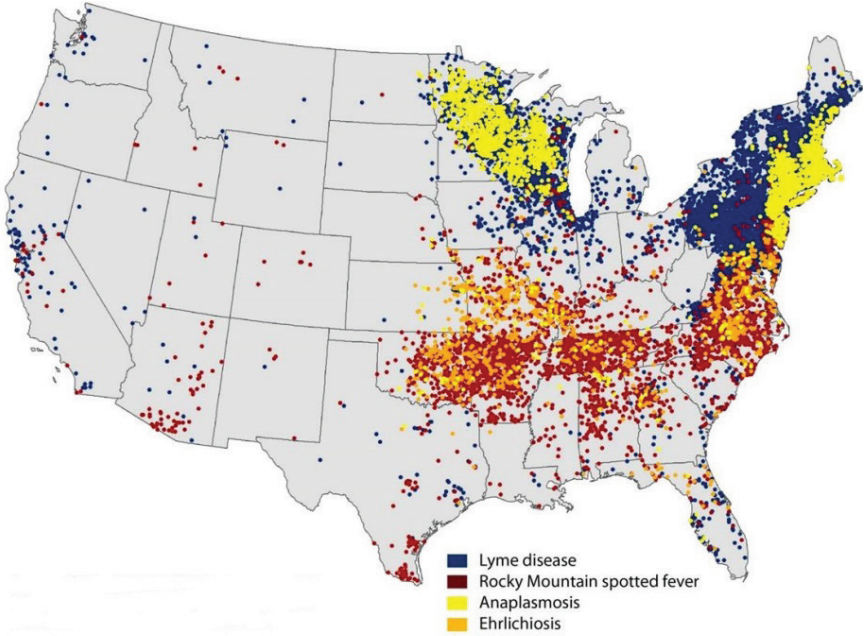

FIGURE A9-6 Distributions of key tick-borne diseases, 2013.
NOTE: Each dot represents county of residence and does not necessarily indicate location of exposure.
SOURCE: Centers for Disease Control and Prevention, 2014.

the etiologic agent of anaplasmosis. Adult *Ixodes scapularis* ticks feed and mate on white tailed deer, whose greatly increased numbers in recent decades have likely contributed to expanding *Ixodes scapularis* tick populations (Spielman et al., 1985). At the same time, suburbanization of woodlands and other habitats has put people in close proximity to deer and ticks. Lyme disease by far has the highest incidence of the tick-borne diseases in the United States. Approximately 35,000 Lyme disease are reported annually (see Figure A9-5) over a widening geographic area (see Figure A9-7). Data indicate that Lyme disease is significantly underreported; the true incidence may be 10 times greater than the number reported (Hinckley et al., 2014).

Unfortunately the relentless increase in diseases spread by *Ixodes scapularis* ticks remains unchecked. Reducing deer populations has been effective in reducing tick populations and human disease in some settings, but large-scale controlled studies have not been done to demonstrate efficacy (Kilpatrick et al., 2014). Four-post deer feeding stations that reduce tick populations on deer have also been used with mixed results (Grear et al., 2014; Hoen et al., 2009). Other host-targeted approaches include using bait boxes to apply acaricides to mice, feed antibiotics to mice, or vaccinate mice (Gomes-Solecki et al., 2006; Piesman,

2006). While some of these approaches have yielded promising results in the laboratory and small-scale field studies, efficacy on reducing human disease has not yet been studied in controlled trials. Acaricides are commonly applied around the perimeter of homes in an attempt to reduce tick abundance and human disease. Unfortunately, a large recent placebo-controlled study showed that this approach substantially reduced tick populations in treated areas but failed to reduce tick exposure or tick-borne disease incidence (CDC, unpublished data). Since *Borrelia burgdorferi* is not transmitted to humans unless *Ixodes scapularis* has been attached for at least 24 hours, tick checks and removing attached ticks can be an effective preventive measure. However, the nymphal ticks are very small and easily missed. A human vaccine for Lyme disease was introduced and subsequently taken off the market, with the manufacturer citing poor sales (Poland, 2011).

Rocky Mountain spotted fever (*Rickettsia rickettsii*) is a significant pathogen in the United States, particularly because of its severe and fatal course if left untreated. Its incidence has increased in recent years (see Figure A9-5), but it is not

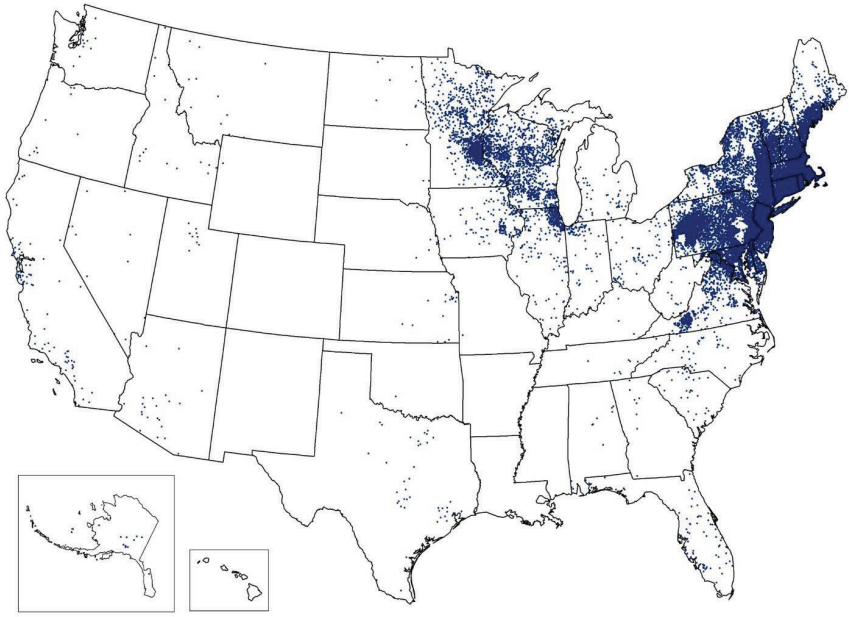

FIGURE A9-7 Cases of Lyme disease in 1996 and 2013.
NOTE: Each dot is placed in the county of residence and does not necessarily indicate the location of exposure.
SOURCE: Centers for Disease Control and Prevention (http://www.cdc.gov/lyme/stats/index.html).

clear to what extent this increase is due to a true increase, increased recognition, or confusion with other Rickettsial diseases. Diagnosis largely rests on serologic tests for which cross-reactivity among the rickettsia can create diagnostic uncertainty. Several tick species vector Rocky Mountain spotted fever, which account for its wide geographic distribution. Tick control is impractical in most areas as cases are widely dispersed geographically and temporally. One exception has been the emergence of Rocky Mountain spotted fever on Native American reservations in Arizona, where RMSF incidence exceeds that of the rest of the United States by at least 10 times (Holman et al., 2009). Transmission in this instance is related to brown dog ticks (*Rhipicephalus sanguineous*) and uncontrolled dog populations (Nicholson et al., 2006). The lack of other tick vectors and vertebrate hosts facilitates disease control, or even elimination, in these settings. Interventions to reduce stray dogs, apply tick-control dog collars to all dogs, and apply acaricides around homes have resulted in greater than 95 percent reductions in tick populations (Drexler et al., 2014). The sustainability of this program and its effect on human disease incidence has not yet been determined.

Several other tick-borne bacterial pathogens are emerging or have been newly discovered in the United States. Ehrlichiosisis is caused by at least three different ehrlichial species in the United States: *Ehrlichia chaffeensis*, *Ehrlichia ewingii,* and a third *Ehrlichia* species provisionally called *Ehrlichia muris*-like (EML) (Paddock and Yabsley, 2007; Pritt et al., 2011). The *Amblyomma americanum* tick is the primary vector of both *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in the United States, and the geographic range of this tick is expanding northward along the Atlantic coast, and in mid-Atlantic and Midwestern states (Cortinas and Spomer, 2013; Springer et al., 2014). The incidence of ehrlichiosis is increasing and is a growing public health concern (see Figure A9-4). The causes of this increase are not understood. Reported fatality rates range from 1-4 percent. *Anaplasma phagocytophylum*, the cause of human anaplasmosis, has a lower fatality rate and like other pathogens spread by *Ixodes scapularis*, is increasing and expanding in distribution (see Figure A9-4). *Anaplasma phagocytophylum* has also been identified rarely in *Ixodes pacificus* ticks (Western black-legged tick).

Borrelia miyamotoi infection has been described in several patients in the United States and has been found both in *Ixodes scapularis* and *Ixodes pacificus* ticks (Gugliotta et al., 2013; Krause et al., 2013; Padgett et al., 2014). The incidence and clinical importance of this infection are unknown. Several new Rickettsial infections have been discovered in recent years. *Rickettsia parkerii,* vectored by *Amblyomma maculatum* (Gulf Coast tick), causes a systemic illness with an eschar at the site of the tick bite in coastal areas of the eastern and southern United States (Paddock et al., 2004). Rickettsia 364D, vectored by *Dermacentor occidentalis* (Pacific Coast tick), also causes a febrile illness with an eschar at the site of the tick bite in coastal regions of Northern California (Johnston et al., 2013).

Tick-Borne Parasitic Infections

While parasitic vector-borne diseases are major scourges worldwide, only *Babesia microti*, the cause of babesiosis, carries significant public health impact in the United States (Vannier and Krause, 2012). Babesiosis has only been reportable since 2011, but as with other pathogens spread by *Ixodes scapularis*, data suggest increasing incidence (see Figure A9-5). *Babesiosia microti* can be transmitted by blood transfusion and has become a transfusion transmission concern in endemic areas (Herwaldt et al., 2011). Babesiosis can be fatal if untreated.

What Is Next?

Three major factors alone or in combination are likely to drive future vector-borne disease trends in the United States: (1) importation of exotic pathogens and vectors, (2) evolving epidemiology and ecology of recognized pathogens currently endemic to the United States, and (3) discovery of new pathogens already endemic to the United States.

Importation of Novel Pathogens and Vectors

Increasing travel and trade undoubtedly will introduce new vectors and pathogens to the United States. The introduction of West Nile virus to the New York City area in 1999 was not predicted. Similarly, accurate prediction of the arrival of new pathogens will be a formidable challenge. However, monitoring global trends in vector-borne disease distribution will be of benefit, as demonstrated by the observation that the global expansion of chikungunya would likely result in the virus' introduction into the Western Hemisphere. This allowed health agencies in the region to establish laboratory and other response capacity beforehand. Nevertheless, nobody could have predicted that it would first take hold on the tiny island of Saint Martin, particularly by an Asian genotype virus likely originating from Southeast Asia or the Western Pacific. Based on India having been the primary source of traveler-related chikungunya cases before 2014 (Lindsey et al., 2015), most had expected that the East-Central-South African genotype virus circulating in India would be introduced to the Americas.

The epidemiology and public health significance of newly imported exotic pathogens may be very difficult to predict. For example, the epidemiology of West Nile virus could not have been ascertained at the time of its introduction and only became apparent after more than a decade of observation. Similarly, while it was expected that chikungunya would produce large outbreaks in the Americas, it remains unknown whether the virus will become permanently established in the Western Hemisphere and how long outbreaks will persist and to what extent they will affect the United States.

In addition to exotic viruses, mosquito species not endemic to the United States have been introduced and established populations across the country,

adding new potential vectors to the ecosystems. This trend was first noted over 200 years ago with the introduction of *Aedes aegypti* from Africa, and its subsequent widespread establishment and transmission of yellow fever and dengue viruses throughout the Western Hemisphere. *Aedes albopictus*, the Asian tiger mosquito, has received the most attention because of its peridomestic habitats, human biting tendencies, and competence to transmit several arboviruses found in the United States (Benedict et al., 2007). However, there are other recently introduced mosquito species expanding in different areas of the United States, including two species introduced from Asia (*Aedes japonicus, Aedes togoi*), one from the Caribbean (*Aedes bahamensis*), and one from Australia (*Aedes notoscriptus*) (Belton and Belton, 1990; Kaufman and Fonseca, 2014; O'Meara et al., 1989).

Evolving Epidemiology of Pathogens Endemic to the United States

Of the arboviruses endemic to the contiguous United States, West Nile virus will likely remain of paramount importance as continued unpredictable focal and regional outbreaks. The St. Louis encephalitis virus caused large outbreaks in the Midwest in the mid-1970s for reasons that still defy explanation and thus is of potential concern despite its decreasing incidence in recent years. It is unknown whether the shifting geographic distributions of the La Crosse encephalitis and eastern equine encephalitis viruses will continue, or if the epidemiology of Powassan virus will change as a result of its apparently new association with *Ixodes scapularis*.

For tropical areas of the United States, such as Puerto Rico and the U.S. Virgin Islands, the trend toward larger dengue outbreaks will likely continue as it has in the Caribbean and Latin America in recent years. Accordingly, more traveler-associated cases can be expected, with the potential to cause outbreaks in areas of the contiguous United States with *Aedes aegypti* populations. The distribution of *Aedes aegypti* is expanding in certain areas, such as in California, placing new areas at risk (Gloria-Soria et al., 2014).

As discussed earlier, the expanding geographic distribution and increasing prevalence of *Ixodes scapularis* ticks will likely continue unabated. Thus, increasing incidence of the diseases they carry—Lyme disease, anaplasmosis, Powassan virus, babesiosis, and *Borrelia miyamotoi* infection—is expected. The eventual extent of this geographic expansion and disease incidence are unknown but could dwarf the current reality. One important consideration is that unlike the Midwestern, Mid-Atlantic, and Northeastern states, the reported incidences of Lyme disease and anaplasmosis in the Western coastal states are not increasing, presumably because of differences in the ecology of its western tick vector, *Ixodes pacificus,* compared to *Ixodes scapularis* ticks found in other disease endemic areas.

For Rocky Mountain spotted fever, it is difficult to determine eventual trends as the root causes of recent increases in incidence remain unknown. The one exception is that successful control efforts could abate the increasing incidence of Rocky Mountain spotted fever on Native American reservations in Arizona if they are maintained and expanded.

Newly Discovered Endemic Pathogens

The discovery of an unprecedented number of tick-borne pathogens in recent years has resulted from dedicated efforts of astute clinicians, microbiologists, and entomologists. These efforts have been supplemented by advances in nucleic acid sequencing that allow for the rapid and efficient identification and characterization of new agents. Nevertheless, decades of inattention have led to identification of few novel arboviruses compared to nonvector-borne viruses (Rosenberg, 2015; Rosenberg et al., 2013), suggesting that renewed efforts at identification of previously unidentified vector-borne pathogens could be quite fruitful.

Some novel or previously unrecognized pathogens may have considerable public health significance. The high prevalence of antibodies to Heartland virus in multiple animal species over a broad geographic area in endemic regions for *Amblyomma americanum*, an aggressive human biting tick, suggests substantial potential for human exposure (Bosco-Lauth et al., 2015). The vectors, animal hosts, human incidence, and disease spectrum of the newly recognized Bourbon virus are unknown.

In addition, genetic mutations in domestic or exotic vector-borne pathogens may alter vector competence, host range, or pathogenicity. For example, genetic changes in the West Nile virus have affected avian mortality and augmented temperature-dependent changes in viral replication in vector mosquitoes (Brault et al., 2007; Kilpatrick et al., 2008), while genetic changes in the chikungunya virus increased the fitness of *Aedes albopictus* as a vector (Tsetsarkin et al., 2007).

Are We Prepared?

Considerable investments by the U.S. government for vector-borne disease surveillance and research following the introduction of West Nile virus in 1999 led to substantial short-term improvements in surveillance; our understanding of the epidemiology, ecology, microbiology, and pathogenesis of vector-borne disease; and diagnosis and recognition of endemic and novel agents. However, if we are to detect and respond to new and exotic pathogens and to reverse the increasing incidence trends of endemic vector-borne diseases, existing capacities must be strengthened and new capacities must be developed (see Table A9-1).

TABLE A9-1 Capacities and Needs Required to Prepare for and Respond to Vector-Borne Diseases in the United States

Capacities	Current needs
Surveillance	• Enhance ArboNET surveillance capacity to address new and emerging mosquito- and tick-borne threats to states. • Enhance TickNET surveillance and response system to increase focus on new and emerging tick-borne diseases; develop new methods to address the overwhelming number of Lyme disease cases.
Diagnosis and pathogen recognition	• Develop new methods for serologic confirmation of arboviral diseases and for markers predictive of severe disease. • Develop sensitive and specific methods for early diagnosis of bacterial diseases, such as Lyme disease and the rickettsioses. • Develop and adopt simpler algorithms for Lyme disease diagnosis. • Continue adoption of advanced molecular detection technologies in reference laboratories; additional dedicated surveillance efforts needed to determine public health impact of newly discovered pathogens.
Research and research capacity	• Increased understanding of environmental influences on transmission, pathogen–host interactions, and the microbiologic underpinnings of transmission and virulence are needed for development of effective prevention technologies. • Promote increased collaboration among modelers, ecologists, and epidemiologists to develop improved predictive tools. • Enhance collaboration with academic research and training programs, and provide additional research funding to support field-based entomology and ecology activities and address critical research needs.
Prevention: Vector control	• Develop strategies to improve implementation of vector and other control measures, particularly in urban areas that have experienced West Nile virus outbreaks.

TABLE A9-1 Continued

Capacities	Current needs
	• Greatly expand current efforts to develop effective and scalable pesticide- and nonpesticide-based vector control methods for *Ixodes scapularis* and *Aedes aegypti*. Evaluate effectiveness based on human outcomes. • Develop public health pesticides with new modes of action and improved delivery systems, as well as cost-effective paradigms for bringing them to market. • Evaluate the disease reduction impact of integrated control programs. • Support basic research leading to the development of novel vector control paradigms.
Prevention: Vaccine development and licensure	• Develop cost-effective and practical pathways for bringing new vector-borne disease vaccines to market. • Develop a next-generation Lyme disease vaccine. • Evaluate the cost-benefit of a West Nile virus vaccine.
Prevention: Other modalities	• Develop new repellent active ingredients and formulations that may increase repellent use and provide long lasting protection. • Enhance programs at state and local levels to increase public awareness of vector-borne disease, with the goal of increasing use of personal protective measures, such as repellents and tick checks. • Develop cost-effective and environmentally safe pathogen reduction technologies for blood donation processing that reduce risk of vector-borne pathogens such as dengue, babesia, chikungunya, ehrlichiosis, and anaplasmosis.
Therapeutics	• Develop cost-effective and practical pathways for bringing new vector-borne disease therapeutics to market. • Educate the public and health care providers about the lack of evidence between doxycycline use and dental staining. • Identify better markers of impending dengue hemorrhagic fever, leading to improved clinical management of severe dengue. • Define optimal therapy for persistent symptoms of chikungunya virus.

Surveillance

Surveillance is a foundational capacity required to determine trends in currently endemic, newly emerging, and exotic pathogens. As surveillance may require human, animal, and vector components, considerable technical expertise is required at national, state, and local levels, a capacity that may take years to develop. Two national surveillance systems monitor vector-borne diseases in the contiguous United States.

The ArboNET surveillance system, developed in 2000 to track the spread of West Nile virus across the United States, is the only surveillance system in the world that tracks human arboviral disease cases as well as environmental indicators of arbovirus transmission activity, such as arbovirus infection in mosquito vectors, avian amplifier hosts, veterinary cases, and vectors in real time. It has expanded in scope, now tracking 14 arboviral diseases. However, capacities related to the conduct of entomologic surveillance required for early detection of impending West Nile virus outbreaks, and for comprehensive arbovirus diagnostic testing at state health department laboratories have diminished (Hadler et al., 2014). Retaining capacities at state and local levels is important, particularly in high population centers where control efforts could substantially reduce human morbidity and mortality from West Nile virus.

The TickNET surveillance and prevention effectiveness program is small in scope relative to the large and growing burden of tick-borne diseases. The sheer number of Lyme disease cases and the difficulties in verifying them present a formidable challenge to health departments in highly endemic areas, producing considerable undercounting and surveillance artifact. New surveillance paradigms based on a sampling approach rather than attempting to capture and verify every case need to be considered. In addition, expansion of TickNET surveillance activities to allow added emphasis on other emerging and newly discovered tick-borne diseases will permit improved understanding of their epidemiology and their public health impact.

Diagnosis and Pathogen Recognition

For the arboviral diseases, widespread development of nucleic acid detection testing capacities has greatly improved diagnostic sensitivity and specificity for dengue and chikungunya, particularly during early disease when diagnosis is most clinically relevant. Nucleic acid detection tests have also been adapted to the identification of West Nile virus in mosquitoes and birds, making monitoring of these indicators of impending human risk more readily available. Nevertheless, serologic tests remain an important component of arboviral diagnosis because human diagnostic samples are often obtained after detectable viremia has subsided. The plaque reduction neutralization test (PRNT) is the most specific serologic test and thus remains the gold-standard serologic confirmatory method. However, the PRNT is performed mostly in reference laboratories as it is technically

demanding, involves culture of live virus, and is slow and time consuming. An alternative to the PRNT would be of great benefit, although none has yet been identified.

The diagnosis of bacterial vector-borne diseases, particularly during early disease, remains problematic. Lyme disease diagnostics are complicated by the low sensitivity of serology-based diagnostic tests during early Lyme disease and diagnosis often relies on recognition of the erythema migrans rash, which may not occur, can be atypical, and can mimic the rashes of other diseases, such as the southern tick associated rash illness (STARI). Established serologic diagnostic algorithms currently use the Western blot test as the second tier in a 2-tier algorithm. Western blot results are interpreted according to the presence of a certain number of specific bands; however, difficulties in identifying the bands have led to considerable confusion, uncertainty, and misinterpretation of results. Other promising serologic diagnostic algorithms not involving the Western blot need to be fully evaluated and adopted as a standard. Ultimately, creation of sensitive and specific diagnostic tests for all stages of disease that don't rely on serology would be of considerable benefit.

Early recognition and treatment dramatically reduces disease morbidity and mortality from Rocky Mountain spotted fever; however, initial symptoms are nonspecific and serologic tests of acute- and convalescent-phase sera are often required for definitive diagnosis. Thus, laboratory confirmation of infection is too late to be clinically useful, and serologic cross-reactivity with other Rickettsia may prohibit definitive diagnosis. Development of sensitive nucleic acid or other early detection tests are urgently needed. While ehrlichiosis and anaplasmosis have lower mortality than Rocky Mountain spotted fever, diagnosis of early disease often relies on serologic methods that have limited use in the acute care setting.

Next generation nucleic acid sequencing undoubtedly will continue to be developed as a tool to identify new vector-borne pathogens, particularly when combined with a concerted effort to establish surveillance and research protocols to identify patients with illnesses of unknown etiology following potential vector exposure. Reference laboratories need to be equipped with these new technologies and associated data management and analytic capabilities. When new human pathogens are discovered, epidemiologic investigation and fieldwork are required to identify potential vectors, enzootic transmission cycles, clinical spectrum, and incidence and geographic distribution of disease.

Research and Research Capacity

Improved surveillance combined with field- and laboratory-based research has improved our understanding of environmental influences on vector-borne disease transmission, pathogen-host interactions, and microbiologic basis of transmission and virulence. While a detailed discussion of research needs is

beyond the scope of this report, this research base provides the underpinnings for the development of all prevention measures. However, research in these areas has decreased, particularly for ecological- and field-based investigations, and as a result, academic programs have diminished, particularly those specializing in medical entomology, the vector component of vector-borne disease. A new pipeline of investigators capable of bridging the gap between laboratory and field research will ensure continued development and evaluation of new intervention methods. This will require partnerships with academic research institutions to address staffing and other critical research areas.

The difficulties in predicting vector-borne disease have resulted in new modeling efforts. Additional expertise and collaborations of modelers with epidemiologists, ecologists, and other subject matter experts have resulted in more realistic and robust models of vector-borne disease transmission and improved estimates of disease burden. The eventual usefulness of vector-borne disease modeling and need for further development in this arena requires an improved understanding of transmission ecology and epidemiology, and will be predicated on the demonstrated benefit of these models on public health practice and policy.

Prevention: Vector Control

Unfortunately, our capacities to control vector-borne diseases through vector control measures remain quite limited, and when effective prevention methods do exist, they are often inadequately employed. For example, as previously mentioned, surveillance indicators reflecting infection rates in mosquito vectors can predict West Nile outbreaks with sufficient lead time to mobilize safe and effective control measures in urban areas, yet inadequate surveillance effort, public concerns about pesticides, lack of local control capacity, or inability to mobilize funds quickly often delay or prohibit implementation of control measures when and where they would be most effective. Greater understanding of these barriers may promote development of measures to mitigate them, particularly in large metropolitan areas where West Nile virus prevention and control efforts would have the biggest impact.

Much of the mosquito control capacity in the United States is developed and funded at local levels for reducing the impact of mosquitoes on quality of life, with vector control capacity benefitting the community as a result of this support. Rather than trying to increase vector control capacity across the board, which seems unrealistic in communities without the need or desire to support nuisance mosquito control, a robust surveillance program, coupled with a rapidly deployable national or regional emergency response capacity should be developed to address the often focal and sporadic West Nile virus outbreaks. This would not only benefit West Nile virus control, but could be used to address other new or emerging mosquito-borne diseases, or situations that develop following natural disasters such as hurricanes and floods.

Highly effective, scalable, and cost-effective tick or mosquito control methods proven to reduce human illness for most vector-borne pathogens in contemporary settings do not exist. Given the substantial public health impact of the diseases they vector, surprisingly few resources are devoted to developing and field-testing new pesticide- or nonpesticide-based control measures. Nevertheless, several novel alternatives to pesticide-based approaches or novel pesticide delivery systems for mosquito and tick control have been developed. For *Ixodes scapularis*, several products that do not contain synthetic pesticides, such as entomopathogenic fungi, nootkatone, and reservoir-targeted vaccines have been developed and are being evaluated and novel pesticide delivery systems have been developed and some are in use, such as bait boxes and 4-posters designed to apply pesticides to the vertebrate hosts of ticks. For *Aedes aegypti*, lethal ovitraps, insect growth regulator auto-dissemination devices, and release of *Wolbachia*-infected or genetically-modified mosquitoes that produce non-viable offspring are among approaches currently under development. Extended release tick control collars are a promising approach for Rocky Mountain spotted fever in locations where dogs are the primary reservoir.

Nevertheless, it is yet to be determined if any of these approaches will be sufficiently scalable and effective in reducing human disease to impact the upward trend in vector-borne disease over the long run. Entomologic and ecologic field research and randomized trials with human disease outcomes are needed; however, this research takes considerable time to complete since vector activity follows annual cycles. At the current pace, decades may pass before effective entomologic control measures are developed and proven effective in reducing human illness on a large scale. As explained above, the human resources required to conduct this research are diminishing.

An Institute of Medicine Report from 2003 warned of the diminishing supply of public health pesticides for vector control resulting from the considerable costs of registration and reregistration relative to the limited size of the public health market (IOM, 2003). The situation has not improved and the cost of developing and registering novel active ingredients or formulations, or for repurposing products developed for agriculture to public health uses remains extraordinarily high. Introduction of new classes of pesticides with unique modes of action and new formulations to improve delivery characteristics is essential to overcome resistance to extant pesticides and to provide the options required for successful integrated vector management programs. New programs to support research and development and streamlined pathways to registration would greatly enhance options for vector management.

Prevention: Vaccines

Given the difficulties with developing, implementing, and sustaining entomologic control measures, creation of human vaccines for the most common

vector-borne diseases is an attractive avenue of pursuit. While yellow fever and Japanese encephalitis vaccines have long been effective and cost-efficient prevention modalities for residents of or travelers to endemic areas, no human vaccines are available for vector-borne diseases endemic to the United States. Dengue vaccines are the furthest along in development, with one having completed phase-3 trials (Capeding et al., 2014; Villar et al., 2015). This vaccine would likely be of considerable public health benefit despite its incomplete protection to all four dengue serotypes. Other dengue vaccines in late-stage development might confer better protection, but are years away from becoming commercially available. Because dengue is only endemic in tropical areas of the United States, such as Puerto Rico and the U.S. Virgin Islands, manufacturers may not put priority on vaccine pursuing licensure in the United States. Nevertheless, given the difficulties with *Aedes aegypti* control and substantial dengue public health impact in Puerto Rico, preparations for the introduction of a vaccine in Puerto Rico should continue, including the development of appropriate surveillance tools so that vaccine effectiveness and impact can be assessed.

Licensed West Nile virus equine vaccines have dramatically reduced equine neuroinvasive disease incidence in the United States (Gardner et al., 2007). While human vaccines have been developed and have undergone successful phase-2 clinical trials (De Filette et al., 2012), phase-3 trials have not been attempted because of uncertain market potential for a West Nile virus vaccine and the considerable logistical difficulties in conducting a phase-3 efficacy trial for a sporadic and geographically dispersed disease that largely occurs in rural and suburban settings. Defining the public health cost-benefit for a West Nile vaccine will help determine future market potential and a clear and cost-efficient pathway to licensure must be identified.

The difficulties with *Ixodes scapularis* control and extremely high Lyme disease incidence warrant accelerated development and licensure of a safe and effective next-generation human Lyme disease vaccine that requires fewer inoculations and with long-lasting efficacy (Shen et al., 2011). Unfortunately, controversies surrounding the previous Lyme disease vaccine have undoubtedly reduced manufacturer interest in further vaccine development (Poland, 2011).

Prevention: Other Modalities

Insect repellents, though demonstrated to effectively reduce human–vector contact, are often infrequently used, even during well-publicized outbreaks. Development of new repellent active ingredients as well as improvements in repellent formulation, such as repellent-containing soaps and spatial repellents that are effective both indoors and outdoors, may improve repellent use and might provide additional protection. Also, investigations to determine to what degree repellents must be used to provide public health benefit should be conducted. Expanded programs to increase public awareness of vector-borne disease, with

the goal of increasing use of personal protective measures, could increase the appropriate use of personal protective measures.

Universal viral nucleic acid screening of blood doors has nearly eliminated the threat of transfusion-associated West Nile virus infection. Nevertheless, this screening is costly and does not cover other vector-borne agents of proven or theoretical transfusion-transmission risk, such as babesiosis. Development of effective and practical pathogen reduction techniques for all blood components would obviate the need to screen for multiple pathogens and would help prevent transfusion transmission of newly emerging vector-borne disease pathogens (Petersen and Busch, 2010). For example, pathogen reduction technology was implemented for platelet screening in French overseas territories experiencing chikungunya outbreaks (Petersen and Epstein, 2014).

Therapeutics

Several potential therapeutics for West Nile virus have been developed; however, as with vaccine development, its sporadic and dispersed epidemiology has precluded evaluation of clinical efficacy (Jester et al., 2006). Thus, no clinical trials for treatment of West Nile virus are currently underway. A clear and cost-efficient pathway to licensure is required before further late-stage clinical development will commence. This is a universal problem for all emerging infectious diseases of this kind; solving it would be broadly useful.

Doxycycline is the preferred treatment for Rocky Mountain spotted fever, other rickettsiosis, and Lyme disease; however, concerns about dental staining stemming from the experience with early tetracycline formulations still lead to warnings against its use in children. Evidence suggests that modern doxycycline formulations do not cause dental staining. It is important to educate the public and health care providers about the lack of evidence between doxycycline use and dental staining in both children and adults.

Recognition of impending dengue hemorrhagic fever and close monitoring of fluid and electrolytes markedly reduce morbidity and mortality. Continued efforts to promote effective practice guidelines are needed. In addition, inexpensive and rapid tests that indicate impending dengue hemorrhagic fever would be of considerable clinical benefit.

Chikungunya virus carries considerable morbidity, and therapeutic options for pain management and for reduction of several arthritic sequelae have not been fully evaluated in controlled clinical trials. Given that chikungunya is likely to be epidemic for years to come, controlled clinical trials are needed.

Concluding Remarks

The United States is faced with an unprecedented array of imported vector-borne disease pathogens, substantial increases in endemic vector-borne diseases

of major public health importance, and newly discovered endemic vector-borne diseases of yet to be determined public health significance. The etiologies underlying these trends are likely accelerating. Following the importation of West Nile virus into the United States, capacities to address emerging vector-borne disease threats, particularly the development of arboviral disease surveillance systems, were greatly augmented. Nevertheless, capacities on nearly all fronts—surveillance, basic and applied research, and prevention—have eroded in recent years, at a time when the need has never been greater. Ramping up effective control programs, such as those for West Nile virus, and developing and identifying new scalable methodologies proven effective on reducing human illness from diseases spread by *Aedes aegypti* mosquitoes and *Ixodes scapularis* ticks are needed. The development of innovative, cost-effective paradigms for bringing new public health pesticides, vaccines, and therapeutics to market is a prerequisite for spurring their development and bringing them to market. Without significant advances on all these fronts, the gap between the increasing impact of vector-borne diseases in the United States and our capacity to effectively respond to them will become ever larger.

References

Bacon, R. M., K. J. Kugeler, P. S. Mead, Centers for Disease Control and Prevention. 2008. Surveillance for Lyme disease—United States, 1992-2006. *MMWR Surveillance Summary* 57(10): 1-9.

Belton, P., and O. C. Belton. 1990. *Aedes togoi* comes aboard. *Journal of the American Mosquito Control Association* 6(2):328-329.

Benedict, M. Q., R. S. Levine, W. A. Hawley, and L. P. Lounibos. 2007. Spread of the tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Diseases* 7(1):76-85.

Bosco-Lauth, A. M., N. A. Panella, J. J. Root, T. Gidlewski, R. R. Lash, J. R. Harmon, K. L. Burkhalter, M. S. Godsey, H. M. Savage, W. L. Nicholson, N. Komar, and A. C. Brault. 2015. Serological investigation of Heartland virus (Bunyaviridae: Phlebovirus) exposure in wild and domestic animals adjacent to human case sites in Missouri 2012-2013. *American Journal of Tropical Medicine and Hygiene* 92:1163-1167.

Bouri, N., T. K. Sell, C. Franco, A. A. Adalja, D. A. Henderson, and N. A. Hynes. 2012. Return of epidemic dengue in the United States: Implications for the public health practitioner. *Public Health Report* 127(3):259-266.

Brault, A. C., C. Y. Huang, S. A. Langevin, R. M. Kinney, R. A. Bowen, W. N. Ramey, N. A. Panella, E. C. Holmes, A. M. Powers, and B. R. Miller. 2007. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. *Nature and Genetics* 39(9):1162-1166.

Busch, M. P., S. Caglioti, E. F. Robertson, J. D. McAuley, L. H. Tobler, H. Kamel, J. M. Linnen, V. Shyamala, P. Tomasulo, and S. H. Kleinman. 2005. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. *New England Journal of Medicine* 353(5):460-467.

Capeding, M. R., N. H. Tran, S. R. Hadinegoro, H. I. Ismail, T. Chotpitayasunondh, M. N. Chua, C. Q. Luong, K. Rusmil, D. N. Wirawan, R. Nallusamy, P. Pitisuttithum, U. Thisyakorn, I. K. Yoon, D. van der Vliet, E. Langevin, T. Laot, Y. Hutagalung, C. Frago, M. Boaz, T. A. Wartel, N. G. Tornieporth, M. Saville, A. Bouckenooghe, and C. Y. D. Study Group. 2014. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: A phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 384(9951):1358-1365.

Carney, R. M., S. Husted, C. Jean, C. Glaser, and V. Kramer. 2008. Efficacy of aerial spraying of mosquito adulticide in reducing incidence of West Nile Virus, California, 2005. *Emerging Infectious Diseases* 14(5):747-54.

Carney, R. M., S. C. Ahearn, A. McConchie, C. Glasner, C. Jean, C. Barker, B. Park, K. Padgett, E. Parker, E. Aquino, and V. Kramer. 2011. Early warning system for West Nile virus risk areas, California, USA. *Emerging Infectious Diseases* 17(8):1445-1454.

Centers for Disease Control and Prevention. 2015. Powassan Virus: Statistics and Maps. http://www.cdc.gov/powassan/statistics.html.

Chung, W. M., C. M. Buseman, S. N. Joyner, S. M. Hughes, T. B. Fomby, J. P. Luby, and R. W. Haley. 2013. The 2012 West Nile encephalitis epidemic in Dallas, Texas. *Journal of the American Medical Association* 310(3):297-307.

Cortinas, R., and S. Spomer. 2013. Lone star tick (Acari: Ixodidae) occurrence in Nebraska: Historical and current perspectives. *Journal of Medical Entomology* 50(2):244-251.

Creech, W. B. 1977. St. Louis encephalitis in the United States, 1975. *Journal of Infectious Diseases* 135(6):1014-1016.

De Filette, M., S. Ulbert, M. Diamond, and N. N. Sanders. 2012. Recent progress in West Nile virus diagnosis and vaccination. *Veterinary Research* 43(1):16.

Drexler, N., M. Miller, J. Gerding, S. Todd, L. Adams, F. S. Dahlgren, N. Bryant, E. Weis, K. Herrick, J. Francies, K. Komatsu, S. Piontkowski, J. Velascosoltero, T. Shelhamer, B. Hamilton, C. Eribes, A. Brock, P. Sneezy, C. Goseyun, H. Bendle, R. Hovet, V. Williams, R. Massung, and J. H. McQuiston. 2014. Community-based control of the brown dog tick in a region with high rates of Rocky Mountain spotted fever, 2012-2013. *PLoS One* 9(12):e112368.

El Khoury, M. Y., J. F. Camargo, J. L. White, B. P. Backenson, A. P. Dupuis 2nd, K. L. Escuyer, L. Kramer, K. St George, D. Chatterjee, M. Prusinski, G. P. Wormser, and S. J. Wong. 2013. Potential role of deer tick virus in Powassan encephalitis cases in Lyme disease-endemic areas of New York, U.S.A. *Emerging Infectious Diseases* 19(12):1926-1933.

Gardner, I. A., S. J. Wong, G. L. Ferraro, U. B. Balasuriya, P. J. Hullinger, W. D. Wilson, P. Y. Shi, and N. J. MacLachlan. 2007. Incidence and effects of West Nile virus infection in vaccinated and unvaccinated horses in California. *Veterinary Research* 38(1):109-116.

Gibney, K. B., S. Robinson, J. P. Mutebi, D. E. Hoenig, B. J. Bernier, L. Webber, C. Lubelczyk, R. J. Nett, and M. Fischer. 2011. Eastern equine encephalitis: An emerging arboviral disease threat, Maine, 2009. *Vector Borne Zoonotic Diseases* 11(6):637-639.

Gloria-Soria, A., J. E. Brown, V. Kramer, M. Hardstone Yoshimizu, and J. R. Powell. 2014. Origin of the dengue fever mosquito, *Aedes aegypti*, in California. *PLoS Neglected Tropical Diseases* 8(7):e3029.

Gomes-Solecki, M. J., D. R. Brisson, and R. J. Dattwyler. 2006. Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait. *Vaccine* 24(20):4440-4449.

Grear, J. S., R. Koethe, B. Hoskins, R. Hillger, L. Dapsis, and M. Pongsiri. 2014. The effectiveness of permethrin-treated deer stations for control of the Lyme disease vector *Ixodes scapularis* on Cape Cod and the islands: A five-year experiment. *Parasitic Vectors* 7:292.

Gugliotta, J. L., H. K. Goethert, V. P. Berardi, and S. R. Telford, 3rd. 2013. Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *New England Journal of Medicine* 368(3):240-5.

Hadler, J. L., D. Patel, K. Bradley, J. M. Hughes, C. Blackmore, P. Etkind, L. Kan, J. Getchell, J. Blumenstock, J. Engel, Control Centers for Disease and Prevention. 2014. National capacity for surveillance, prevention, and control of West Nile virus and other arbovirus infections—United States, 2004 and 2012. *Morbidity and Mortality Weekly Report* 63(13):281-284.

Healy, J. M., W. K. Reisen, V. L. Kramer, M. Fischer, N. P. Lindsey, R. S. Nasci, P. A. Macedo, G. White, R. Takahashi, L. Khang, and C. M. Barker. 2015. Comparison of the efficiency and cost of West Nile virus surveillance methods in California. *Vector Borne Zoonotic Diseases* 15(2):147-155.

Herwaldt, B. L., J. V. Linden, E. Bosserman, C. Young, D. Olkowska, and M. Wilson. 2011. Transfusion-associated babesiosis in the United States: A description of cases. *Annals of Internal Medicine* 155(8):509-519.

Hinckley, A. F., N. P. Connally, J. I. Meek, B. J. Johnson, M. M. Kemperman, K. A. Feldman, J. L. White, and P. S. Mead. 2014. Lyme disease testing by large commercial laboratories in the United States. *Clinical Infectious Diseases* 59(5):676-681.

Hoen, A. G., L. G. Rollend, M. A. Papero, J. F. Carroll, T. J. Daniels, T. N. Mather, T. L. Schulze, K. C. Stafford 3rd, and D. Fish. 2009. Effects of tick control by acaricide self-treatment of white-tailed deer on host-seeking tick infection prevalence and entomologic risk for *Ixodes scapularis*-borne pathogens. *Vector Borne Zoonotic Diseases* 9(4):431-438.

Holman, R. C., J. H. McQuiston, D. L. Haberling, and J. E. Cheek. 2009. Increasing incidence of Rocky Mountain spotted fever among the American Indian population in the United States. *American Journal of Tropical Medicine and Hygiene* 80(4):601-605.

IOM (Institute of Medicine). 2003. *Microbial threats to health: Emergence, detection, and response*. Washington, DC: The National Academies Press.

Jester, P. M., S. J. Tilden, Y. Li, R. J. Whitley, and W. M. Sullender. 2006. Regulatory challenges: Lessons from recent West Nile virus trials in the United States. *Contemporary Clinical Trials* 27(3):254-259.

Johnston, S. H., C. A. Glaser, K. Padgett, D. A. Wadford, A. Espinosa, N. Espinosa, M. E. Eremeeva, K. Tait, B. Hobson, S. Shtivelman, C. Hsieh, and S. L. Messenger. 2013. Rickettsia spp. 364D causing a cluster of eschar-associated illness, California. *Pediatric Infectious Disease Journal* 32(9):1036-1039.

Kaufman, M. G., and D. M. Fonseca. 2014. Invasion biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Annual Review of Entomology* 59:31-49.

Kendrick, K., D. Stanek, C. Blackmore, Centers for Disease Control and Prevention. 2014. Notes from the field: Transmission of chikungunya virus in the continental United States—Florida, 2014. *Morbidity and Mortality Weekly Report* 63(48):1137.

Kilpatrick, A. M., and S. E. Randolph. 2012. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet* 380(9857):1946-1955.

Kilpatrick, A. M., M. A. Meola, R. M. Moudy, and L. D. Kramer. 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathogens* 4(6):e1000092.

Kilpatrick, H. J., A. M. LaBonte, and K. C. Stafford. 2014. The relationship between deer density, tick abundance, and human cases of Lyme disease in a residential community. *Journal of Medical Entomology* 51(4):777-784.

Kosoy, O., A. J. Lambert, D. J. Hawkinson, D. M. Pastula, C. S. Goldsmith, D. C. Hunt, and J. E. Staples. 2015. Novel *Thogotovirus* species associated with febrile illness and death, United States, 2014. *Emerging Infectious Diseases* 21:5.

Krause, P. J., S. Narasimhan, G. P. Wormser, L. Rollend, E. Fikrig, T. Lepore, A. Barbour, and D. Fish. 2013. Human *Borrelia miyamotoi* infection in the United States. *New England Journal of Medicine* 368(3):291-293.

Lei, X. Y., M. M. Liu, and X. J. Yu. 2015. Severe fever with thrombocytopenia syndrome and its pathogen SFTSV. *Microbes and Infection* 17(2):149-154.

Leparc-Goffart, I., A. Nougairede, S. Cassadou, C. Prat, and X. de Lamballerie. 2014. Chikungunya in the Americas. *Lancet* 383(9916):514.

Lindsey, N. P., H. E. Prince, O. Kosoy, J. Laven, S. Messenger, J. E. Staples, and M. Fischer. 2015. Chikungunya virus infections among travelers—United States, 2010-2013. *American Journal of Tropical Medicine and Hygiene* 92(1):82-87.

McMullan, L. K., S. M. Folk, A. J. Kelly, A. MacNeil, C. S. Goldsmith, M. G. Metcalfe, B. C. Batten, C. G. Albarino, S. R. Zaki, P. E. Rollin, W. L. Nicholson, and S. T. Nichol. 2012. A new phlebovirus associated with severe febrile illness in Missouri. *New England Journal of Medicine* 367(9):834-841.

Mohammed, H. P., M. M. Ramos, A. Rivera, M. Johansson, J. L. Munoz-Jordan, W. Sun, and K. M. Tomashek. 2010. Travel-associated dengue infections in the United States, 1996 to 2005. *Journal of Travel Medicine* 17(1):8-14.

Muehlenbachs, A., C. R. Fata, A. J. Lambert, C. D. Paddock, J. O. Velez, D. M. Blau, J. E. Staples, M. B. Karlekar, J. Bhatnagar, R. S. Nasci, and S. R. Zaki. 2014. Heartland virus-associated death in Tennessee. *Clinical Infectious Diseases* 59(6):845-850.

Nash, D., F. Mostashari, A. Fine, J. Miller, D. O'Leary, K. Murray, A. Huang, A. Rosenberg, A. Greenberg, M. Sherman, S. Wong, and M. Layton. 2001. The outbreak of West Nile virus infection in the New York City area in 1999. *New England Journal of Medicine* 344(24):1807-1814.

Nicholson, W. L., C. D. Paddock, L. Demma, M. Traeger, B. Johnson, J. Dickson, J. McQuiston, and D. Swerdlow. 2006. Rocky Mountain spotted fever in Arizona: Documentation of heavy environmental infestations of *Rhipicephalus sanguineus* at an endemic site. *Annals of the New York Academy of Science* 1078:338-341.

O'Meara, G. F., V. L. Larson, D. H. Mook, and M. D. Latham. 1989. *Aedes bahamensis*: Its invasion of south Florida and association with *Aedes aegypti*. *Journal of the American Mosquito Control Association* 5(1):1-5.

Paddock, C. D., and M. J. Yabsley. 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. *Current Topics in Microbiology and Immunology* 315):289-324.

Paddock, C. D., J. W. Sumner, J. A. Comer, S. R. Zaki, C. S. Goldsmith, J. Goddard, S. L. McLellan, C. L. Tamminga, and C. A. Ohl. 2004. *Rickettsia parkeri*: A newly recognized cause of spotted fever rickettsiosis in the United States. *Clinical Infectious Diseases* 38(6):805-811.

Padgett, K., D. Bonilla, A. Kjemtrup, I. M. Vilcins, M. H. Yoshimizu, L. Hui, M. Sola, M. Quintana, and V. Kramer. 2014. Large scale spatial risk and comparative prevalence of *Borrelia miyamotoi* and *Borrelia burgdorferi* sensu lato in *Ixodes pacificus*. *PLoS One* 9(10):e110853.

Pan American Health Organization (PAHO). 2014. Chikungunya: PAHO/WHO Data, Maps and Statistics. http://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=7928&Itemid=40931&lang=en.

Park, S. W., M. G. Han, S. M. Yun, C. Park, W. J. Lee, and J. Ryou. 2014. Severe fever with thrombocytopenia syndrome virus, South Korea, 2013. *Emerging Infectious Diseases* 20(11):1880-1882.

Pastula, D. M., G. Turabelidze, K. F. Yates, T. F. Jones, A. J. Lambert, A. J. Panella, O. I. Kosoy, J. O. Velez, M. Fisher, E. Staples, Centers for Disease Control and Prevention. 2014. Notes from the field: Heartland virus disease - United States, 2012-2013. *Morbidity and Mortality Weekly Report* 63(12):270-271.

Pealer, L. N., A. A. Marfin, L. R. Petersen, R. S. Lanciotti, P. L. Page, S. L. Stramer, M. G. Stobierski, K. Signs, B. Newman, H. Kapoor, J. L. Goodman, and M. E. Chamberland. 2003. Transmission of West Nile virus through blood transfusion in the United States in 2002. *New England Journal of Medicine* 349(13):1236-1245.

Petersen, L. R., and M. P. Busch. 2010. Transfusion-transmitted arboviruses. *Vox Sanguinis* 98(4): 495-503.

Petersen, L. R., and J. S. Epstein. 2014. Chikungunya virus: New risk to transfusion safety in the Americas. *Transfusion* 54(8):1911-1915.

Petersen, L. R., and M. Fischer. 2012. Unpredictable and difficult to control—the adolescence of West Nile virus. *New England Journal of Medicine* 367(14):1281-1284.

Petersen, L. R., and E. B. Hayes. 2008. West Nile virus in the Americas. *Medical Clinics of North America* 92(6):1307-1322, ix.

Petersen, L. R., A. C. Brault, and R. S. Nasci. 2013. West Nile virus: Review of the literature. *Journal of the American Medical Association* 310(3):308-315.

Piesman, J. 2006. Strategies for reducing the risk of Lyme borreliosis in North America. *International Journal of Medical Microbiologist* 296(Suppl 40):17-22.

Poland, G. A. 2011. Vaccines against Lyme disease: What happened and what lessons can we learn? *Clinical Infectious Diseases* 52(Suppl 3):s253-s258.

Pritt, B. S., L. M. Sloan, D. K. Johnson, U. G. Munderloh, S. M. Paskewitz, K. M. McElroy, J. D. McFadden, M. J. Binnicker, D. F. Neitzel, G. Liu, W. L. Nicholson, C. M. Nelson, J. J. Franson, S. A. Martin, S. A. Cunningham, C. R. Steward, K. Bogumill, M. E. Bjorgaard, J. P. Davis, J. H. McQuiston, D. M. Warshauer, M. P. Wilhelm, R. Patel, V. A. Trivedi, and M. E. Eremeeva. 2011. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *New England Journal of Medicine* 365(5):422-429.

Ramos, M. M., H. Mohammed, E. Zielinski-Gutierrez, M. H. Hayden, J. L. Lopez, M. Fournier, A. R. Trujillo, R. Burton, J. M. Brunkard, L. Anaya-Lopez, A. A. Banicki, P. K. Morales, B. Smith, J. L. Munoz, S. H. Waterman, and Dengue Serosurvey Working Group. 2008. Epidemic dengue and dengue hemorrhagic fever at the Texas-Mexico border: Results of a household-based seroepidemiologic survey, December 2005. *American Journal of Tropical Medicine and Hygiene* 78(3):364-369.

Reisen, W. K., H. D. Lothrop, S. S. Wheeler, M. Kennsington, A. Gutierrez, Y. Fang, S. Garcia, and B. Lothrop. 2008. Persistent West Nile virus transmission and the apparent displacement St. Louis encephalitis virus in southeastern California, 2003-2006. *Journal of Medical Entomology* 45(3):494-508.

Rosenberg, R. 2015. Detecting the emergence of novel, zoonotic viruses pathogenic to humans. *Cellular and Molecular Life Sciences* 72(6):1115-1125.

Rosenberg, R., M. A. Johansson, A. M. Powers, and B. R. Miller. 2013. Search strategy has influenced the discovery rate of human viruses. *Proceedings of the National Academy of Science of the United States of America* 110(34):13961-13964.

Ruktanonchai, D. J., S. Stonecipher, N. Lindsey, J. McAllister, S. K. Pillai, K. Horiuchi, M. Delorey, B. J. Biggerstaff, T. Sidwa, J. Zoretic, R. Nasci, M. Fischer, and S. L. Hills. 2014. Effect of aerial insecticide spraying on West Nile virus disease—north-central Texas, 2012. *American Journal of Tropical Medicine and Hygiene* 91(2):240-245.

Saito, T., K. Fukushima, K. Umeki, and K. Nakajima. 2015. Severe fever with thrombocytopenia syndrome in Japan and public health communication. *Emerging Infectious Diseases* 21(3):487-489.

Savage, H. M., M. S. Godsey Jr., A. Lambert, N. A. Panella, K. L. Burkhalter, J. R. Harmon, R. R. Lash, D. C. Ashley, and W. L. Nicholson. 2013. First detection of heartland virus (Bunyaviridae: Phlebovirus) from field collected arthropods. *American Journal of Tropical Medicine and Hygeine* 89(3):445-452.

Sharp, T. M., N. M. Roth, J. Torres, K. R. Ryff, N. M. Perez Rodriguez, C. Mercado, M. D. Pilar Diaz Padro, M. Ramos, R. Phillips, M. Lozier, C. S. Arriola, M. Johansson, E. Hunsperger, J. L. Munoz-Jordan, H. S. Margolis, B. R. Garcia, Centers for Disease Control and Prevention. 2014. Chikungunya cases identified through passive surveillance and household investigations—Puerto Rico, May 5-August 12, 2014. *Morbidity and Mortality Weekly Report* 63(48):1121-1128.

Shen, A. K., P. S. Mead, and C. B. Beard. 2011. The Lyme disease vaccine—a public health perspective. *Clinical Infectious Diseases* 52(Suppl 3):s247-s252.

Spielman, A., M. L. Wilson, J. F. Levine, and J. Piesman. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annual Review of Entomology* 30:439-460.

Springer, Y. P., L. Eisen, L. Beati, A. M. James, and R. J. Eisen. 2014. Spatial distribution of counties in the continental United States with records of occurrence of *Amblyomma americanum* (Ixodida: Ixodidae). *Journal of Medical Entomology* 51(2):342-351.

Stramer, S. L., C. T. Fang, G. A. Foster, A. G. Wagner, J. P. Brodsky, and R. Y. Dodd. 2005. West Nile virus among blood donors in the United States, 2003 and 2004. *New England Journal of Medicine* 353(5):451-459.

Sutherst, R. W. 2004. Global change and human vulnerability to vector-borne diseases. *Clinical Microbiology Reviews* 17(1):136-173.

Tsetsarkin, K. A., D. L. Vanlandingham, C. E. McGee, and S. Higgs. 2007. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathogens* 3(12):e201.

Vannier, E., and P. J. Krause. 2012. Human babesiosis. *New England Journal of Medicine* 366(25):2397-2407.

Vega-Rua, A., K. Zouache, R. Girod, A. B. Failloux, and R. Lourenco-de-Oliveira. 2014. High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of chikungunya virus. *Journal of Virology* 88(11):6294-6306.

Villar, L., G. H. Dayan, J. L. Arredondo-Garcia, D. M. Rivera, R. Cunha, C. Deseda, H. Reynales, M. S. Costa, J. O. Morales-Ramirez, G. Carrasquilla, L. C. Rey, R. Dietze, K. Luz, E. Rivas, M. C. Miranda Montoya, M. Cortes Supelano, B. Zambrano, E. Langevin, M. Boaz, N. Tornieporth, M. Saville, F. Noriega, and C. Y. D. Study Group. 2015. Efficacy of a tetravalent dengue vaccine in children in Latin America. *New England Journal of Medicine* 372(2):113-23.

Yendell, S. J., M. Fischer, and J. E. Staples, 2015. Colorado tick fever in the United States, 2002-2012. *Vector Borne Zoonotic Diseases* 15:311-316.

A10

ARBOVIRUS EVOLUTION, VECTOR COMPETENCE, AND VIRULENCE MODELS—CHANGING PATTERNS OF INFECTION

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Abstract

Viruses that are transmitted by arthropods, especially mosquitoes, have emerged very recently as major causes of public health concern: dengue and chikungunya viruses are transmitted by some of the most common mosquito vectors that cohabit with and bite humans, and new virus variants are being transmitted at increased rates throughout the world. Not only are these viruses spreading, along with humans that travel and infect other mosquitoes, but the most virulent variants are being naturally selected for and causing increased disease severity. Here we summarize the approaches used to measure these evolving arbovirus characteristics, what we might expect in the near future, and what we are doing to try to understand the mechanisms of their evolution and transmission, in order to design effective control measures and provide accurate input for mathematical models of disease dynamics.

Introduction

In the context of this workshop, it is important to discuss how mosquito-viruses have been evolving, so that we might find some patterns to guide our expectations of any type of emergence. Here we discuss what is currently known about two of the most important arthropod-borne viruses (arboviruses), dengue

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(DENV) and chikungunya (CHIKV), which are continuing to spread globally, and being introduced to new populations (both mosquitoes and humans) that are susceptible and are clinically inexperienced with the diseases these viruses cause. Although these viruses belong to different virus genera (*Flavivirus* and *Alphavirus,* respectively) and contain different genes and have different structures, they are both transmitted by bite, by the same two mosquito species, *Aedes aegypti* and *Aedes albopictus* (females only, as they require blood for egg development), forming mosquito–human–mosquito cycles of virus amplification and transmission. Both viruses contain single-stranded RNA genomes that are normally prone to higher rates of mutation than DNA viruses; however, these viruses evolve at lower rates than other RNA-containing viruses, presumably because they are constrained in maintaining their ability to replicate in humans and mosquitoes, with differing host cell receptors, cell biology, and mechanisms of immunity.

These two arboviruses produce large numbers of virus particles in the human host's blood (viremia) after infection by mosquito bite (via saliva) and are therefore infectious to other biting vectors, such as mosquitoes, ticks, and flies. However, these two viruses are capable of replicating and spreading throughout the bodies and salivary glands of only the two above-mentioned *Aedes* mosquitoes, without causing mortality; thus, these mosquitoes are serving as exponential biological amplifiers for the viruses. Therefore, when we study the evolution of these viruses, we must evaluate how they replicate, disseminate, and are transmitted via saliva by mosquitoes; this is referred to as vector competence. In contrast, for human hosts, we need to determine rates of infection and replication, or pathogenesis and virulence in individuals with differing immune status, genetic predispositions to viral infections, and possibly other underlying diseases. Thus, for detecting differences between evolving viruses in humans, we require systems in which to measure virulence, or the effects of viral replication and the damage this causes in different human cells (tropism), leading to overall pathogenesis and differing degrees of disease; these factors would also have an impact on how often the human viremic host could infect other vectors. And, unfortunately for us, no other animal responds to DENV or CHIKV like humans, so we have no straightforward models in which to study replication, immunity, and clinical presentations.

To test these principles of arbovirus evolution and the ultimate effects on disease emergence, we need to measure evolutionary pressures and effects in all three organisms (virus, mosquito, and humans), requiring us to rely on very diverse assay systems: for the viruses, we study their genetic changes via nucleotide sequencing, by generating phylogenetic trees, and by generating structures, with reflected protein changes that might change tropism or escape immunity; for humans, we study epidemiological links to specific virus antigenic (serotypes) or genetic variants (genotypes), how the viruses behave in primary human cell culture, or in new, complex animal models of disease and virulence; and for mosquitoes, we can work with field-collected or colonized mosquitoes, which are

bred in the laboratory under controlled environments. Because of the complexity of these systems, it is important to note that the outcomes of these measurements and comparisons should always be done in systems that mimic the natural cycles of virus transmission; otherwise, these studies could lead to incorrect conclusions. These studies are necessary to determine if vaccination and treatment strategies, for which there are none currently licensed, can be directed at the most virulent virus variants that cause the majority of the disease outbreaks. This information can also help to strategize disease control approaches, which are even more critical when dealing with mosquito vectors that cannot be eradicated. The importance of these studies is also highlighted by the fact that these data are needed to derive any of the mathematical models of disease dynamics also discussed in this workshop, which depend on accuracy of input for realistic prediction outcomes.

Dengue Viruses

Disease Characteristics and Viral Genetic Variability

Currently, dengue viruses are the most prevalent of all the arboviruses, causing an estimated 400 million human infections per year, in over 100 countries worldwide; however, these infections produce clinical disease, dengue fever (DF), in only around 10–15 percent of those infected (attack rate) (for reviews see Bhatt et al., 2013; Guzman et al., 2010). The most recent incursion of new virus variants into the Americas, during the 1980s, caused the first massive epidemics of dengue hemorrhagic fever (DHF), which is the most severe form of the disease, and can be fatal in 5–20 percent of patients. Because dengue viruses differ by around 25 percent in their exterior proteins, we classify them into four different antigenic groups or serotypes; this also means that immunity to one serotype does not protect against infection by another serotype. In fact, this insufficient "secondary" immune response increases the possibility of getting more severe disease, or DHF. So, in addition of having to measure direct destruction of cells by the virus, we also have to measure the effects of immunologic responses gone wrong; these events were first described as antibody-dependent enhancement of disease, but we now know that other immune responses, such as cytokines/chemokines secreted during an inflammatory response, lead to platelet and endothelial cell activation that cause vascular leakage and hemorrhaging (for a review, see Rothman, 2011). In addition to having four different serotypes, each of these serotype viruses show variability in their genomes' sequences, and can be classified into genotypes; there are three to five genotypes within each serotype, and these have specific geographic distributions, epidemiology, virulence, and/or transmission abilities associated with each of them (for review see Rico-Hesse, 2003). Therefore, it is very important to measure these evolving differences between strains or variants, because these characteristics can be closely associated with potential for spread and human clinical disease presentations.

Phylogenetic trees DENV contain approximately 11,000 nucleotides of RNA, and produce 8 different proteins, in the order 5′-capsid-membrane-envelope-non-structural proteins 1 through 5-3′; in addition, they have around 100 nucleotides at the beginning (5′) of their genome, and 400 nucleotides at the end (3′), that do not code for any proteins (known as untranslated region; UTR). We showed some time ago that nucleotide sequences from certain areas of the DENV genome could be used to construct evolutionary trees that could help us separate out variants from different regions of the world and demonstrate their times and routes of spread (Rico-Hesse, 1990; Rico-Hesse et al., 1997). Since then, numerous studies have been published comparing different areas of the viral genome or complete genome sequences for various DENV strains, and are continuously being updated along with epidemiologic characteristics. To this end, we will focus on the interpretation of one type of phylogenetic tree, for DENV of serotype 2, to demonstrate the first linkages of genotypes with certain epidemiologic and virulence characteristics.

In Figure A10-1, a phylogenetic tree constructed with the nucleotide sequences from the envelope gene of numerous DENV-2 strains, and representatives of the other three serotypes, is shown. Studies of hundreds of virus strains showed that for DENV-2, there were four distinct genotypic groups (simplified here) that correlated with the geographic origins of the patient samples, with sequence variations of about 6 percent or more (Rico-Hesse, 2007). Here we have highlighted two genotypic groups, the American and Southeast Asian (SE Asian) genotypes because these groups differed in their clinical disease presentations; that is, samples from patients with DF fell into all genotypes, but those from DHF patients fell into only one of the genotypes (SE Asian).

The American genotype had been circulating in the American continent before the 1980s, without causing major epidemics or severe disease (DHF); in 1979–1980, the SE Asian genotype was introduced, most probably via returning Cuban military personnel who had been infected in Vietnam, after the war (Rico-Hesse et al., 1997). This SE Asian genotype virus introduction was directly associated with the first occurrences of DHF in the Americas, in spite of having numerous serotypes circulating before this time; this genotype, with its high virulence characteristic has since spread to the rest of the world, and has seemed to displace the native genotypes in other continents also (e.g., Africa) (Messina et al., 2014).

In our case, the American genotype viruses have not been isolated on this continent since 1995, in northern Mexico (see bottom black highlight in Figure A10-1) and in northern Peru, in 2000 (Cruz et al., 2013), and the viruses isolated on the Mexican border with Texas, in 2005, are now SE Asian genotype viruses (see top black highlight in Figure A10-1). Thus, a virus variant that evolved within serotype 2 (maintaining its antigenic structure) became more virulent to humans and was more transmissible than the variants native to this and other continents. Unfortunately, because we had no animal models of disease at

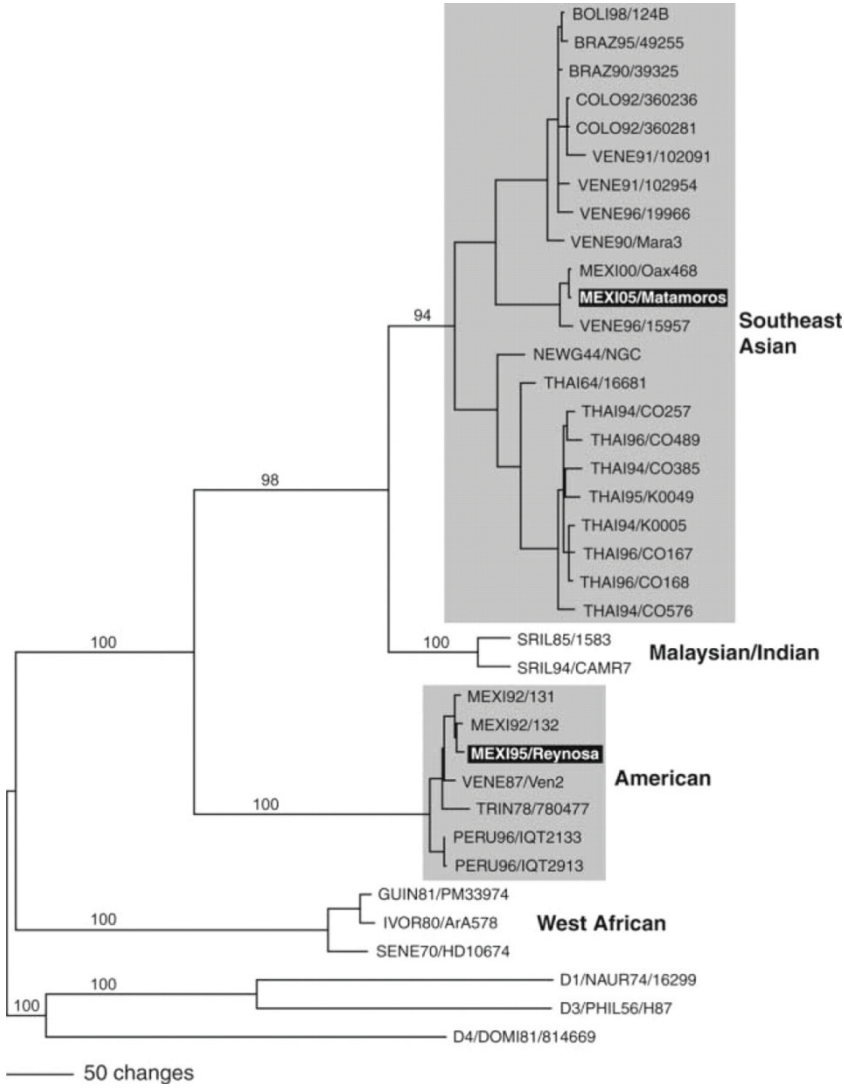

FIGURE A10-1 Phylogenetic tree of selected DENV-2 strains, using complete E gene sequences, and representatives of the other three serotypes to root the tree. The genetic distance between the virus strains is proportional to the scale, representing 50 nucleotides; values above branches represent percent statistical support. Viruses are grouped into four genotypes, with the Southeast Asian and American genotypes shaded. Each virus is labeled with the first four letters of the country in which it was isolated, followed by the last two digits of the year of isolation, and the strain number or name.
SOURCE: Rico-Hesse, 2007. Reproduced with permission from Oxford University Press, on behalf of *Clinical Infectious Diseases*.

the time, we could not prove the direct link of infection by the SE Asian genotype to higher virulence and transmission.

This same effect, of infection by a specific genotype and more severe disease and transmission, has been shown for a Sri Lanka variant of DENV serotype 3 (Messer et al., 2002, 2003), but has not yet been demonstrated for genotypes of DENV serotypes 1 and 4. It is also important to point out that several reports have suggested that DENV was undergoing intragenomic recombination events, with the production of virions with nucleotide or antigenic properties of several genotypes or serotypes (Holmes et al., 1999; Holmes, 2006), but these studies were shown to be a result of technical errors in sequencing methods, and subsequent errors in the GenBank sequence postings. Therefore, the more virulent genotypes we discuss here are a result of independent mutation events that have led to the production of distinct virus lineages that can outcompete other members of the same antigenic groups (DENV-2 and DENV-3), and they have spread throughout the world. However, we still have not determined the exact parts of the virus genome that lead to these epidemiologic or virulence characteristics. Our studies with infectious clones and recombinant laboratory techniques have allowed us to pinpoint some parts of the virus that we believe can lead to increases in replication that translate into higher virion production, increased mosquito infectivity and vector competence, and increased human virulence and pathogenesis.

Virus replication in target cells Owing to the lack of animal models of disease, and only indirectly, by observation of human clinical presentations (during prospective epidemiologic studies), it has been very difficult to measure which factors lead to increased DENV replication and subsequent disease. If we focus on the production of DF, and not on the complex and little understood effects of our own immune system in causing DHF, we can measure differences in replication abilities of DENV in their primary target cells, taken directly from uninfected individuals. Some human tropism studies in biopsied materials from patients with or without DF, and some autopsy specimens from those dying of DHF, had shown that dendritic cells and macrophages were the first cells to be infected and replicated DENV at higher rates (Wu et al., 2000; Jessie et al., 2004). However, because of ethical reasons, there have been very few early studies of what happens to humans right after being infected by mosquito bite (Sabin, 1952). Some of these studies are being analyzed in more detail (Snow et al., 2014), but there are too few subjects and too many variables to be able to identify specific factors such as the exact dose of DENV needed to produce disease. Also, these "volunteers" were not biopsied or analyzed pathologically to determine the exact events leading to the sites of DENV replication after infection. In our laboratory, we measured the replication abilities of DENV of different genotypes, in monocyte-derived dendritic cells (DCs) from unidentified blood donors (Cologna et al., 2005).

In these cells, cultured in vitro (or ex vivo), we controlled for the numbers of cells and the dose of DENV used to infect, and we compared the infection and production abilities of 19 low-passaged (less than 4 passages from the patient's sample) virus strains, representing the SE Asian (n = 12) and American (n = 7) genotypes of DENV-2 (see Figure A10-2). First we measured the number of cells infected by each genotype (by detection of expressed viral protein) (see Figure A10-2A), and then the number of virions produced by the same cells (using RNA genomes as a surrogate) (see Figure A10-2B). Surprisingly, the number of cells infected was higher for the less virulent, American genotype strains, and this varied by blood donor. However, when the numbers of viral RNA genomes (DENV are notoriously difficult to measure as infectious particles) were compared across donors, all donor samples produced much higher amounts of SE Asian genotype viruses. This means that American viruses may infect more target cells, but they produce less progeny viruses after a single round of replication (at 48 hours post-infection), and that some donors (not previously exposed to DENV) have some innate, probably genetic, cellular factors that prevent DENV replication. These differences in replication ability are independent of any measurable immune system response (adaptive immunity), since we are growing them in purified DC cultures. Therefore, segregation of DENV strains into nucleotide variant groups, or genotypes, helps us define evolutionary differences that increase the probability of the virus to infect human cells, produce viremia, and cause disease.

DENV determinants of replication To study the specific structures of the DENV RNA genome or expressed proteins that might determine differences in replication ability, we constructed chimeras of SE Asian and American genotype viruses, using recombinant DNA techniques (Cologna and Rico-Hesse, 2003). The sites for possible replication and/or virulence determinants were selected first by complete genome nucleotide sequencing of six SE Asian genotype strains and six American genotype strains (Leitmeyer et al., 1999). Their viral RNA nucleotide sequences were determined by chemical analysis (primer-extension Sanger sequencing off of viral RNA, with no cloning) from patient serum samples, so as not to introduce mutations during passage in cells, either by biological or enzymatic amplification techniques.

When these sequences were aligned, we found consistent differences across many different areas of the genome, but only one difference in an encoded amino acid, at E390 (N to D), which changed the charge and thus probably the structure of the proteins on the outside of the DENV virion. However, two other consistent differences occurred at sites in the untranslated parts of the genome, in the 5' UTR (two nucleotides) and in the 3' UTR (14 nucleotides, including 10 deletions) that we hypothesized could significantly alter the folding patterns of these RNA regions, thus, altering their ability to initiate or control replication and possibly switch to template translation, in order to control virion production.

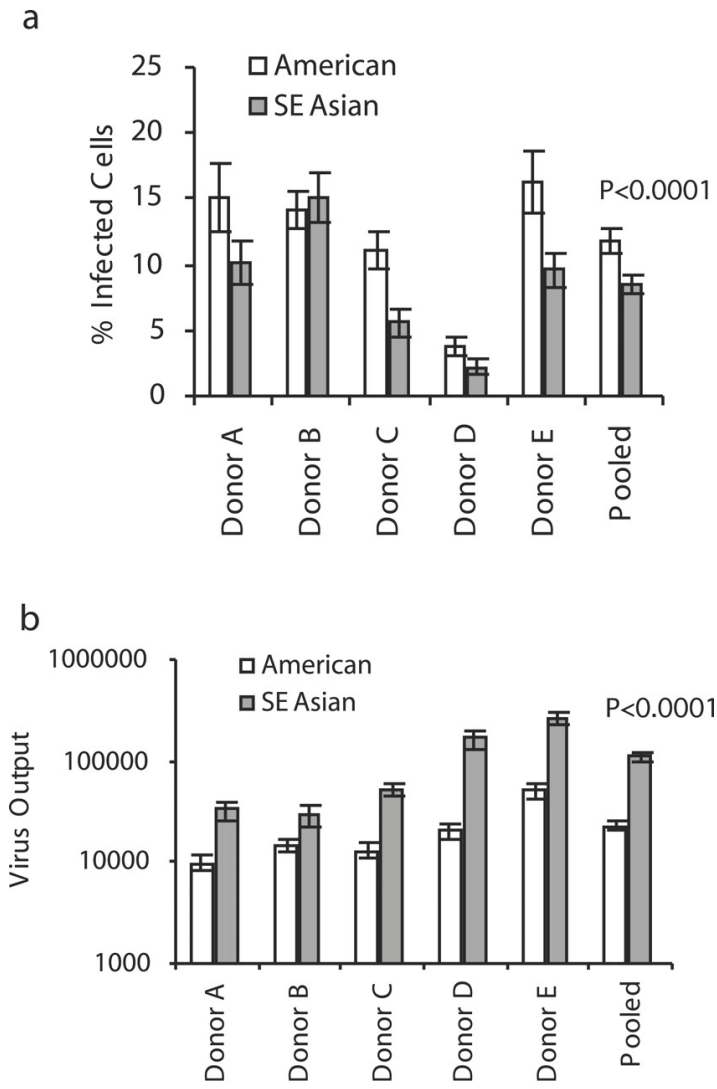

FIGURE A10-2 DENV infectivity and output in human dendritic cells. A. Infected DC cultures were examined by flow cytometry with a DENV-specific MAb, to determine the number of infected cells. B. Virus output was estimated by dividing the number of genome equivalents in culture supernatants by the number of infected cells. Graphs were generated for individual donors and for pooled data from five donors (A–E).

NOTE: Within each graph, the white bars represent the mean results of seven American genotype viruses, and the gray bars represent the results from 12 SE Asian genotype viruses. Error bars are SE, with infections in triplicate.

SOURCE: Cologna et al., 2005. Copyright © 2005, American Society for Microbiology. All Rights Reserved. Reproduced with permission from ASM Journals.

We later expanded these in silico studies, to determine the RNA folding patterns of viruses from all four genotypes, and found that strains from each genotype of DENV-2 had differing degrees of complexity in their folding patterns (see Figure A10-3), including overlaps (or pseudoknots), and these patterns seemed to correlate with rates of replication and/or virulence (Rico-Hesse, 2009). This suggested that these untranslated portions of the genome could in fact directly control initiation of replication, especially since they are the binding sites for

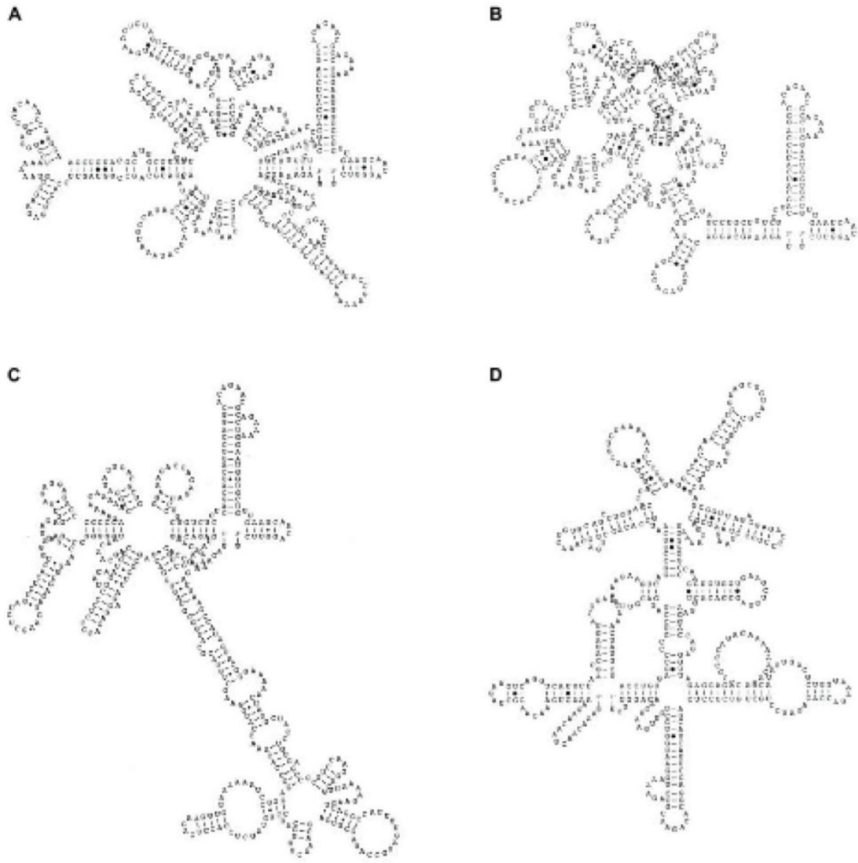

FIGURE A10-3 Predicted folding patterns of the 3'UTR of DENV-2 viruses representing each of the four genotypes, shown in order of complexity, with many pseudoknots predicted for the first two. A = SE Asian, B = Indian Subcontinent, C = American, D = West African. The complete 3'UTR for each strain (400+ nt) was entered and analyzed in RNASTAR software, which uses a processive analysis to estimate RNA folding patterns. SOURCE: Rico-Hesse, 2009. Reproduced with permission from Future Medicine Ltd.

RNA polymerase, are responsible for genome circularization, to form replication intermediates (dsRNA), and for initiation of translation of the DENV polyprotein, thus switching from RNA replication to virion production. These hypotheses are currently being evaluated using novel and complex approaches in cell biology, viral chimeras, and biochemical and physical structure analyses.

Our studies with chimeras of SE Asian and American genotype viruses, with substitutions in the 5'UTR, EN390D amino acids, and 3'UTR, demonstrated that all three of these sites are important determinants of virus replication in primary human cell cultures (macrophages and DCs) (Cologna and Rico-Hesse, 2003). We inserted these American genotype sites into an infectious background clone of a SE Asian genotype virus, showing that replication and output rates were similar to wild-type American genotype virus in human cells. It remains for us to do the reverse experiment, by inserting SE Asian sites into an American genotype virus background to determine if replication and output can reach those of wild-type SE Asian viruses. However, modification of these structures did not affect their growth in C6/36 mosquito cell cultures. It should be noted that the C6/36 cell line is a single cell type and therefore does not represent the whole mosquito; these cell lines are known to be defective in some innate, cellular immune responses and therefore may not represent virus tropism in mosquitoes.

In 2003, when these studies were performed, the emphasis was placed on the exterior proteins of the virus as determinants of tropism and replication, meaning viral attachment sites on host cells could control the eventual output of virus. But, as we mentioned above, the number of infected cells does not actually determine the output of virus for the DENV-2 viruses we have studied. In fact, these studies helped us focus further on the role of the 3'UTR, its folding pseudoknots, and cellular factors for controlling levels of virion production from infected human cells. This is currently a very novel concept in the areas of virus replication and pathogenicity.

DENV determinants of virulence in humanized mice In 2005 we reported on the first animal model of DENV disease (DF), where NOD/SCID (immunodeficient) mice that were engrafted with hematopoietic stem cells derived from human umbilical cord blood (CB-hu-mice) were infected by injection of one DENV-2 strain, K0049 (low passage, SE Asian genotype). These mice developed viremia and the same clinical signs of disease as humans (fever, thrombocytopenia, erythema) (Bente et al., 2005). These humanized mice develop many components of the human immune system, including macrophages, dendritic cells, mast cells, and some lymphocytes (B and T cells), but they do not make specific antibodies to the infecting virus, nor do they produce educated T cells (required for DHF). Figure A10-4 outlines the procedures used to prepare these CB-hu-mice. Many other types of humanized mice have since been developed, and this is a rapidly changing field of research (for review see Brehm et al., 2013).

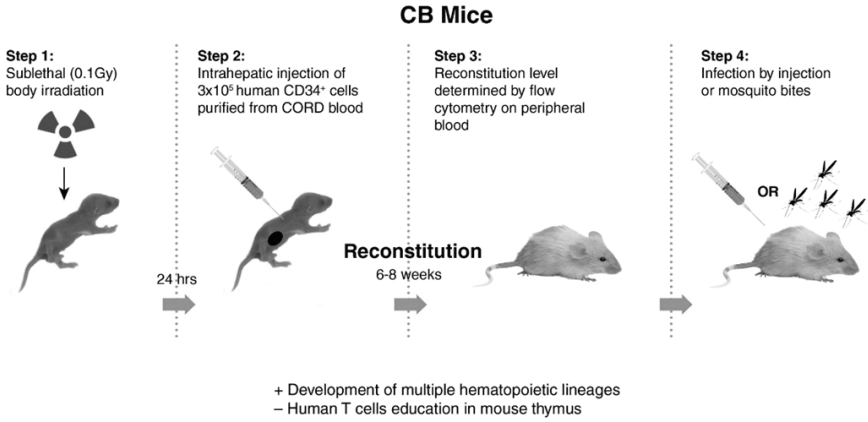

FIGURE A10-4 Preparation of humanized mice, using umbilical cord blood hematopoietic stem cells (CB-hu-mice), and methods of infection with DENV.
NOTE: Advantages (+) over other mouse models, factors missing (–) compared to other humanized mice.

After our initial report, we tested injection of eight different DENV-2 viruses (10^6 PFU equivalents, subcutaneously), representing the four genotypes, to demonstrate consistent differences in virulence of these viruses, in humanized mice (Mota and Rico-Hesse, 2009). However, we used a newer, more immunodeficient strain of mice, called NOD/SCID/IL2r gamma null (NSG), as recipients of the human umbilical cord blood stem cells (Figure A10-4), as these proved to be much better at attaining higher engraftment levels, and are currently still being used by many investigators attempting to study human-restricted pathogens. As can be seen in Figure A10-5, the representative viruses we selected produced statistically distinct viremias for each of the virus genotypes, with the SE Asian viruses highest and longest viremias, and the American genotype viruses lower (see Figure A10-5B), but not the shortest viremias (West African sylvatic genotype, Figure A10-5D), thus supporting our previous studies of epidemiologic relationships and growth in primary human target cells.

Other clinical signs were notably different, depending on the infecting DENV genotype, and statistical differences could be seen in fever, erythema, and thrombocytopenia levels. In addition, we performed studies of virus tropism, with a SE Asian genotype virus, demonstrating replication in numerous human cells, by flow cytometry and immunohistochemistry. These studies showed DENV replication in differentiated human B cells and in numerous, unidentified cells in the humanized mouse bone marrow (Mota and Rico-Hesse, 2011). It remains to be seen if we can identify all of the different types of human cells infected in these mice, as the numbers of these cells may be too small to enumerate, including those in lymph nodes, which are atrophied in these immunodeficient mice.

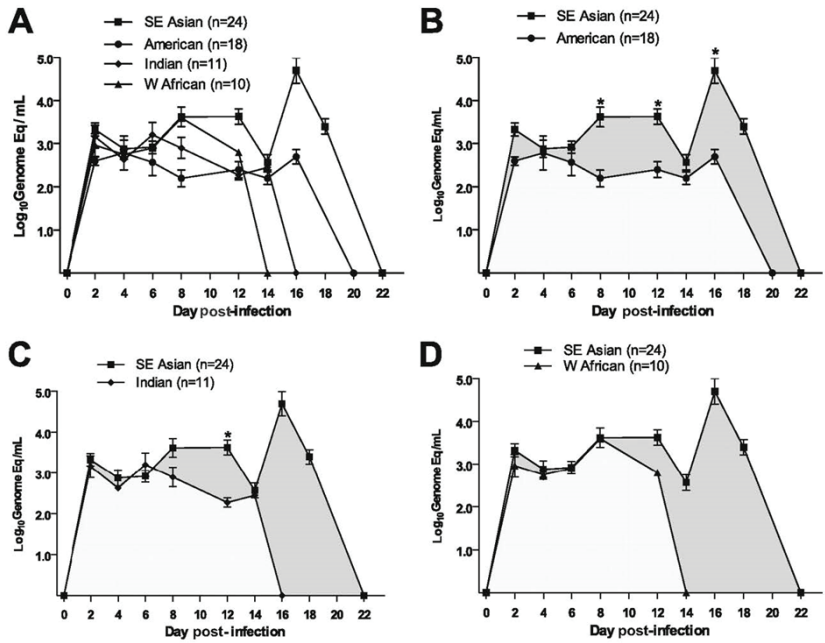

FIGURE A10-5 Comparison of viremia levels in humanized mice (CB-hu-mice) infected by inoculation of approximately six logs PFU of eight different viruses, representing the four genotypes of DENV-2. Viral genome copies were determined in sera every 2 days by a quantitative RT-PCR that has a threshold of detection at 340 viral genome copies/mL. NOTE: Error bars are SE, and n = number of infected mice per virus genotype. SOURCE: Mota and Rico-Hesse, 2009. Reproduced with permission from American Society for Microbiology.

Further improvements of this animal model may lead to a better understanding of DENV tropism for human cells, and their contribution to pathogenesis of DF and DHF.

DENV determinants of pathogenesis, including mosquito saliva To further develop the humanized mouse model of DENV disease, we initiated studies using only one strain of DENV-2, K0049, from the most virulent, SE Asian genotype, but this time by infecting the CB-hu-mice by the natural route of infection, mosquito bite (Cox et al., 2012). This entails inoculating (with very small amounts of virus, < 40 PFU eq.) female *Aedes aegypti* mosquitoes reared in the laboratory, keeping them in a biosafety level-3 lab facility for the extrinsic incubation period (7–9 days), and having them bite mouse footpads (best mimics of human epithelium), in order to transmit the virus via saliva.

These studies are extremely difficult to perform, as they require special containment facilities for both immunodeficient mice and mosquitoes, and then working with infectious, live mosquitoes in the BSL3. These studies also required titrations of how many mosquitoes are needed to bite each CB-hu-mouse, to get 100 percent development of DF; the final tally was four mosquitoes per mouse. However, our results were extremely novel, and not only did the mice have higher and more extended viremias when infected via mosquito bite (Figure A10-6), but they were able to make specific antibodies to the virus (IgM only; no class switching without T cells), and even to the mosquito saliva proteins. The erythema and thrombocytopenia were also higher, suggesting that the mosquito bite delivery has extreme effects on the virus as an antigen (opsonization), on hemostasis (probably on platelets and endothelial cells also), and these mice cannot clear the virus with their deficient immune system until after 54 days.

There are many factors that remain to be tested in CB-hu-mice, including the role of specific mosquito saliva proteins on the effects to virus and host cells, and the innate immune response, but fortunately, these mice are ready to be used in

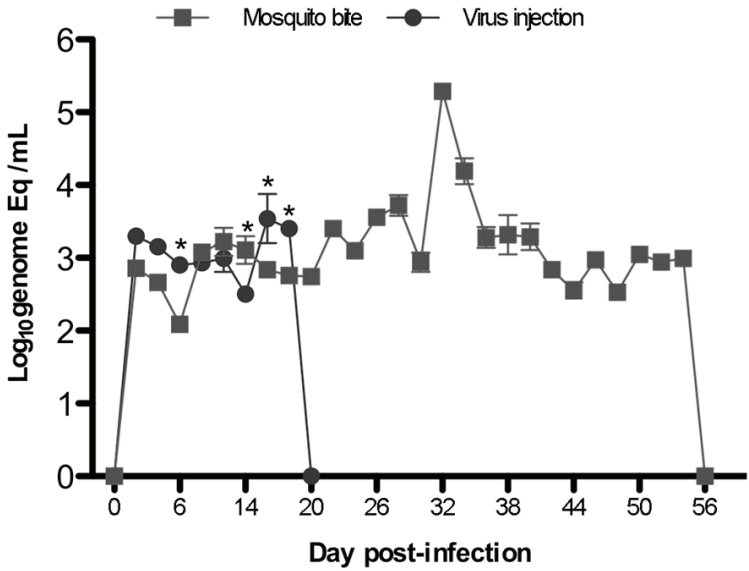

FIGURE A10-6 Comparison of viremias (measured by RNA equivalents in serum, by quantitative RT-PCR) in CB-hu-mice infected with DENV-2, strain K0049, by each of two routes: by subcutaneous injection of six log PFU equivalents, or by infected mosquito bites (4–5 mosquitoes per mouse). Asterisks denote statistically significant differences for time points where there were data for both types of infection.
SOURCE: Cox et al., 2012. Reproduced with permission from American Society for Microbiology.

tests of antivirals or other treatments, to suppress DF clinical disease. However, these results also put into question the validity of any animal tests that use injection of virus, without the mosquito saliva components that occur during natural DENV transmission.

DENV determinants of mosquito infection and transmission We began studies of DENV-2 strain replication, dissemination, and transmission dynamics in *Aedes aegypti* mosquitoes in the late 1990s in parallel with virus virulence studies (Armstrong and Rico-Hesse, 2001). In the case of DENV, this species of mosquito has been proven to be more competent than the *Aedes albopictus* mosquito, although the latter was the main vector of DENV epidemics in Hawaii and in several countries along the Mediterranean. *Aedes aegypti* populations (collected in the field) have also been shown to differ genetically and show differences in vector competence, for differing DENV strains (Armstrong and Rico-Hesse, 2003).

Our studies have shown that although DENV-2 of the four different genotypes bind to mosquito midguts at the same rates (Cox et al., 2011), their levels of replication and dissemination are extremely varied, thus suggesting that viral infection via receptors on the mosquito midgut is not the constraining factor for virus dissemination, but rather, the rates of replication and virion production in mosquito cells. In fact, for SE Asian and American genotype viruses in low passage colonies ($F < 4$) of mosquitoes established from the field (McAllen, Texas), there is an up to 60-fold difference in the potential for these mosquitoes to be able to transmit virus (SE Asian >>American). See Figure A10-7 for a comparison of vectorial capacity (Anderson and Rico-Hesse, 2006).

When we infected these mosquitoes orally (by imbibing blood in a chamber), with both SE Asian and American viruses in the same amounts in spiked blood, as a direct competition experiment, the SE Asian viruses were able to disseminate into the salivary glands by day 7 versus day 10 for American genotype viruses, in mosquitoes from varied geographical collection sites (Cologna et al., 2005). This means that the SE Asian viruses have an exponentially higher degree of transmission efficiency over American genotype viruses, and this could explain their more recent displacement of the native genotype viruses on several continents. Thus, specific DENV-2 variants have evolved and adapted to cause more viremia in human hosts (virulence) and to be more infectious and transmitted at much higher rates by their main mosquito vector (vector competence), which could explain their changing patterns of infection and transmission around the world.

These results suggest that we should better prepare for DENV emergence by focusing control efforts on those viruses that have been shown to belong to the genotypes that show increased virulence and transmission characteristics (SE Asian for DENV-2; Sri Lankan for DENV-3). We should also focus our attention on these variants to understand the complex interaction between infection and pathogenesis in the human host and to better devise vaccines and treatments that would protect against the most dangerous genotypes.

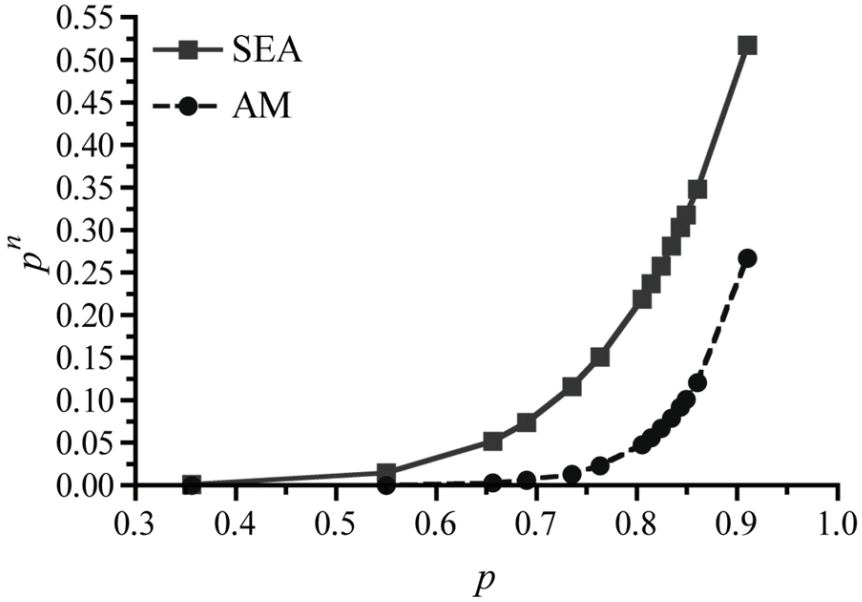

FIGURE A10-7 Vectorial capacity of field-collected (McAllen, Texas) *Aedes aegypti* mosquitoes for viruses belonging to the SE Asian genotype and American genotype of DENV-2. The probability (*P*) of these mosquitoes to transmit the SE Asian viruses is up to 60-fold higher than for American genotype viruses, depending on day after mosquito infection (extrinsic incubation).
SOURCE: Anderson and Rico-Hesse, 2006. Reproduced with permission from *American Journal of Tropical Medicine and Hygiene*.

Chikungunya Viruses

Disease Characteristics and Viral Genetic Variability

The recent introduction and establishment of transmission of CHIKV in the Americas, first detected in December 2013, has made the study of this virus a priority for our nation. So far, autochthonous transmission has been demonstrated in Florida, and it is suspected that this virus will establish itself soon in the remainder of the U.S. Gulf Coast. Historically, French scientists had been the only ones prioritizing research on this virus, mainly because their overseas laboratories (Pasteur Institutes) were located in areas of endemic CHIKV transmission, and because many of their citizens were returning sick from vacationing in their former colonies (for review see Thiberville et al., 2013). However, the disease caused by this virus, chikungunya fever, and its related, severe arthralgias, have now become a concern to most of the developed countries, due to rapid travel

and introduction of viremic humans to even temperate climates, where the main vectors of CHIKV are present, both *Aedes aegypti* and *Aedes albopictus*.

The main problem with dealing with these two efficient viral vectors is that these mosquitoes differ in their biting habits (albopictus bites other animals in addition to humans), their habitat (albopictus can live farther away from urban areas), and their tolerance to low temperatures (infected albopictus eggs can overwinter in some of the coldest climates). In addition, the virus itself differs immensely from DENV, in that it infects other parts of the human body, including keratinocytes and fibroblasts, but causes almost identical symptoms and signs of disease as DENV, which make its extremely difficult to distinguish these two diseases. But more importantly, CHIKV causes severe, long lasting, and debilitating arthralgias in children and older adults, and it has an extremely high attack rate of 90 percent, which means it causes disease in 9 out of 10 infected individuals (versus 10 percent for DENV) (Schwartz and Albert, 2010).

Although there is only one serotype of virus, and infection produces lifelong protection against reinfection, the majority of the world's population is currently susceptible to CHIKV, and this virus can be transmitted by mosquitoes that live in most parts of the globe. Therefore, we expect CHIKV to become the most prevalent and important emerging arbovirus of our lifetime.

Phylogenetic Trees and Structures

The CHIKV genome consists of approximately 12,000 nucleotides of RNA, which encode 9 proteins, in the order 5′-nonstructural proteins 1-4-capsid-envelope proteins1-3–protein 6k-3′; in addition, there are two UTRs on each end of around 75 nucleotides each, and a poly(A) tail on the 3′ end. Numerous studies have used comparison of nucleotide sequences from either the E2 or E1 envelope genes to generate phylogenetic trees of evolutionary relationships of CHIKV strains from around the world (Lanciotti et al., 1998; Powers et al., 2000). The most recent phylogenies, using complete coding genome sequences, consistently classify CHIKV into three genotypes that correspond with geographic origin: the West African, Asian, and Eastern Central Southern African (ECSA) (Thiberville et al., 2013). These investigators have also derived routes of spread of these genotypes, and most seem to have emerged from West Africa in the 1950s and 1960s, to other African regions (ECSA) and Asia, where independent cycles of evolution formed the new genotypes.

So far, most researchers have been concerned about the recent expansion of the ECSA genotype into islands in the Indian Ocean and from there to the Indian subcontinent, causing massive epidemics in 2004–2008. These events prompted concern around the world, with the first major funding initiatives to produce CHIKV vaccines and antivirals, and to investigate the viral biology and structural determinants of virulence. In contrast with DENV, where numerous structures of virions at various stages of maturation and resolution have been determined, and

from all four serotypes, for CHIKV there is no available complete virion structure (only for a virus-like-particle) (Sun et al., 2013).

We have shown this here, in Figure A10-8, with some of the purported sites of biological significance highlighted on the glycoprotein envelope protein (E1, E2, E3) trimer structure, as described by others (Voss et al., 2010). By using these predicted virion structures as a backdrop, we can see that three of the most important virus neutralization, virulence, and mosquito transmission determinants are all exposed on the end of the glycoprotein trimer (Figure A10-8B). Unfortunately, for DENV, the structures described to date do not show simple, consistent sites for some of these biological characteristics, and this may be complicated by the fact that those viruses show such varied antigenic structure (serotypes) and virulence characteristics.

Virus Replication in Target Cells

Although numerous reports describe growth of different strains of CHIKV in keratinocytes and fibroblasts in culture, a recent report disputes those results, and concludes that keratinocytes first serve as an antiviral defense on the skin, with specific innate immune factors secreted (Bernard et al., 2014). However, there are ample indications that fibroblasts, epithelial, and endothelial cells are infected and produce virus progeny soon after infection. In fact, these cells are probably producing the majority of the inflammatory response in joints and muscles, as they produce immune factors of activation and recruitment such as cytokines and chemokines (Sourisseau et al., 2007). In addition, because primary infections by any CHIKV strain seem to induce lifelong immunity to disease, it is most probable that patients make very high amounts of antibodies (and specific T cells and plasmablasts) that can serve to neutralize any incoming virus during a secondary infection. Such a strong neutralizing epitope has been described in a monoclonal antibody derived from patient's plasma, and its primary binding site on the virion has been defined on E2, across the I121 and W64 amino acids (see Figure A10-8B).

The current problem with understanding the complete cycle of CHIKV replication and disease causation is that there are no detectable infectious virus particles in the joints or other organs of animals (mice and nonhuman primates) infected and studied after viremia disappearance, during the expected chronic sequellae. Immunodeficient and very young mice (Couderc et al., 2008) and some primates (Labadie et al., 2010) have been shown to develop CHIKV disease similar to humans, but none of these models fully mimics the route of inoculation and complete pathological picture as in humans.

The most recent study, in wild-type mice, suggests that a single amino acid on the E2 glycoprotein (E282), and some epistatic site differences (see Figure A10-8B), may determine the amount of dissemination and arteritis in this model; however, these mice do not entirely reflect the pathological processes

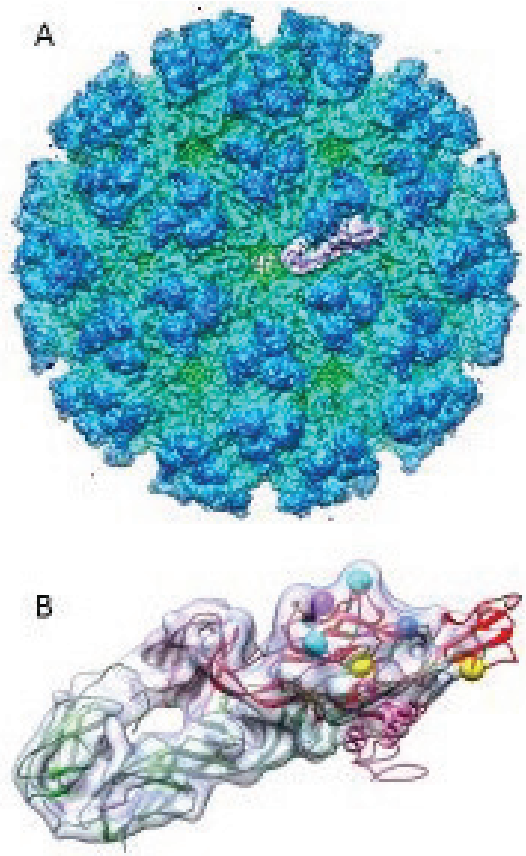

FIGURE A10-8 Chikungunya virion and glycoprotein structures, including sites of purported biological activities. (A). Cryo electron density map of chikungunya virus-like-particles, low pass filtered to 10Å and radial colored (green: 220Å to blue: 420Å). Purple density corresponds to a single E1-E2-E3 glycoprotein complex. (B). X-ray crystal structure of E1(green)-E2(red)-E3(pink) docked into the cryo electron density map shown in A. Residue 82 (purple) of E2 is known to be associated with dissemination and arthritis in mice. Residues 121 and 64 (cyan) of E2 are responsible for neutralization by a human MAb. Residue 226 (blue) of E1 has been shown to affect mosquito transmission efficiency. Finally, residues 211 and 60 (yellow) of E2 have been shown to exert epistatic effects on residue 226 (blue) of E1.
SOURCE: Cryo electron density map modified from Sun et al., 2013 (EMDB-5577); X-ray crystal structure was modified from Voss et al., 2010 (PDB-3N42).

occurring in humans, as they develop footpad and hind limb swelling only, without showing clinical signs (viremia, rash, fever, weight loss) and recumbence as in humans (Ashbrook et al., 2014). Thus, we are currently at a lack of complete animal models in which to study the pathogenesis of CHIKV and DENV, including the immune mechanisms and factors that might lead to severe disease presentations.

CHIKV Determinants of Pathogenesis, Including Mosquito Saliva

We propose to use a newer—although more complicated—type of humanized mouse to study immunopathogenesis of both DENV and CHIKV to attempt to develop disease that fully mimics human signs and postviremia induction of severe sequellae (DHF with secondary dengue, arteritis/arthritis in chikungunya fever). The preparation of these mice, known as bone marrow-liver-thymus humanized mice (BLT-hu-mice), is outlined in Figure A10-9. In this case, the adult NSG mice, the same strain as used for CB-hu-mice, are surgically implanted (suprarenal capsule) with human stem cells derived from fetal liver and thymus, for a much more complete reconstitution of the human immune system, including adaptive immunity (for a review see Brehm et al., 2013). These mice attain high levels of engraftment of the mouse bone marrow, substituting human cells, and because of the presence of human thymus, they are able to continually produce functional B and T cells, and thus make specific human antibodies, with class switching (IgM and IgG antibodies).

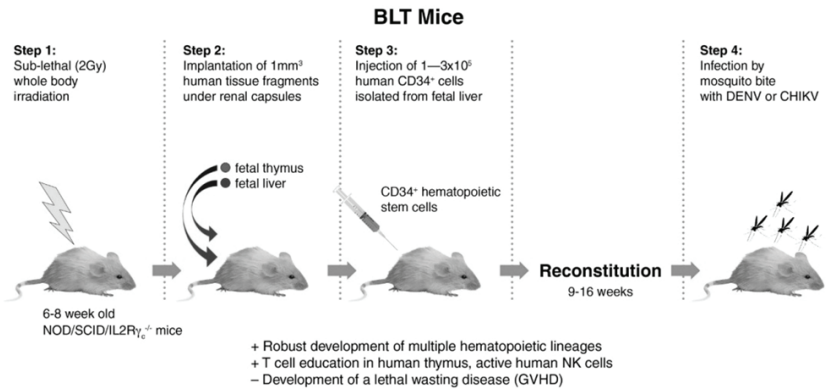

FIGURE A10-9 Preparation of humanized mice, using fetal tissues and hematopoietic stem cells (BLT-hu-mice: bone marrow, liver, and thymus), and methods of infection with DENV or CHIKV.
NOTE: Advantages (+) over other mouse models; disadvantages (-) compared to other humanized mice.

Although these mice are extremely difficult to produce, they theoretically allow for the measurement of human immune responses, including innate and adaptive factors, which could possibly lead to the models necessary for studying severe DENV and CHIKV disease. These animals could also potentially be used to determine the effects of mosquito saliva proteins, as these have been reported to be important mediators of infection by CHIKV, in cell culture and in wild-type mice (Thangamani et al., 2010). Use of these mice could allow us to perform natural route of infection experiments with CHIKV, which we have shown drastically modify the effects of infection and immunity to DENV.

CHIKV Determinants of Mosquito Infection and Transmission

Previous studies using infectious clones of CHIKV (ECSA genotype) and their recombinant modifications implicated a site in the E1 glycoprotein (A226V) as a determinant of switching vector competence from *Aedes aegypti* to *Aedes albopictus* (Tsetsarkin et al., 2007, 2009). This would have created a very significant problem for the expansion of CHIKV disease into entirely new areas and new human populations, or a new mode of vector adaptation. However, more recent experiments using over 35 different colonies of *Aedes albopictus* and *Aedes aegypti* mosquito strains, and testing CHIKV strains with the E1226A and E1226V mutation in the ECSA genotype backgrounds, and the new, Asian genotype virus that has been introduced recently to the Americas, have not shown consistent statistically significant differences in vector competence for either species of mosquito (three groups showed lower dissemination in *albopictus*) (Vega-Rua et al., 2014). However, these studies failed to include sufficient Asian genotype virus strains to fully evaluate their rates of transmission by either mosquito species, and these are the CHIKV genotype variants that concern us most now.

Another concern is that there is evidence that *Aedes albopictus* can replicate and transmit both DENV and CHIKV simultaneously, after oral infection (Vazeille et al., 2010); therefore, we need to evaluate the dynamics of transmission and virulence when both viruses are infecting mosquitoes and human hosts simultaneously. This adds a major degree of complexity to the measurements of evolution and virulence that should be taken into account for various assay systems and ultimately, in the way that arbovirus dynamics and emergence mechanisms can be modeled effectively.

Acknowledgments

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References

Anderson, J. R., and R. Rico-Hesse. 2006. *Aedes aegypti* vectorial capacity is determined by the infecting genotype of dengue virus. *American Journal of Tropical Medicine Hygiene* 75(5):886-892.

Armstrong, P. M., and R. Rico-Hesse. 2001. Differential susceptibility of *Aedes aegypti* to infection by the American and Southeast Asian genotypes of dengue type 2 virus. *Vector Borne Zoonotic Diseases* 1(2):159-168.

Armstrong, P. M., and R. Rico-Hesse. 2003. Efficiency of dengue serotype 2 virus strains to infect and disseminate in *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene* 68(5):539-544.

Ashbrook, A. W., K. S. Burrack, L. A. Silva, S. A. Montgomery, M. T. Heise, T. E. Morrison, and T. S. Dermody. 2014. Residue 82 of the chikungunya virus E2 attachment protein modulates viral dissemination and arthritis in mice. *Journal of Virology* 88(21):12180-12192.

Bente, D. A., M. W. Melkus, J. V. Garcia, and R. Rico-Hesse. 2005. Dengue fever in humanized NOD/SCID mice. *Journal of Virology* 79(21):13797-13799.

Bernard, E., R. Hamel, A. Neyret, P. Ekchariyawat, J. P. Moles, G. Simmons, N. Chazal, P. Despres, D. Misse, and L. Briant. 2014. Human keratinocytes restrict chikungunya virus replication at a post-fusion step. *Virology* 476C:1-10.

Bhatt, S., P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, et al. 2013. The global distribution and burden of dengue. *Nature* 496(7446):504-507.

Brehm, M. A., N. Jouvet, D. L. Greiner, and L. D. Shultz. 2013. Humanized mice for the study of infectious diseases. *Current Opinions in Immunology* 25(4):428-435.

Cologna, R., and R. Rico-Hesse. 2003. American genotype structures decrease dengue virus output from human monocytes and dendritic cells. *Journal of Virology* 77(7):3929-3938.

Cologna, R., P. M. Armstrong, and R. Rico-Hesse. 2005. Selection for virulent dengue viruses occurs in humans and mosquitoes. *Journal of Virology* 79(2):853-859. doi: 10.1128/JVI.79.2.853-859.2005.

Couderc, T., F. Chretien, C. Schilte, O. Disson, M. Brigitte, F. Guivel-Benhassine, Y. Touret, G. Barau, N. Cayet, I Schuffenecker, P. Despres, F. Arenzana-Seisdedos, A. Michault, M.L. Albert, and M. Lecuit. 2008. A mouse model for chikungunya: Young age and inefficient type-I interferon signaling are risk factors for severe disease. *PLoS Pathogens* 4(2):e29.

Cox, J., H. E. Brown, and R. Rico-Hesse. 2011. Variation in vector competence for dengue viruses does not depend on mosquito midgut binding affinity. *PLoS Neglected Tropical Diseases* 5(5):e1172.

Cox, J., J. Mota, S. Sukupolvi-Petty, M. S. Diamond, and R. Rico-Hesse. 2012. Mosquito bite delivery of dengue virus enhances immunogenicity and pathogenesis in humanized mice. *Journal of Virology* 86(14):7637-7649.

Cruz, C. D., B. M. Forshey, D. S. Juarez, C. Guevara, M. Leguia, T. J. Kochel, and E. S. Halsey. 2013. Molecular epidemiology of American/Asian genotype DENV-2 in Peru. *Infections Genetics, and Evolution* 18:220-228.

Guzman, M. G., S. B. Halstead, H. Artsob, P. Buchy, J. Farrar, D. J. Gubler, E. Hunsperger, A. Kroeger, H. S. Margolis, E. Martinez, M. B. Nathan, J. L. Pelegrino, C. Simmons, S. Yoksan, and R. W. Peeling. 2010. Dengue: A continuing global threat. *Nature Reviews Microbiology* 8(12 Suppl):S7-S16.

Holmes, E. C. 2006. The evolutionary biology of dengue virus. *Novartis Foundation Symposia* 277:177-187; discussion 87-92, 251-253.

Holmes, E. C., M. Worobey, and A. Rambaut. 1999. Phylogenetic evidence for recombination in dengue virus. *Molecular Biology and Evolution* 16(3):405-409.

Jessie, K., M. Y. Fong, S. Devi, S. K. Lam, and K. T. Wong. 2004. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. *Journal of Infectious Diseases* 189(8):1411-1418.

Labadie, K., T. Larcher, C. Joubert, A. Mannioui, B. Delache, P. Brochard, L. Guigand, L. Guigand, L. Dubreil, P. Lebon, B. Verrier, X. de Lamballerie, A. Suhrbier, Y. Cherel, R. Le Grand, and P. Roques. 2010. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *Journal of Clinical Investigations* 120(3):894-906.

Lanciotti, R. S., M. L. Ludwig, E. B. Rwaguma, J. J. Lutwama, T. M. Kram, N. Karabatsos, B. C. Cropp, and B. R. Miller. 1998. Emergence of epidemic O'nyong-nyong fever in Uganda after a 35-year absence: Genetic characterization of the virus. *Virology* 252(1):258-268.

Leitmeyer, K. C., D. W. Vaughn, D. M. Watts, R. Salas, I. Villalobos, Chacon de, C. Ramos, and R. Rico-Hesse. 1999. Dengue virus structural differences that correlate with pathogenesis. *Journal of Virology* 73(6):4738-4747.

Messer, W. B., U. T. Vitarana, K. Sivananthan, J. Elvtigala, L. D. Preethimala, R. Ramesh, N. Withana, D. J. Gubler, and A. M. De Silva. 2002. Epidemiology of dengue in Sri Lanka before and after the emergence of epidemic dengue hemorrhagic fever. *American Journal of Tropical Medicine and Hygiene* 66(6):765-773.

Messer, W. B., D. J. Gubler, E. Harris, K. Sivananthan, and A. M. de Silva. 2003. Emergence and global spread of a dengue serotype 3, subtype III virus. *Emerging Infectious Diseases* 9(7):800-809.

Messina, J. P., O. J. Brady, T. W. Scott, C. Zou, D. M. Pigott, K. A. Duda, S. Bhatt, L. Katzelnick, R. Howes, K. Battle, C. Simmons, and S. Hays. 2014. Global spread of dengue virus types: Mapping the 70 year history. *Trends in Microbiology* 22(3):138-146.

Mota, J., and R. Rico-Hesse. 2009. Humanized mice show clinical signs of dengue fever according to infecting virus genotype. *Journal of Virology* 83(17):8638-8645.

Mota, J., and R. Rico-Hesse. 2011. Dengue virus tropism in humanized mice recapitulates human dengue fever. *PLoS One* 6(6):e20762.

Powers, A. M., A. C. Brault, R. B. Tesh, and S. C. Weaver. 2000. Re-emergence of Chikungunya and O'nyong-nyong viruses: Evidence for distinct geographical lineages and distant evolutionary relationships. *Journal of General Virology* 81(Pt 2):471-479.

Rico-Hesse, R. 1990. Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. *Virology* 174(2):479-493.

Rico-Hesse, R. 2003. Microevolution and virulence of dengue viruses. *Advances in Virus Research* 59:315-341.

Rico-Hesse, R. 2007. Dengue virus evolution and virulence models. *Clinical Infectious Diseases* 44(11):1462-1466.

Rico-Hesse, R. 2009. Dengue virus markers of virulence and pathogenicity. *Future Virology* 4(6):581.

Rico-Hesse, R., L. M. Harrison, R. A. Salas, D. Tovar, A. Nisalak, C. Ramos, J. Boshell, M. T. de Mesa, R. M. Nogueira, and A. T. da Rosa. 1997. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology* 230(2):244-251.

Rothman, A. L. 2011. Immunity to dengue virus: A tale of original antigenic sin and tropical cytokine storms. *Nature Reviews Immunology* 11(8):532-543.

Sabin, A. B. 1952. Research on dengue during World War II. *American Journal of Tropical Medicine and Hygiene* 1(1):30-50.

Schwartz, O., and M. L. Albert. 2010. Biology and pathogenesis of chikungunya virus. *Nature Reviews of Microbiology* 8(7):491-500.

Snow, G. E., B. Haaland, E. E. Ooi, and D. J. Gubler. 2014. Research on dengue during World War II revisited. *American Journal of Tropical Medicine and Hygiene* 91(6):1203-1217.

Sourisseau, M., C. Schilte, N. Casartelli, C. Trouillet, F. Guivel-Benhassine, D. Rudnicka, N. Sol-Foulon, et al. 2007. Characterization of reemerging chikungunya virus. *PLoS Pathogens* 3(6):e89.

Sun, Y., J. Yan, H. Mao, L. Zhang, Q. Lyu, Z. Wu, W. Zheng, C. Feng, and Y. Zhang. 2013. Characterization of the complete genome of chikungunya in Zhejiang, China, using a modified virus discovery method based on cDNA-AFLP. *PLoS One* 8(12):e83014.

Thangamani, S., S. Higgs, S. Ziegler, D. Vanlandingham, R. Tesh, and S. Wikel. 2010. Host immune response to mosquito-transmitted chikungunya virus differs from that elicited by needle inoculated virus. *PLoS One* 5(8):e12137.

Thiberville, S. D., N. Moyen, L. Dupuis-Maguiraga, A. Nougairede, E. A. Gould, P. Roques, and X. de Lamballerie. 2013. Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research* 99(3):345-370.

Tsetsarkin, K. A., D. L. Vanlandingham, C. E. McGee, and S. Higgs. 2007. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathogens* 3(12):e201.

Tsetsarkin, K. A., C. E. McGee, S. M. Volk, D. L. Vanlandingham, S. C. Weaver, and S. Higgs. 2009. Epistatic roles of E2 glycoprotein mutations in adaption of chikungunya virus to *Aedes albopictus* and *Ae. aegypti* mosquitoes. *PLoS One* 4(8):e6835

Vazeille, M., L. Mousson, E. Martin, and A. B. Failloux. 2010. Orally co-infected *Aedes albopictus* from La Reunion Island, Indian Ocean, can deliver both dengue and chikungunya infectious viral particles in their saliva. *PLoS Neglected Tropical Diseases* 4(6):e706.

Vega-Rua, A., K. Zouache, R. Girod, A. B. Failloux, and R. Lourenco-de-Oliveira. 2014. High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of Chikungunya virus. *Journal of Virology* 88(11):6294-6306.

Voss, J. E., M. C. Vaney, S. Duquerroy, C. Vonrhein, C. Girard-Blanc, E. Crublet, A. Thompson, G. Bricogne, and F. A. Rey. 2010. Glycoprotein organization of chikungunya virus particles revealed by X-ray crystallography. *Nature* 468(7324):709-712.

Wu, S. J., G. Grouard-Vogel, W. Sun, J. R. Mascola, E. Brachtel, R. Putvatana, M. K. Louder, et al. 2000. Human skin Langerhans cells are targets of dengue virus infection. *Nature Medicine* 6(7):816-820.

A11

VECTOR-BORNE DISEASE EMERGENCE AND SPREAD IN THE EUROPEAN UNION[1]

*Jan C. **Semenza***[2]

The emergence and spread of vector-borne diseases (VBD) in Europe is a function of biotic (living organisms in an ecosystem), abiotic (nonliving elements in an ecosystem) and socioeconomic drivers of disease. Permissive circumstances that coincide in time and space can trigger an outbreak of VBD. Anticipating and elucidating such an outbreak requires a systems perspective. In a foresight study, the European Centre for Disease Prevention and Control mapped the interrelated and interdependent nature of disease drivers in order to predict the abrupt emergence of infectious disease threats by 2020 in Europe (Suk and Semenza, 2011). The most significant infectious disease drivers for Europe were grouped into three broad categories: globalization and environmental change, social and

[1] Modified by author from Int. J. Environ. Res. Public Health 2015, 12(6), 6333–6351, doi:10.3390/ijerph120606333 for inclusion in this workshop summary.

[2] European Centre for Disease Prevention and Control.

demographic change, and the public health system. Their relation to VBD is briefly described below.

Globalization and Environmental Change

These two factors are recognized as significant disease drivers. They include the steadily expanding reach of travel and trade and population movements. Global disease dispersal is aided by a dense network of air traffic and shipping routes (Semenza et al., 2014; Thomas et al., 2014). They have facilitated the arrival, establishment, and spread of invasive pathogens to novel geographic destinations, including dengue, malaria, chikungunya, and West Nile (Randolph and Rogers, 2010). Approximately 60 percent of human pathogens are estimated to be of zoonotic origin, in that they can be transmitted from animals to humans (Karesh et al., 2012). Thus, land use can indirectly determine the spread of zoonotic diseases through different exposure pathways in urban, suburban, and rural settings with a wider range of animal habitats such as pastures, arable fields, and managed forests (Karesh et al., 2012; Patz et al., 2004). Urbanization, urban sprawl, and high-population densities have also been associated with VBD emergence (Jones et al., 2008). Habitat encroachment and habitat destruction can result in displacement of wild animals into novel environments that can have a bearing on exposure patterns to infectious pathogens. Climatic conditions are also significant drivers of VBD as some of the vectors are cold-blooded; thus, climate change can shift the geographical ranges of VBD transmission (McMichael, 2013b; Lindgren et al., 2012; Confalonieri et al., 2007; Altizer et al., 2013; Semenza and Menne, 2009; Semenza et al., 2012).

Social and Demographic Change

The human world is currently experiencing shifts in demographic profiles, social inequality, and lifestyles. Socially and economically disadvantaged groups suffer disproportionally from infectious diseases in Europe (Semenza and Giesecke, 2008; Suhrcke et al., 2011). In the 1990s, during times of economic hardship, individuals in Central and Eastern Europe resorted to mushroom harvesting in wooded areas, and thereby increased their risk of tick-borne encephalitis (Stefanoff et al., 2012). The economic crisis in Kosovo in 1999–2000 resulted in the abandonment of food stores with the subsequent rise in rodent populations which led to the emergence of tularemia (Reintjes et al., 2002). The 2007 mortgage foreclosures in the Californian housing market resulted in many abandoned homes with swimming pools, increasing breeding habitats for mosquitoes, which was linked to an unexpectedly early seasonal increase in West Nile virus cases (Reisen et al., 2008).

The Public Health System

This includes surveillance and reporting, research and development, animal and food safety and health care. However, current surveillance systems might not be adequately equipped to cope with the arrival and dispersal of tropical pathogens commonly associated with warmer temperatures (Lindgren et al., 2012; Semenza and Domanović, 2013). Contamination of blood products from donors infected with known, unexpected, or unknown pathogens represents a significant threat to the blood supply and thus to public health. Current microbial blood-safety practices might not be adequate to cope with global environmental change (Semenza and Domanović, 2013). Research and development of novel surveillance systems and of pathogen-reduction technologies for the blood supply might reduce the risk from these emerging threats (Semenza et al., 2013; Lindgren et al., 2012; Semenza and Domanović, 2013). Access to health care is an important determinant for early treatment for a VBD and can help interrupt the outbreak by removing a host from the transmission cycle.

Eight plausible threat scenarios facing the European Union by 2020 were formulated, based on the line-listing of different drivers for infectious diseases from the ECDC foresight study described above (Suk and Semenza, 2011). These threat scenarios were selected based on the plausibility of the event, potential severity of the scenario in terms of burden of disease, and relevance of the scenario to multiple EU member states. They were primarily intended for prioritization of public health interventions and health policy decision making. These plausible scenarios were used to develop tangible steps to mitigate the potential public health fallout from such infectious disease threats (Suk and Semenza, 2011). One plausible scenario included a VBD outbreak triggered by environmental/climate change, travel and tourism, global trade, and social inequality. The VBD scenario considered a threat from the introduction of new disease vectors, which creates new opportunities for disease transmission; expanded ability of vectors to transmit pathogens (e.g., by mutation); and a shift in the transmission range of diseases, hosts, and vectors due to socioeconomic factors and climate change.

The drivers and plausible scenarios for 2020 were compared to the threat events that actually occurred between 2008 and 2013 and detected through ECDC's epidemic intelligence activities (Semenza et al., 2016). ECDC conducts epidemic intelligence by monitoring all events (e.g., from media reports) that could potentially be a threat to public health in Europe. Event-based data collected at ECDC are verified and archived on a daily basis and used to generate the Communicable Disease Threats Reports (CDTR); these are weekly reports summarizing information gathered through epidemic intelligence regarding communicable disease threats of concern to the EU. Data and epidemiological information for each event were extracted from the CDTR, epidemiological reports and communications, ECDC rapid risk assessments, threat assessments, mission reports, and scientific publications. The threat events were sorted into 10 threat categories:

1. Vector and rodent-borne diseases,
2. Food and water-borne diseases,
3. Other zoonosis,
4. Vaccine-preventable diseases,
5. Multidrug-resistant diseases,
6. Health care-associated infections,
7. Injecting drug-use associated diseases,
8. Sexually transmitted infections,
9. Pandemic influenza,
10. Air-borne infections.

A total of 116 threats, 10 threat categories, and 17 drivers in 3 driver categories, were recorded (see Figure A11-1). Vector- and rodent-borne disease events were the second most common threat events in the database with 27 individual events. The event-based database did not comprehensively capture mortality and morbidity (ECDC has a dedicated repository for notifiable infectious diseases), but 64 deaths and 4,748 illnesses were attributed to those 27 individual VBD

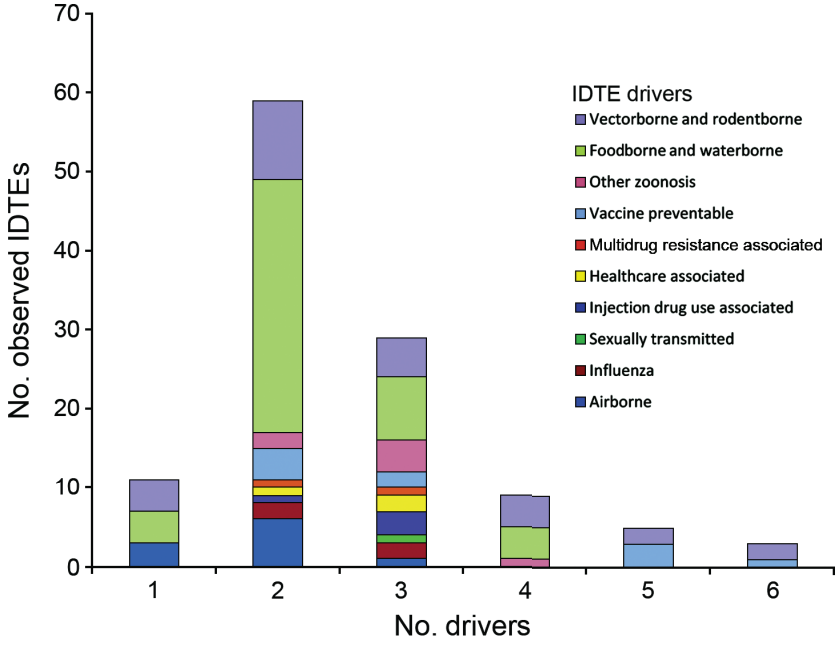

FIGURE A11-1 Number of observed infectious disease threat events (IDTEs) in relation to number of drivers for each IDTE group, Europe, 2008-2013.
NOTE: This figure is a new visualization of the same data set that was presented at the workshop.
SOURCE: Semenza et al., in press with *Emerging Infectious Diseases*. Data from ECDC.

events. These events included the large dengue outbreak in Madeira (Lourenco and Recker, 2014) (over 2,000 cases) and the West Nile fever outbreak in Southeast Europe with over 260 cases in Greece alone (Paz et al., 2013).

Based on a logistic regression analysis of all the drivers for the vector and rodent-borne diseases category, the natural environment, climate, and lifestyle scored as the top three drivers. "Natural environment" was the sole driver in four events, a codriver in two events with climate, and one of four drivers in four events. The majority of these events were West Nile fever outbreaks where environmental and climatic determinates obviously play an important role (Paz and Semenza, 2013). The lifestyle driver pertained to a large outbreak of hantavirus in Germany in 2010 where the bank vole populations had increased substantially due to excessive seed production the previous year (Faber et al., 2010); human behavior favouring exposure and potentially increased dust production following dry and warm weather were attributed to the outbreak.

Comparing the scenarios of the 2020 foresight study (Suk and Semenza, 2011) with the threat events that actually occurred between 2008 and 2013 reveals some similarities. The large dengue outbreak in Madeira was sparked by the importation of viremic air traffic passengers in an environment where the vector had recently been introduced. Environmental and climatic conditions contributed to the upsurge of WNF in Southeast Europe (Paz and Semenza, 2013). Social inequality was a factor in the emergence of malaria in Greece in 2009 where migrant workers from endemic countries were part of the malaria transmission cycle (Sudre et al., 2013). However, regardless of the accuracy of such foresight studies VBD continue to emerge and spread in the European Union. Traditional public health strategies might not suffice to cope with the public health challenges associated with global environmental change. ECDC has developed a pragmatic approach to tackle these emerging threats which are described below.

The European Environment and Epidemiology (E3) Network

Many environmental drivers can be considered epidemic precursors of disease (see Figure A11-2). Monitoring changes in environmental conditions can help anticipate, or even forecast, an upsurge of disease (Lindgren et al., 2012; Semenza and Menne, 2009; Semenza et al., 2013). However, traditional environmental and infectious disease epidemiology is hampered by a number of shortcomings when it comes to the public health challenges from global environmental change (McMichael, 2013a). Environmental, climatic, or epidemiologic data often lack historic baseline measurements which make comparison and extrapolation difficult. The effects of global environmental change do not adhere to typical effect-response relationships which traditional epidemiologic methods have been refined (and perfected) to measure. The pathways tend to be more complex and sometimes protracted; they can be direct but more often than not indirect, diffuse or delayed (Butler and Harley, 2010). Estimating future health risks requires

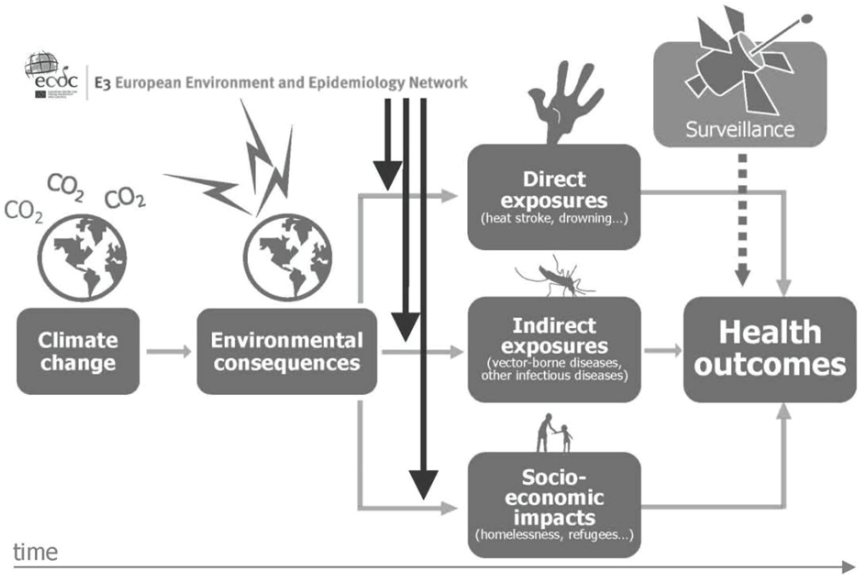

FIGURE A11-2 European Environment and Epidemiology (E3) Network.
SOURCE: © European Centre for Disease Prevention and Control (ECDC) (www. ecdc. europa.eu).

interdisciplinary collaborations to develop scenario-based models. Moreover, detection of health endpoints with traditional surveillance methods suffers from significant time lags because of delays in case identification, diagnosis, and reporting, which can result in exposure misclassification and confounding. A geographic shift in infectious diseases might also lead to an expansion of the disease burden into new areas and might therefore be missed. Thus, these changes in the risk profile for human populations call for novel approaches to assess interconnected and interdependent risks (Altizer et al., 2013; Woolhouse, 2011).

Rapid developments in geographic information systems over the last decades have facilitated the management and use of spatial data for analytic epidemiology. A number of tools are now available over the web to explore and map spatial data that adhere to the standards of geographic data (e.g., INSPIRE directive). Risk models can then be used for the quantitative estimation of dynamic risks by taking into account changes in disease drivers. With this approach, future risk under different scenarios can be estimated.

The European Centre for Disease Prevention and Control (ECDC) has recognised the need for a proactive approach to deal with global environmental change (Lindgren et al., 2012; Semenza and Menne, 2009; Semenza et al., 2013). ECDC has developed an infrastructure coined the European Environment and

Epidemiology (E3) Network to help monitor environmental and climatic conditions related to infectious disease threats (see Figure A11-2) (Semenza et al., 2013; Semenza and Menne, 2009). The hub is composed of a data repository, a geoportal for data visualization and dissemination, and online tools that support the analysis of environmental, climatic and social drivers of infectious diseases (see Figure A11-3) (ECDC, 2014). The E3 Network provides technical support for the reporting, monitoring, analysis, and mapping of data and enhances the analytical capacity of existing resources in Europe. Results have been disseminated to policy makers, public health practitioners, European Union and international agencies, other governmental sectors, and nongovernmental organisations.

With the E3 Network, climatic, weather, and environmental data can be merged and integrated with health data in order to provide support tools for decision makers (see Figure A11-4) (Lindgren et al., 2012; Semenza and Menne, 2009; Semenza et al., 2013). Easy and rapid linkage of data for novel analyses provides novel opportunities to deal with the complex nature of interconnected and interdependent risks. Such an approach can rapidly identify geographic areas of increased risk at a certain point in time. It can also define high-risk populations that are particularly vulnerable to exposure and guide public health interventions.

FIGURE A11-3 E3 geoportal of the European Environment and Epidemiology (E3) Network.
SOURCE: © ECDC.

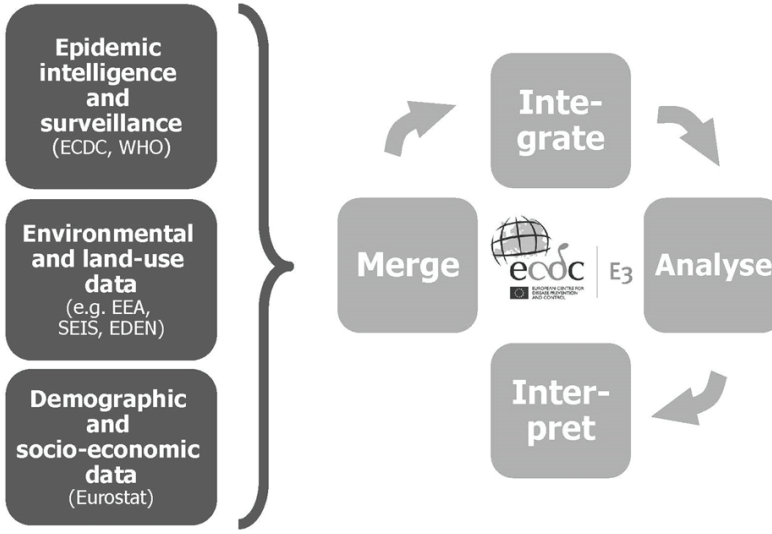

FIGURE A11-4 European Environment and Epidemiology (E3) Network.
SOURCE: © ECDC.

Information from these analyses can provide lead time for outreach to the public and engagement of health care providers. It can also be used to set public health policies and inform civil society about potential consequences of global environmental change on public health.

The initial building-block of the E3 Network was a set of data that was assembled through a research project of the Directorate-General for Research and Innovation of the European Commission entitled Emerging Diseases in a Changing European Environment project (EDEN). The processing of these data sets, and those continuously assembled from other sources, with regular outputs from advanced scientific analysis, serve as a reference point (ECDC, 2014). It also supports data exchanges and scientific collaborations between member states, researchers, ECDC experts, and other authorised users across geographical and political boundaries in the European Community, with particular interest in the area of climatic change adaptation, landscape epidemiology, and emerging disease threats. The data of the E3 Network have been used in a number of case studies, three of which are briefly described below.

Intercepting Vector-Borne Disease Emergence and Spread: Case Studies

Environmental Suitability of Malaria Transmission in Greece

E3 data were used to predict the environmental suitability of malaria transmission in Greece (Sudre et al., 2013). In the past, malaria was endemic in Greece, but due to a successful malaria control and elimination programmes the country was declared malaria free in 1974 (Sabatinelli et al., 2001; Danis et al., 2011). Yet, importation of malaria has continued to occur accompanied by sporadic autochthonous transmission (Vakali et al., 2012; Kampen et al., 2002, 2003). A cluster of six locally acquired *Plasmodium vivax* cases without travel history to an endemic area was discovered in 2009; a total of 267 malaria cases were noted by Greek health authorities between 2009 and 2012, although some reported a travel history (Danis et al., 2011).

Nevertheless, the continuing transmission of *P. vivax* by indigenous vectors in areas with permissive environmental and climatic conditions could potentially signal the reemergence of malaria in Greece. Delineating specific areas environmentally suitable for transmission could direct and focus malaria control efforts. To assess the location of exposure of locally acquired malaria for such a suitability map, a standardized questionnaire was administered to each malaria case in Greece by a health officer. Cases with a travel history were excluded from this analysis as the goal was to delineate the areas environmentally suitable for autochthonous malaria transmission in Greece. By defining the environmental and climatic profile of areas with active transmission cycles between 2009 and 2012 other areas at risk for malaria re-emergence in Greece could then be defined.

Georeferenced environmental and climatic information for Greece and several other data sources were retrieved from the E3 Network data repository and other sources and processed for spatial modeling (Scharlemann et al., 2008; Earth Resources Observation and Science Center USGS EROS, 2005; European Environment Agency, 2011; The Joint Research Centre, 2009). They included demographic indicators, land cover categories, altitude, seasonal variations of vegetation, temperature, and so on. Using nonlinear discriminant analysis (NLDA) available in eRiskMapper version 1.1.4, a disease risk map was developed of areas suitable for persistent malaria transmission (Sudre et al., 2013) (see Figure A11-5).

Areas of environmental suitability for malaria transmission were characterized by warmer temperatures; low elevation; intensive, year round irrigated agriculture with complex cultivation patterns. Elevated temperatures (both nighttime and daytime temperature parameters were predictive in this model) can accelerate mosquito and parasite development which are likely contributing factors to mosquitoes presence and potentially to malaria transmission. This suitability map matched the historical distribution of malaria in Greece, particularly in the Peloponnese, the west coast of Central Greece and Epirus, and the east part of central Greece.

FIGURE A11-5 Areas latently hospitable and environmentally permissive for persistent malaria transmission, Greece, 2009–2012. Map showing areas predicted to be environmentally suitable for malaria transmission.
NOTE: Values from 0 to 0.5 (dark to light green) indicate conditions not favorable for malaria transmission (based on locally acquired cases); yellow to dark red areas delineate conditions increasingly favorable for transmission (values from 0.5 to 1).
SOURCE: Sudre et al., 2013.

This map was shared with public health practitioners in Greece responsible for integrated preparedness and response activities. Using EU structural funds, transmission was eventually interrupted in 2013 through targeted epidemiological and entomological surveillance, vector control activities, and awareness rising among the general population and health workers, in the areas environmentally suitable for transmission.

Environmental Determinants of West Nile Fever

Transmission of WNF is determined by environmental/climatic and biological drivers (Randolph and Rogers, 2010; Paz and Semenza, 2013). For sustained transmission to take hold at a given place and time, susceptible birds have to come in contact with infected birds in the presence of competent vectors. The avian transmission cycle is then amplified by local birds at which point the transmission can spill over to dead-end hosts such as humans or horses through

bridge vectors that feed on both birds and humans/horses (Paz and Semenza, 2013). A crucial aspect of WNV amplification among competent insect vectors and vertebrate hosts is also their population densities which determine the intensity of transmission. Vector population densities depend on temperature which accelerates the growth rates (Reisen et al., 2006). Moreover, elevated temperature decreases the timing between blood meals but accelerates viral replication rates and thus the transmission of WNV (Andrade et al., 2011). Thus, permissive weather and environmental conditions are responsible for sustained local transmission whereas migratory birds and short distance vector transportation affect the geographic dispersion.

In Europe, several WNF outbreaks have been linked with elevated ambient temperature (Paz and Albersheim, 2008; Savage et al., 1999; Paz et al., 2013). For example, Southeastern Europe was afflicted by a heat wave at the end of July to mid-August of 2010 which was followed by an outbreak of WNF cases (Paz et al., 2013). Temperature deviations above the 30 year mean struck Russia (deviations > 9°C), Romania (> 5°C), Turkey (> 5°C), and Greece (> 3°C) where 419, 57, 47, and 262 cases of WNF were reported, respectively (Figure A11-6). A number of meteorological variables were examined but temperature was the most significant one: in 'colder' countries of more northern latitudes a statistically significant correlation between number of WNF cases and temperature was observed, with time lags of up to 4 weeks from the onset of the temperature raise; in contrast, warmer and more southern countries presented correlations without these time lags (Paz et al., 2013). It has been noted that eruptions of WNF in previously unaffected areas tend to occur in years when summer temperatures deviate from the norm and that continued transmission can occur the following years even at normal summer temperatures (Reisen et al., 2006).

The notion that the initial outbreak is associated with a heat wave but not the subsequent ones has been observed in a number of settings (Epstein, 2001; Paz et al., 2013; Reisen et al., 2006; Pecoraro et al., 2007; Soverow et al., 2009).

To examine other environmental variables as predictors of WNF risk (Ozdenerol et al., 2013) we tested the contribution of remotely sensed temperature, the state of vegetation and water bodies, and bird migratory routes. The analysis was performed at the district level where each district was considered "infected" if WNF human cases were reported there that year, and as "non-infected" otherwise.

The number of WNF cases from 2002 to 2011 was assembled from ECDC surveillance data, peer-reviewed papers and the grey literature to fit the models. ECDC surveillance data for 2012 and 2013 were used for external validation. We used univariate and multivariate logistic regression models to assess the probability of a district to be categorized as WNV positive. The status of infection was set as the response variable, and anomalies of temperature, wetlands, and bird migratory routes were set as explanatory variables. In the final multivariate logistic regression model, parameters of WNV risk at district level for 2002–2011 were:

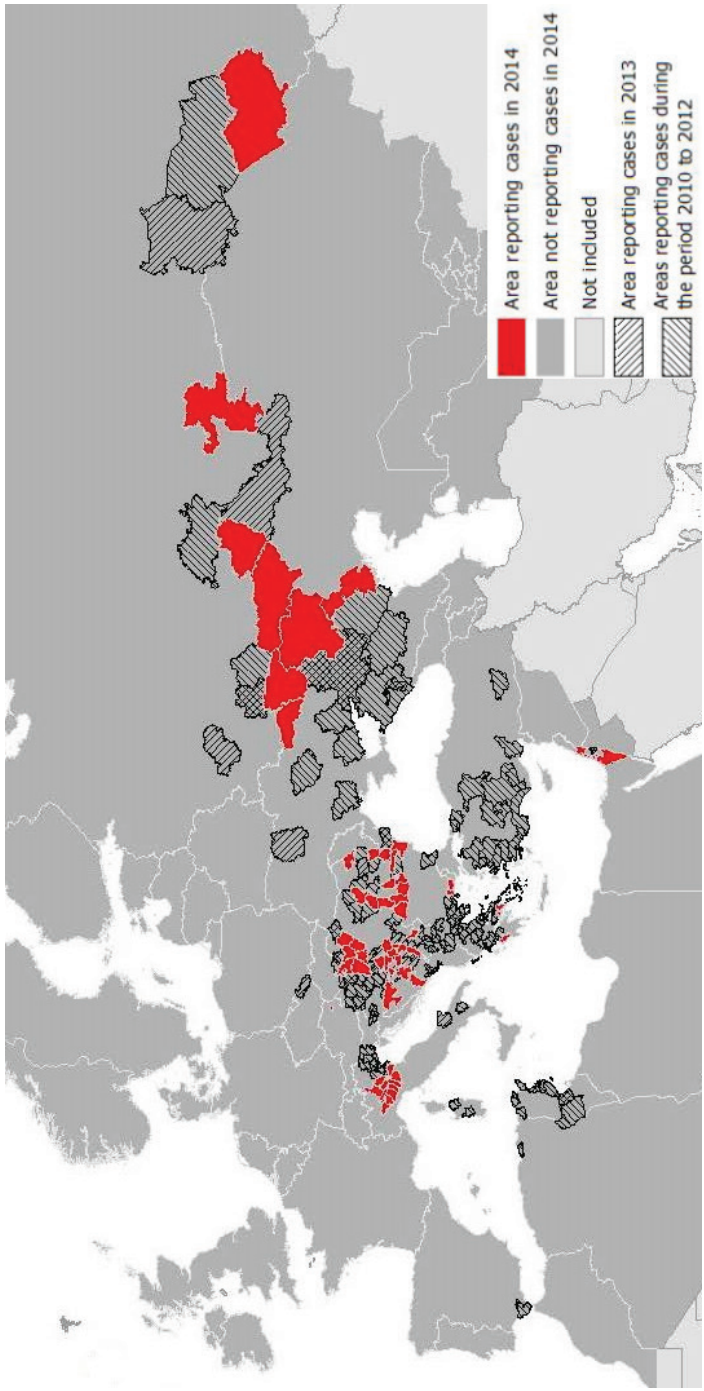

FIGURE A11-6 Distribution of WNF cases by affected areas, European region and Mediterranean basin.
NOTE: Transmission season 2014 and previous transmission seasons; accessed November 20, 2014.
SOURCE: ECDC.

July temperature anomalies, the anomaly of the Modified Normalized Difference Water Index (MNDWI) (Xu, 2006) in early June, an outbreak the previous year, the size of the human population, wetlands and the type of avian flyways. Model validation with 2012 and 2013 data showed a good level of prediction; thus, July temperature anomalies and MNDWI can be considered determinants for WNV transmission in Europe. These models indicate that risk maps for WNV transmission can be assembled with up-to-date anomalies of July temperatures for a given year along with the MNDWI (Semenza et al., 2016).

These two environmental determinants lend themselves for an integration of environmental monitoring in public health surveillance systems of human cases, serological surveillance of domestic and wild avifauna, and entomological surveillance (Ozdenerol et al., 2013; Kwan et al., 2012; Semenza and Zeller, 2014). Figure A11-7 displays the spatial heterogeneity of the probability of WNV infection per district in 2012 and 2013 as predicted by this model (Tran et al., 2014). Central and Eastern Europe, Turkey, Israel, and Tunisia were predicted to have higher risk values for 2012. In comparison with Figure A11-6, WND cases were notified in all of the predicted high-risk areas, except in Ukraine, and Turkey. Tunisia, Northern Italy, Northern Greece, Central Europe, and South Russia scored the highest predicted values in agreement with main areas of transmission in 2013 (see Figure A11-7). These findings indicate that the variables in this model can in part describe the variability in WNV transmission in Europe at the district level. Applying temperature anomalies for July can produce short-term and even long-term predictive maps of the probability of WNV infections.

Needless to say, besides these environmental/climatic drivers, biological drivers such as the presence and abundance of avian hosts and mosquito vectors of WNV need to be better described in order to more accurately predict the transmission of WNF in Europe (Paz and Semenza, 2013).

Dengue Dispersal Through Air Traffic

Dengue is by far the most significant mosquito-borne viral disease affecting humans globally, but there is currently no efficacious vaccine available for dengue (Simmons et al., 2012; Capeding et al., 2014). Tens of millions of cases occur annually resulting in approximately 20,000–25,000 deaths predominantly in children (Gubler, 2002; Mackenzie et al., 2004; Simmons et al., 2012; WHO, 2013; Bhatt et al., 2013). Transmission occurs largely in tropical and subtropical regions of the world, threatening almost half of the world's population (WHO, 2013). In continental Europe limited outbreaks have occurred in areas infested by two of the mosquito vectors, *Aedes albopictus* and *Aedes aegypti*. *Aedes aegypti* is the predominant mosquito vector that transmits the dengue virus to humans, whereas *Aedes albopictus* is a less effective vector (Lambrechts et al., 2010).

Ae. aegypti is not present on continental Europe but was first reported in 2005 on the Portuguese island of Madeira and has subsequently invaded the

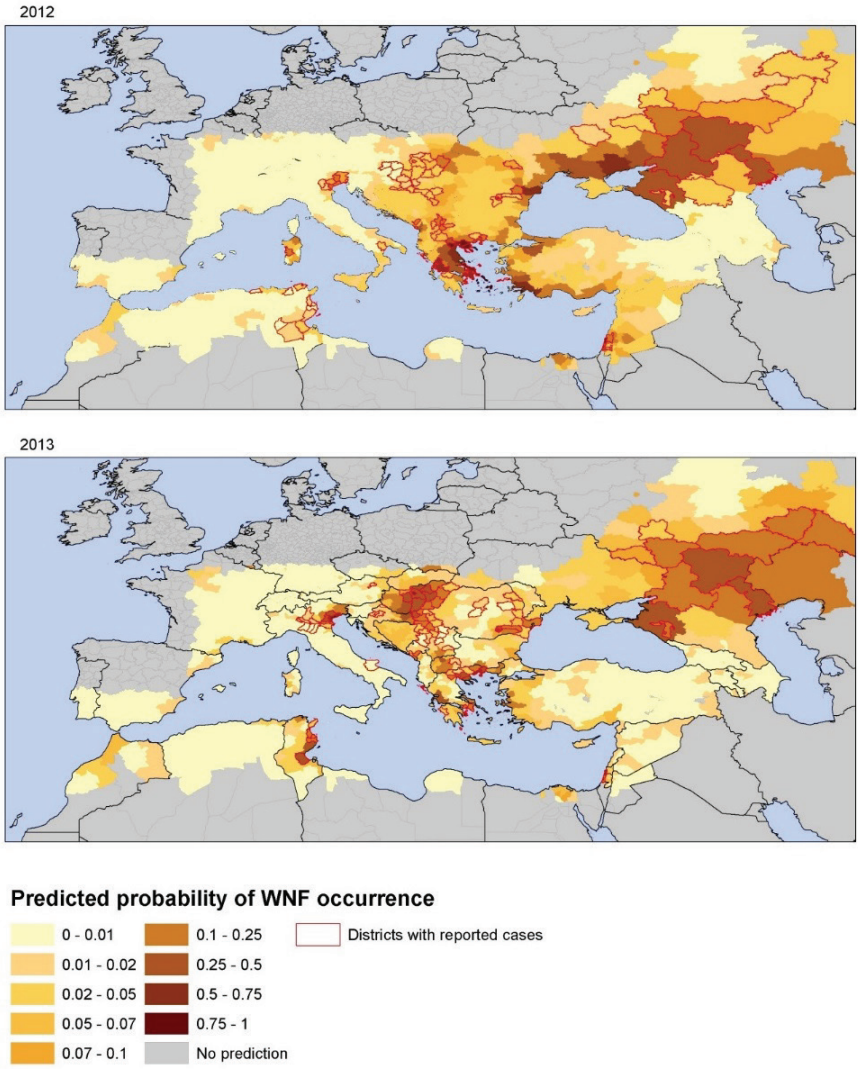

FIGURE A11-7 Map of predicted probability of WNV infection based on environmental predictors, Europe and neighbouring countries, 2012 and 2013.
SOURCE: Tran et al., 2013. Reproduced with permission from BioMed Central.

southern part of the island (ECDC, 2012). In Europe, *Ae. albopictus* has been reported in at least 15 countries (either established or recently recorded) and continues to broaden its reach. The development period for *Ae. albopictus* begins in April and dwindles off in October/November based on entomological monitoring activities in the Mediterranean (Giatropoulos et al., 2012; Tran et al., 2013; Zitko and Merdic, 2014); however, the time of peak activity for *Ae. albopictus* are the summer months.

Travellers from the tropics or subtropics, can be considered at risk for dengue virus (DENV) infection (Gardner et al., 2012). Through international air travel, infected travellers can arrive in Europe during their viremic period, and be bitten by local *Aedes* mosquitoes (Vaughn et al., 2000). These infected mosquitos can subsequently transmit DENV locally and trigger an outbreak. In Europe, transmission has in fact occurred in areas where *Aedes* mosquitoes are present (La Ruche et al., 2010; Gjenero-Margan et al., 2011). In 2010, two dengue cases without recent travel history or blood transfusion were recognized in Southern France (La Ruche et al., 2010) and two other dengue cases in Croatia (Gjenero-Margan et al., 2011). Thus, for the first time in decades, local transmission had occurred in Europe. In 2012, an epidemic of over 2,000 dengue cases erupted in Madeira, Portugal, in areas where *Ae. aegypti* is known to be present (ECDC, 2012).

With the goal to quantify the risk of dengue importation in areas where local transmission could occur (due to the presence of the vector) we took into account the global disease burden and seasonality of dengue, the volume and seasonal fluctuations of travellers originating from dengue-affected areas, and the seasonality and distribution of competent mosquito populations within Europe (Semenza et al., 2014). We developed a model based on 2010 data that relates air travellers from dengue affected areas to dengue importation to Europe. Over 5.8 million air passengers entered Europe from dengue-affected areas in 2010; country-level arrival by month is illustrated in Figure A11-8 (Semenza et al., 2014). The final European destinations were mapped as a function of the volumes of global air travellers arriving from areas with dengue activity during 2010; the spatial extent of the *Ae. albopictus* distribution (from the E3 data repository) was overlaid (see Figure A11-9). Milan and Rome received over half, and Barcelona 14 percent of these travellers that enter Europe from dengue-active/affected areas (Semenza et al., 2014).

Imported dengue cases were significantly related to the monthly number of travellers arriving from dengue-affected locations. We developed a hierarchical multivariate model for imported dengue cases in 2010: the adjusted incidence rate ratio was 1.09 with a 95 percent confidence interval of [1.01–1.17] for every 10,000 traveller increase (Semenza et al., 2014). This corresponds to a 9 percent increase in the incidence of imported cases for every additional 10,000 travellers arriving from dengue-affected areas, all other predictors in the model being constant. In August, September, and October the rate ratio was 1.70 (95% CI:

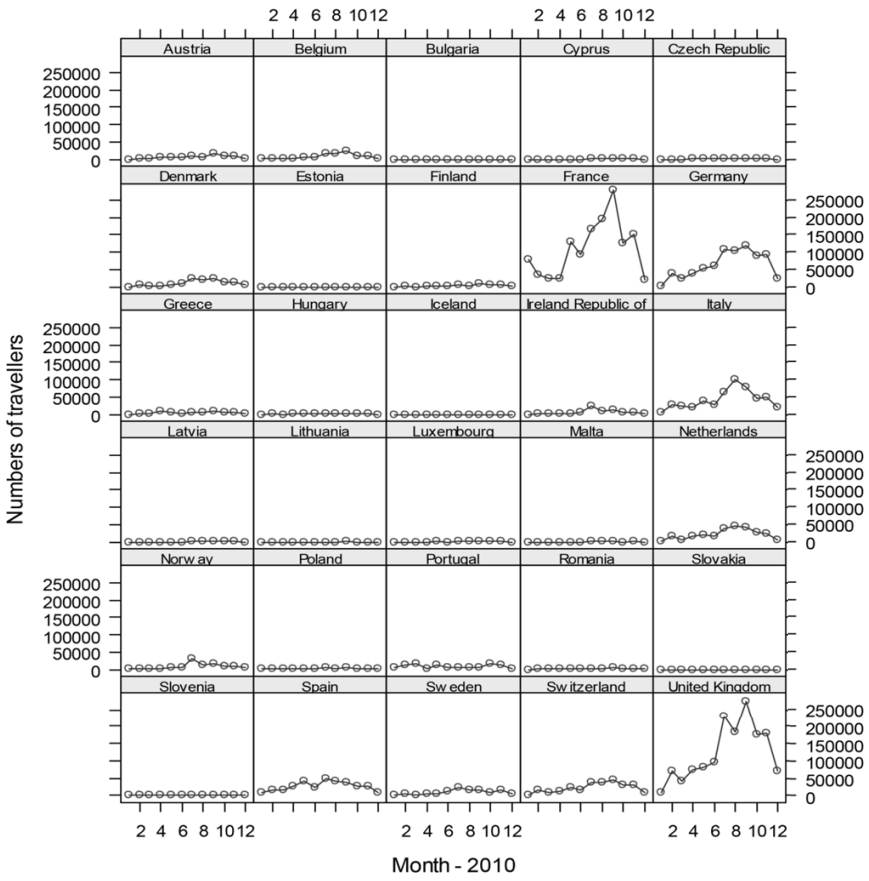

FIGURE A11-8 Country-level destination of international air travellers from dengue-affected areas, by month, 2010.
SOURCE: Semenza et al., 2014. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

1.23–2.35), 1.46 (95% CI: 1.02–2.1), and 1.35 (95% CI: 1.01–1.81), respectively (Semenza et al. 2014).

This empirical model for 2010 aims to quantify the association between the number of monthly in-coming travellers with the number of monthly dengue importations at the country level. The main driver of dengue importation and its pattern into EU countries can be described with high spatial and temporal resolution international air traffic data (see Figure A11-9) (Semenza et al., 2014). Moreover, the model accounts for dengue seasonality in the origin countries since

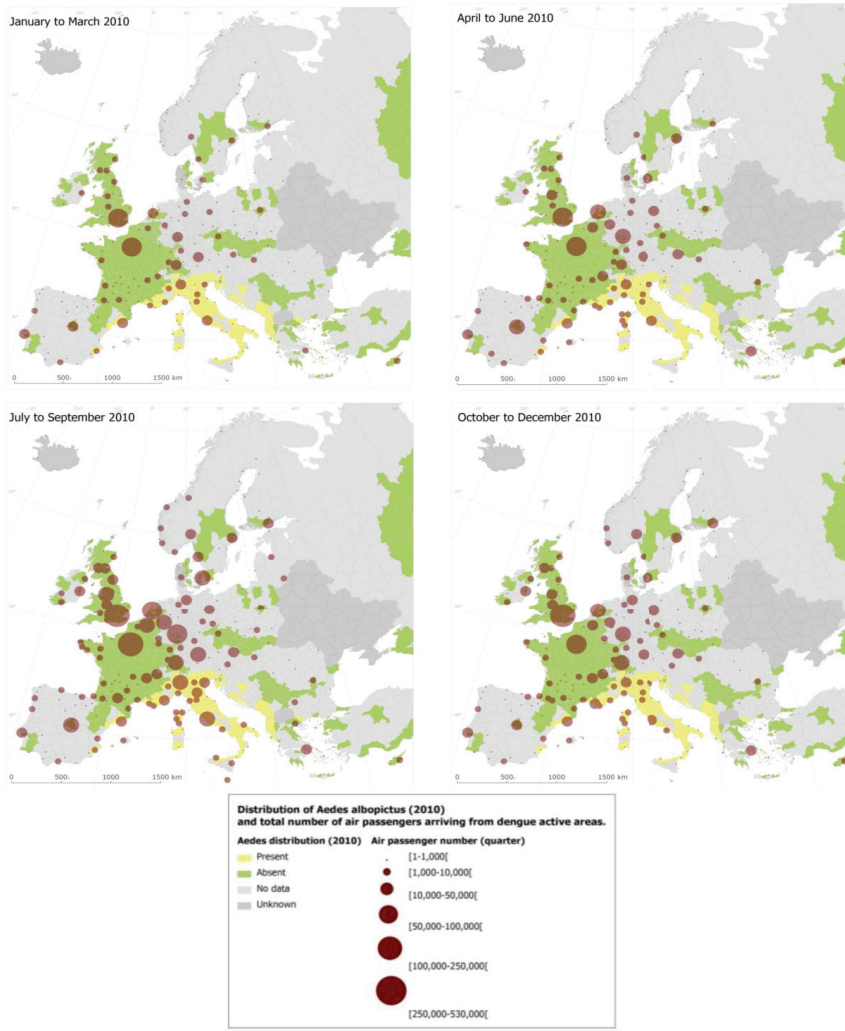

FIGURE A11-9 Airport-level final destination of international travellers from dengue-affected areas by quarter for 2010, overlaid with the presence of *Ae. albopictus*, 2010. SOURCE: Semenza et al., 2014. Available from *PLoS Neglected Tropical Diseases* under Creative Commons license.

dengue presence was recorded by month and documents that the importation risk for 2010 was the highest between August and October.

Disease dispersal through international air traffic is the inevitable consequence of globalization. Pathogen introduction is difficult to intercept, and public

health has to resort to early detection, rapid response, and effective control measures to contain potential dengue establishment and spread (Hufnagel et al., 2004; Semenza and Zeller, 2014). The approach presented here could be translated to other settings in support of integrated surveillance of human cases and vectors (Lindgren et al., 2012; Semenza et al., 2014; Suk and Semenza, 2014). Such empirical models lend themselves to guide public health responses and can be developed into early warning systems of emerging risks (Nichols et al., 2014; Semenza et al., 2013).

Conclusion

Vector-borne diseases are a threat to global public health, including Europe. Mounting an effective public health response to these threats obviously includes awareness rising among the general public, public health practitioners, and policy makers about disease vectors and their relationship with infectious diseases. Exposure prevention through personal protection and vector control are essential activities of effective public health practice. However, intercepting the emergence and spread of vector-borne diseases can contain escalating human and financial costs of a potential epidemic. Monitoring environmental/climatic precursors of these outbreaks through early warning systems can help predict the emergence and spread of vector-borne diseases (Nichols et al., 2014; Semenza et al., 2013). Forecasts and predictions can be developed by linking the monitoring of environmental/climatic precursors to dedicated disease surveillance systems with integrated vector surveillance of invasive and endemic vector species as described in this chapter.

In recognition of the above, the European Commission emphasises the need to strengthen public health preparedness, including surveillance and monitoring. Specifically, DG SANCO acknowledges the importance of the E3 Network:

> By connecting these sources of information, the E3 network should bolster European early warning for climate-related disease events. It should also enable forecasting and risk mapping of infectious disease incidence in relation to environmental changes. (Commision of the European Communities, 2009)

Monitoring the upstream environmental, climatic, and socioeconomic drivers of disease can provide the lead time for a swift public health response in order to contain human and financial costs associated with VBD emergence and spread in the European Union.

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References

Altizer, S., R. S. Ostfeld, P. T. J. Johnson, S. Kutz, and C. D. Harvell. 2013. Climate change and infectious diseases: From evidence to a predictive framework. *Science* 341(6145):514-519.

Andrade, C. C., P. D. Maharaj, W. K. Reisen, and A. C. Brault. 2011. North American West Nile virus genotype isolates demonstrate differential replicative capacities in response to temperature. *Journal of General Virology* 92(Pt 11):2523-2533.

Bhatt, S., P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, O. Sankoh, M. F. Myers, D. B. George, T. Jaenisch, G. R. Wint, C. P. Simmons, T. W. Scott, J. J. Farrar, and S. I. Hay. 2013. The global distribution and burden of dengue. *Nature* 496(7446):504-507.

Butler, C. D., and D. Harley. 2010. Primary, secondary and tertiary effects of eco-climatic change: The medical response. *Postgrad Medical Journal* 86(1014):230-234.

Capeding, M. R., N. H. Tran, S. R. S. Hadinegoro, H. I. H. J. M. Ismail, T. Chotpitayasunondh, M. N. Chua, C. Q. Luong, K. Rusmi, D. N. Wirawan, R. Nallusamy, P. Pitisuttithum, U. Thisyakorn, I. Yoon, D. Vliet, E. Langevin, T. Laot, Y. Hutagalung, C. Frago, M. Boaz, A. Wartel, N. G. Tornieporth, M. Saville, and A. Bouckenooghe. 2014. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: A phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 384(9951):1358-1365.

Commision of the European Communities. 2009. Human, animal and plant health impacts of climate change. http://ec.europa.eu/health/ph_threats/climate/docs/com_2009-147_en.pdf (accessed December 1, 2014).

Confalonieri, U., B. Menne, R. Akhtar, K. L. Ebi, M. Hauengue, R. S. Kovats, B. Revich, and A. Woodward. 2007. Human health, In *Climate change 2007: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson. Cambridge, UK: Cambridge University Press.

Danis, K., A. Baka, A. Lenglet, W. Van Bortel, I. Terzaki, M. Tseroni, M. Detsis, E. Papanikolaou, A. Balaska, S. Gewehr, G. Dougas, T. Sideroglou, A. Economopoulou, N. Vakalis, S. Tsiodras, S. Bonovas, and J. Kremastinou. 2011. Autochthonous *Plasmodium vivax* malaria in Greece, 2011. *European Surveillance* 16(42)pii:19993.

Earth Resources Observation and Science Center USGS EROS. 2005. Global 30 Arc-Second Elevation Dataset (GTOPO30).

ECDC (European Centre for Disease Prevention and Control). 2012. Dengue outbreak in Madeira, Portugal 2012. http://www.ecdc.europa.eu/en/press/news/Lists/News/ECDC_DispForm.aspx?List=32e43ee8%2De230%2D4424%2Da783%2D85742124029a&ID=866&RootFolder=%2Fen%2Fpress%2Fnews%2FLists%2FNews (accessed August 1, 2014).

ECDC. 2014. Tender specifications for analysis of environmental drivers of infectious diseases framework service contract. http://e3devint.ecdcnet.europa.eu/SitePages/Vibrio%20Risk%20Map.aspx (accessed December 1, 2014).

Epstein, P. R. 2001. West Nile virus and the climate. *Journal of Urban Health* 78(2):367-371.

European Environment Agency. 2011. Corine Land Cover 2000 seamless vector data—version 15 (08/2011). Copenhagen, Denmark: European Environment Agency.

Faber, M. S., R. G. Ulrich, C. Frank, S. O. Brockmann, G. M. Pfaff, J. Jacob, D. H. Kruger, and K. Stark. 2010. Steep rise in notified hantavirus infections in Germany, April 2010. *Euro Surveillance* 15(20) pii=19574.

Gardner, L. M., D. Fajardo, S. T. Waller, O. Wang, and S. Sarkar. 2012. A predictive spatial model to quantify the risk of air-travel-associated dengue importation into the United States and Europe. *Journal of Tropical Medicine* 2012:103679.

Giatropoulos, A., N. Emmanouel, G. Koliopoulos, and A. Michaelakis. 2012. A study on distribution and seasonal abundance of *Aedes albopictus* (Diptera: Culicidae) population in Athens, Greece. *Journal of Medical Entomology* 49(2):262-269.

Gjenero-Margan, I., B. Aleraj, D. Krajcar, V. Lesnikar, A. Klobucar, I. Pem-Novosel, S. Kurecic-Filipovic, S. Komparak, R. Martic, S. Duricic, L. Betica-Radic, J. Okmadzic, T. Vilibic-Cavlek, A. Babic-Erceg, B. Turkovic, T. Avsic-Zupanc, I. Radic, M. Ljubic, K. Sarac, N. Benic, and G. Mlinaric-Galinovic. 2011. Autochthonous dengue fever in Croatia, August-September 2010. *European Surveillance* 16(9).

Gubler, D. J. 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Archives of Medical Research* 33(4):330-342.

Hufnagel, L., D. Brockmann, and T. Geisel. 2004. Forecast and control of epidemics in a globalized world. *Proceedings of the National Academy of Sciences of the United States of America* 101(42):15124-15129.

The Joint Research Centre. 2009. *Raster data on population density using Corine Land Cover 2000 inventory*. Copenhagen, Denmark: European Environment Agency.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451(7181):990-993.

Kampen, H., E. Maltezos, M. Pagonaki, K. P. Hunfeld, W. A. Maier, and H. M. Seitz. 2002. Individual cases of autochthonous malaria in Evros Province, northern Greece: Serological aspects. *Parasitology Research* 88(3):261-266.

Kampen, H., J. Proft, S. Etti, E. Maltezos, M. Pagonaki, W. A. Maier, and H. M. Seitz. 2003. Individual cases of autochthonous malaria in Evros Province, northern Greece: Entomological aspects. *Parasitology Research* 89(4):252-258.

Karesh, W. B., A. Dobson, J. O. Lloyd-Smith, J. Lubroth, M. A. Dixon, M. Bennett, S. Aldrich, T. Harrington, P. Formenty, E. H. Loh, C. C. MacHalaba, M. J. Thomas, and D. L. Heymann. 2012. Ecology of zoonoses: Natural and unnatural histories. *Lancet* 380(9857):1936-1945.

Kwan, J. L., B. K. Park, T. E. Carpenter, V. Ngo, R. Civen, and W. K. Reisen. 2012. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004-2010. *Emerging Infectious Diseases* 18(8):1298-1306.

La Ruche, G., Y. Souares, A. Armengaud, F. Peloux-Petiot, P. Delaunay, P. Despres, A. Lenglet, F. Jourdain, I. Leparc-Goffart, F. Charlet, L. Ollier, K. Mantey, T. Mollet, J. P. Fournier, R. Torrents, K. Leitmeyer, P. Hilairet, H. Zeller, W. Van Bortel, D. Dejour-Salamanca, M. Grandadam, and M. Gastellu-Etchegorry. 2010. First two autochthonous dengue virus infections in metropolitan France, September 2010. *European Surveillance* 15(39):19676.

Lambrechts, L., T. W. Scott, and D. J. Gubler. 2010. Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission. *PLoS Neglected Tropical Diseases* 4(5):e646.

Lindgren, E., Y. Andersson, J. E. Suk, B. Sudre, and J. C. Semenza. 2012. Public health. Monitoring EU emerging infectious disease risk due to climate change. *Science* 336(6080):418-419.

Lourenco, J., and M. Recker. 2014. The 2012 Madeira dengue outbreak: Epidemiological determinants and future epidemic potential. *PLoS Neglected Tropical Diseases* 8(8):e3083.

Mackenzie, J. S., D. J. Gubler, and L. R. Petersen. 2004. Emerging flaviviruses: The spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine* 10(12 Suppl):S98-S109.

McMichael, A. J. 2013a. Impediments to comprehensive research on climate change and health. *International Journal of Environmental Research and Public Health* 10(11):6096-6105.

McMichael, A. J. 2013b. Globalization, climate change, and human health. *New England Journal of Medicine* 368(14):1335-1343.

Nichols, G. L., Y. Andersson, E. Lindgren, I. Devaux, and J. C. Semenza. 2014. European monitoring systems and data for assessing environmental and climate impacts on human infectious diseases. *International Journal of Environmental Research and Public Health* 11(4):3894-3936.

Ozdenerol, E., G. N. Taff, and C. Akkus. 2013. Exploring the spatio-temporal dynamics of reservoir hosts, vectors, and human hosts of West Nile virus: A review of the recent literature. *International Journal of Environmental Research and Public Health* 10(11):5399-5432.

Patz, J. A., P. Daszak, G. M. Tabor, A. A. Aguirre, M. Pearl, J. Epstein, N. D. Wolfe, A. M. Kilpatrick, J. Foufopoulos, D. Molyneux, and D. J. Bradley. 2004. Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. *Environmental Health Perspectives* 112(10):1092-1098.

Paz, S., and I. Albersheim. 2008. Influence of warming tendency on *Culex pipiens* population abundance and on the probability of West Nile fever outbreaks (Israeli Case Study: 2001-2005). *Ecohealth* 5(1):40-48.

Paz, S., and J. C. Semenza. 2013. Environmental drivers of West Nile fever epidemiology in Europe and Western Asia--a review. *International Journal of Environmental Research and Public Health* 10(8):3543-3562.

Paz, S., D. Malkinson, M. S. Green, G. Tsioni, A. Papa, K. Danis, A. Sirbu, C. Ceianu, K. Katalin, E. Ferenczi, H. Zeller, and J. C. Semenza. 2013. Permissive summer temperatures of the 2010 European West Nile fever upsurge. *PLoS One* 8(2):e56398.

Pecoraro, H. L., H. L. Day, R. Reineke, N. Stevens, J. C. Withey, J. M. Marzluff, and J. S. Meschke. 2007. Climatic and landscape correlates for potential West Nile virus mosquito vectors in the Seattle region. *Journal of Vector Ecology* 32(1):22-28.

Randolph, S. E., and D. J. Rogers. 2010. The arrival, establishment and spread of exotic diseases: Patterns and predictions. *Nature Review Microbiology* 8(5):361-371.

Reintjes, R., I. Dedushaj, A. Gjini, T. R. Jorgensen, B. Cotter, A. Lieftucht, F. D'Ancona, D. T. Dennis, M. A. Kosoy, G. Mulliqi-Osmani, R. Grunow, A. Kalaveshi, L. Gashi, and I. Humolli. 2002. Tularemia outbreak investigation in Kosovo: Case control and environmental studies. *Emerging Infectious Diseases* 8(1):69-73.

Reisen, W. K., Y. Fang, and V. M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology* 43(2):309-317.

Reisen, W. K., R. M. Takahashi, B. D. Carroll, and R. Quiring. 2008. Delinquent mortgages, neglected swimming pools, and West Nile virus, California. *Emering Infectious Diseases* 14(11):1747-1749.

Sabatinelli, G., M. Ejov, and P. Joergensen. 2001. Malaria in the WHO European Region (1971-1999). *European Surveillance* 6(4):61-65.

Savage, H. M., C. Ceianu, G. Nicolescu, N. Karabatsos, R. Lanciotti, A. Vladimirescu, L. Laiv, A. Ungureanu, C. Romanca, and T. F. Tsai. 1999. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *American Journal of Tropical Medicine and Hygiene* 61(4):600-611.

Scharlemann, J. P., D. Benz, S. I. Hay, B. V. Purse, A. J. Tatem, G. R. Wint, and D. J. Rogers. 2008. Global data for ecology and epidemiology: A novel algorithm for temporal Fourier processing MODIS data. *PLoS One* 3(1):e1408.

Semenza, J. C. 2015. Prototype early warning systems for vector-borne diseases in Europe. *International Journal of Environmental Research and Public Health* 12(6):6333-6351; doi:10.3390/ijerph120606333.

Semenza, J. C., and D. Domanović. 2013. Blood supply under threat. *Nature Climate Change* 3:432-435.

Semenza, J. C., and J. Giesecke. 2008. Intervening to reduce inequalities in infections in Europe. *American Journal of Public Health* 98(5):787-792.

Semenza, J. C., and B. Menne. 2009. Climate change and infectious diseases in Europe. *Lancet Infectious Diseases* 9(6):365-375.

Semenza, J. C., and H. Zeller. 2014. Integrated surveillance for prevention and control of emerging vector-borne diseases in Europe. *European Surveillance* 19(13).

Semenza, J. C., J. E. Suk, V. Estevez, K. L. Ebi, and E. Lindgren. 2012. Mapping climate change vulnerabilities to infectious diseases in Europe. *Environmental Health Perspectives* 120(3):385-392.

Semenza, J. C., B. Sudre, T. Oni, J. E. Suk, and J. Giesecke. 2013. Linking environmental drivers to infectious diseases: The European environment and epidemiology network. *PLoS Neglected Tropical Diseases* 7(7):e2323.

Semenza, J. C., B. Sudre, J. Miniota, M. Rossi, W. Hu, D. Kossowsky, J. E. Suk, W. Van Bortel, and K. Khan. 2014. International dispersal of dengue through air travel: Importation risk for Europe. *PLoS Neglected Tropical Diseases* 8(12):e3278.

Semenza, J. C., E. Lindgren, L. Balkanyi, L. Espinosa, M. S. Almqvist, P. Penttinen, J. Rocklöv. Determinants and Drivers of Infectious Disease Threat Events in Europe. 2016. *Emerging Infectious Diseases* 22(4): 581-589.

Semenza, J. C., A. Tran, L. Espinosa, B. Sudre, D. Domanovic, S. Paz. Climate change projections of West Nile virus infections in Europe: Implications for blood safety practices. *Environmental Health* (in press).

Simmons, C. P., J. J. Farrar, V. Nguyen v, and B. Wills. 2012. Dengue. *New England Journal of Medicine* 366(15):1423-1432.

Soverow, J. E., G. A. Wellenius, D. N. Fisman, and M. A. Mittleman. 2009. Infectious disease in a warming world: How weather influenced West Nile virus in the United States (2001-2005). *Environmental Health Perspectives* 117(7):1049-1052.

Stefanoff, P., M. Rosinska, S. Samuels, D. J. White, D. L. Morse, and S. E. Randolph. 2012. A national case-control study identifies human socio-economic status and activities as risk factors for tick-borne encephalitis in Poland. *PLoS One* 7(9):e45511. doi: 10.1371/journal.pone.0045511.

Sudre, B., M. Rossi, W. Van Bortel, K. Danis, A. Baka, N. Vakalis, and J. C. Semenza. 2013. Mapping environmental suitability for malaria transmission, Greece. *Emerging Infectious Diseases* 19(5):784-786. doi: 10.3201/eid1905.120811.

Suhrcke, M., D. Stuckler, J. E. Suk, M. Desai, M. Senek, M. McKee, S. Tsolova, S. Basu, I. Abubakar, P. Hunter, B. Rechel, and J. C. Semenza. 2011. The impact of economic crises on communicable disease transmission and control: A systematic review of the evidence. *PLoS One* 6(6):e20724.

Suk, J. E., and J. C. Semenza. 2011. Future infectious disease threats to Europe. *American Journal of Public Health* 101(11):2068-2079.

Suk, J. E., and J. C. Semenza. 2014. From global to local: Vector-borne disease in an interconnected world. *European Journal of Public Health* 24(4):531-532.

Thomas, S. M., N. B. Tjaden, S. van den Bos, and C. Beierkuhnlein. 2014. Implementing cargo movement into climate based risk assessment of vector-borne diseases. *International Journal of Environmental Research and Public Health* 11(3):3360-3374.

Tran, A., G. L'Ambert, G. Lacour, R. Benoit, M. Demarchi, M. Cros, P. Cailly, M. Aubry-Kientz, T. Balenghien, and P. Ezanno. 2013. A rainfall- and temperature-driven abundance model for *Aedes albopictus* populations. *International Journal of Environmental Research and Public Health* 10(5):1698-1719.

Tran, A., B. Sudre, S. Paz, M. Rossi, A. Desbrosse, V. Chevalier, and J. C. Semenza. 2014. Environmental predictors of West Nile fever risk in Europe. *International Journal of Health Geographics* 13(1):1-11.

Vakali, A., E. Patsoula, G. Spanakos, K. Danis, E. Vassalou, N. Tegos, A. Economopoulou, A. Baka, A. Pavli, C. Koutis, C. Hadjichristodoulou, and T. Kremastinou. 2012. Malaria in Greece, 1975 to 2010. *Eurosurveillance* 17(47):pii=20322.

Vaughn, D. W., S. Green, S. Kalayanarooj, B. L. Innis, S. Nimmannitya, S. Suntayakorn, T. P. Endy, B. Raengsakulrach, A. L. Rothman, F. A. Ennis, and A. Nisalak. 2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *Journal of Infectious Diseases* 181(1):2-9.

WHO (World Health Organization). 2013. Dengue 2014. http://www.who.int/denguecontrol/en/ (accessed September 21, 2014).

Woolhouse, M. 2011. How to make predictions about future infectious disease risks. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 366(1573):2045-2054.

Xu, H. Q. 2006. Modification of normalised difference water index (NDWI) to enhance open water features in remotely sensed imagery. *International Journal of Remote Sensing* 27(14):3025-3033.

Zitko, T., and E. Merdic. 2014. Seasonal and spatial oviposition activity of *Aedes albopictus* (Diptera: Culicidae) in Adriatic Croatia. *Journal of Medical Entomology* 51(4):760-768.

A12

DISRUPTION OF INSECT TRANSMISSION OF PLANT VIRUSES[1]

*Anna E. **Whitfield**[2] and Dorith Rotenberg*

Summary

Plant-infecting viruses are transmitted by a diverse array of organisms including insects, mites, nematodes, fungi, and plasmodiophorids. Virus interactions with these vectors are diverse, but there are some commonalities. Generally the infection cycle begins with the vector encountering the virus in the plant and the virus is acquired by the vector. The virus must then persist in or on the vector long enough for the virus to be transported to a new host and delivered into the plant cell. Plant viruses rely on their vectors for breaching the plant cell wall to be delivered directly into the cytosol. In most cases, viral capsid or membrane glycoproteins are the specific viral proteins that are required for transmission and determinants of vector specificity. Specific molecules in vectors also interact with the virus and while there are few-identified to no-identified receptors, candidate recognition molecules are being further explored in these systems. Due to the specificity of virus transmission by vectors, there are defined steps that represent good targets for interdiction strategies to disrupt the disease cycle. This review focuses on new technologies that aim to disrupt the virus–vector interaction and focuses on a few of the well-characterized virus–vector interactions in the field. In closing, we discuss the importance of integration of these technologies with current methods for plant virus disease control.

Introduction

The virus transmission cycle involves host-finding, feeding and acquisition of virus, transport and delivery of virus to a new host plant (see Figure A12-1). Each step in the transmission process provides an opportunity for interdiction. Strategies for disrupting transmission are the focus of this review and we highlight recent biotech-based approaches to reduce vectorial capacity and populationreduction approaches that utilize the specificity of the virus–vector interaction to target insects.

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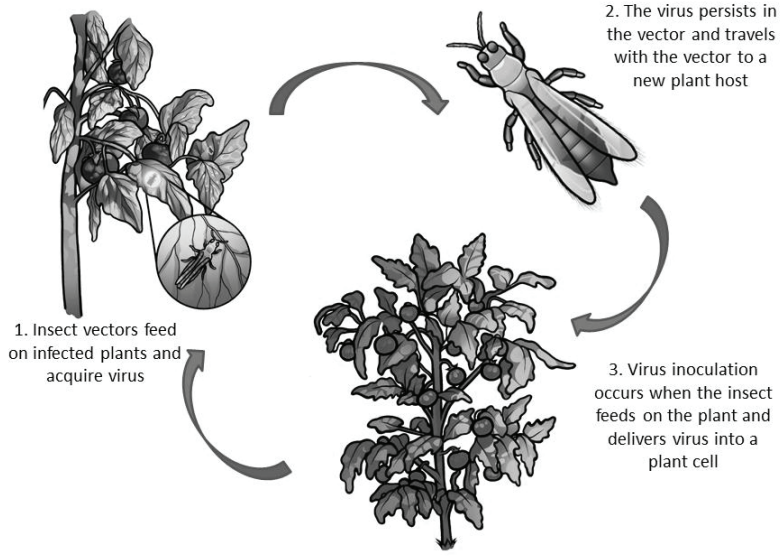

FIGURE A12-1 The transmission cycle for insect-borne plant viruses. Each step in the transmission process represents a unique opportunity for disruption

Overview of the Mechanisms and Methods of Plant Virus Transmission

Plant virus transmission by insects is classified into two major categories: non-circulative and circulative transmission. The non-circulative-externally borne viruses associate with specific cuticular structures of the insect stylet or foregut (see Figure A12-2) and the attached virus particles are lost during the insect molt (reviewed in Ng and Falk, 2006; Blank et al., 2014).

Non-circulative viruses are transmitted in a non-persistent or semi-persistent manner which means that they are acquired within seconds to minutes of feeding and transmitted rapidly as well. Semi-persistent viruses require longer periods to be acquired and transmitted (minutes to hours). By contrast, the circulative or internally-borne viruses require a greater time for acquisition and transmission (hours to days) and must traverse the gut and reach the salivary glands for transmission to occur. These viruses are not lost during insect molts and have a latent period between initial acquisition and transmission. The latent period is synonymous with extrinsic incubation period in animal vector biology. For all types of insect transmission, viral determinants of transmissibility have been defined. For the non-circulative viruses, some viruses bind directly to insect stylets or foreguts and other viruses need the assistance of another viral protein(s) that serves as a bridge between the insect structures and the virion (Chen et al., 2011;

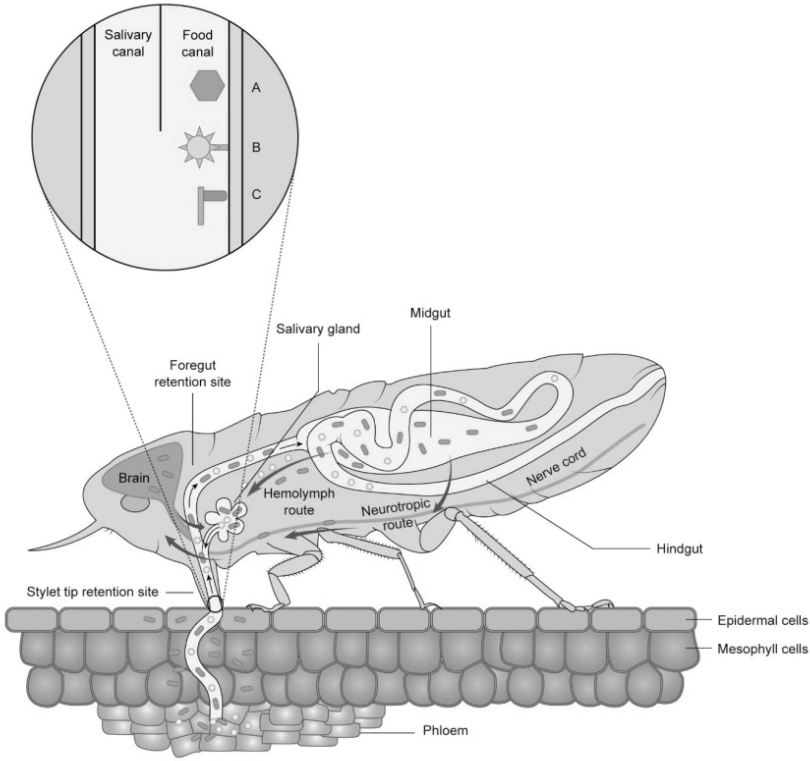

FIGURE A12-2 Viruses localize to different sites in the plant-feeding insect vector depending on their modes of transmission. Non-circulative viruses bind to the insect stylet (see inset) or foregut. Non-propagative circulative (yellow circles) viruses are generally phloem limited and move through the insect body via the midgut or hindgut. Circulative viruses enter the hemolymph and then enter the salivary glands. By contrast, circulative propagative viruses (red ovals) tend to enter the insect through the midgut and replicate in insect tissues. Some propagative viruses are phloem limited while others are widely distributed in plant tissues. The salivary glands are the final destination for circulative transmission, and viruses reach the salivary glands via the hemolymph or other routes such as the nervous tissue (neurotropic route) or through connective tissues. Inset: Magnification of an insect stylet showing the proposed site of virion attachment at the tip of the stylet in the common duct region. Letters designate the different strategies for virion binding and retention in the stylet: capsid strategy, direct binding of coat protein to the stylet **(a)**, helper component strategies for caulimoviruses, two virus proteins serve as a 'bridge' between the virion and the stylet **(b)** and potyviruses, one virus protein (HC-Pro) binds to the aphid stylet and to the virus **(c)**.
SOURCES: Modified from Blanc et al., 2014, and Ammar et al., 2009.

Liu et al., 2006; Lung and Pirone, 1974; Govier and Kassani, 1974). For the circulative viruses, the viral capsid proteins and glycoproteins have been identified as viral determinants of insect transmission (reviewed in Hogenhout et al., 2008). Similarly, for the viruses transmitted by soil-dwelling plant–virus vectors (nematodes, fungi, and plasmodiophorids) the viral coat protein(s) is responsible for binding and retention in the vector (Ohki et al., 2010; Andret-Link et al., 2004; Adams et al., 2001). Despite being transmitted by different mechanisms, the requirement of a viral protein–insect molecular interaction is a consistent theme in transmission by insects and provides a common target for interrupting the transmission process.

Blocking Virus Transmission with Viral Capsid Proteins and Glycoproteins

Viral proteins are required for attachment and/or entry into the insect vector. Therefore, exploiting these proteins for their specific binding affinities to vector tissues is an obvious approach for blocking virus acquisition and transmission. For all the vector-borne plant viruses, a specific viral protein(s) is required for virus transmission. Genomes of plant viruses are quite small, and defining the viral attachment protein(s) (VAP) has been completed for diverse and seemingly intractable virus–vector systems. Using this knowledge, recombinant VAP can be used to (1) reduce transmission of viruses by blocking virus binding and subsequent dissemination in the vector and (2) reducing the vector population using the viral protein to deliver toxic cargo to the insect (see Table A12-1).

Exploiting Viral Proteins to Control Vectors of Circulative Viruses

For circulative viruses, the structural proteins of the viral capsid are the determinants of insect vector specificity (reviewed in Grey et al., 2014). The route of virus dissemination has been well-characterized for members of the family *Luteoviridae* and the coat protein (CP) and the readthrough extension of the coat protein are required for transmission. Luteovirids are small icosahedral virions (25–30 nm) that are composed of a major coat protein and a minor protein that has a carboxy-terminal extension termed the readthrough domain (RTD). Initial virus entry occurs in the insect gut and the specific region for entry varies with virus species, occurring in the midgut or hindgut. Several studies have documented that the coat protein is sufficient for delivery of virus into the hemocoel and the RTD is crucial for transmission. It is thought that the salivary glands are the barrier to transmission of particles with mutations in the RTD (Brault et al., 2000; Peter et al., 2008; Liu et al., 2009). Knowledge of *Pea enation mosaic virus* (PEMV) CP binding and movement through the insect gut was used to target a hemocoel-active toxin to aphids (Bonning et al., 2014). The authors found that a recombinant CP fused to non-viral toxin peptides could be delivered via transcytosis from the aphid gut to the hemocoel to be aphicidal.

TABLE A12-1 A Summary of Strategies Used to Disrupt Plant Virus–Vector Interactions

Disruption strategy	Virus	Vector	Mode of transmission	*In planta* experiments conducted	References
Blocking virus entry into vector using a viral protein	*Rice ragged stunt virus* (RRSV, *Oryzavirus*, *Reoviridae*)	Planthopper, *Nilaparvata lugens*	Circulative, propagative	Yes	Guoying et al., 1999; Shao et al., 2003
	Tomato yellow leaf curl virus (TYLCV, Begomovirus, *Geminiviridae*)	Whitefly, *Bemisia tabaci*	Circulative	No	Wang et al., 2014
	Tomato spotted wilt virus (TSWV, *Tospovirus*, *Bunyaviridae*)	Thrips, *Frankliniella occidentalis*	Circulative, propagative	Yes	Whitfield et al., 2004, 2008; Montero-Astua et al., 2014
Viral coat protein/ toxin fusions	*Pea enation mosaic virus (PEMV, Enamovirus*, *Luteoviridae*)	Aphids, *Acyrthosiphon pisum*, *Myzus persicae*, *Aphis glycines* and *Rhopalosiphum padi* [a]	Circulative	Yes	Bonning et al., 2014
Insect-gut binding peptide/toxin fusions	PEMV[b]	Aphids, *A. pisum*, *M. persicae*	Circulative	No	Liu et al., 2010; Chogule et al., 2013
Targeting vector proteins that interact with virus	*Rice stripe virus* (RSV, *Tenuivirus*[c])	Planthopper, *Laodelphax striatellus* [d]	Circulative, propagative	No	Huo et al., 2014

[a] *A. glycines* and *R. padi* do not transmit PEMV but were susceptible to the PEMV CP-toxin fusion protein.
[b] Peptide identified to bind aphid vector guts and reduced PEMV access to insect hemocoel.
[c] Member of a floating virus genus; tenuiviruses are closely related to the family Bunyaviridae however they lack an envelope.
[d] Disrupted transovarial transmission by RNA-interference of vitellogenin.

The benefit of using this system is that luteovirids are transmitted specifically by aphids. Additionally, the insect gut is not the major barrier to luteovirids entry into the insect and the salivary gland appears to be a more significant barrier to aphid transmission of these viruses. Additionally, the CP-toxin fusion killed non-vector aphids but had no apparent effect on an off-target lepidopteran species, *Heliothis virescens*. Begomoviruses are transmitted in a similar circulative manner by whitefly vectors and the viral CP was shown to bind to whitefly midguts and reduce the amount of virus in whiteflies in feeding experiments (Wang et al., 2014). The ability of viral CPs to bind to insect guts and block virus entry indicates that preventing virus entry and delivering toxic peptides may prove to be transmission inhibition-based approaches for other viruses that circulate through the insect body.

An alternative strategy to CP-mediated transport of toxins to aphid vectors has been documented with the use of aphid gut-binding peptides. A bio-panning approach identified a 12 amino acid peptide that bound to pea aphid guts (Liu et al., 2010). Interestingly, this peptide, GBP3.1, reduced PEMV abundance in the vector for up to 70 minutes after acquisition of the peptide. Although the primary amino acid sequence of GBP3.1 was dissimilar to the PEMV CP sequence, structural similarity was identified between the peptide and a specific surface loop of the viral protein, suggesting that reduced virus abundance may have resulted in competitive binding for gut molecules between the peptide and the virus.

The utility of this aphid binding peptide has been exploited to expand the target range of a *Bacillus thuringiensis* cytolytic toxin, Cyt2Aa (Chougule et al., 2013). The GBP3.1 peptide was incorporated into the surface loops of the toxin and the modified toxin bound aphid membranes. The modified toxin retained activity and was found to be toxic to *Acyrthosiphon pisum* and *Myzus persicae*. Modification of insect-specific toxins with the addition of aphid-binding peptides and/or virus CP is a promising new control strategy for vector and non-vector aphids.

Disruption of Transmission of Circulative, Propagative Viruses Using Viral Proteins

Tomato spotted wilt virus (TSWV) is an enveloped negative strand RNA virus and the type member of the genus *Tospovirus* within the family Bunyaviridae. Tospoviruses are transmitted in a circulative-propagative manner exclusively by thrips vectors, including *Frankliniella occidentalis*, the western flower thrips (Whitfield et al., 2005). Efficient transmission to plants requires that thrips acquire TSWV during the larval stages to transmit as adults. When vector competent larval thrips feed on infected tissue, the virus enters the insect midgut, initiates a high titer infection in the gut, and then disseminates to the salivary gland tissues. The virus traverses several membrane barriers en route from the vector midgut to salivary glands (Kritzman et al., 2002; Nagata et al., 2002;

Moritz et al., 2004), and virus titer was documented to be positively correlated with the number of TSWV transmission events by individual female and male thrips (Rosenberg et al., 2009). Collectively, these studies highlight the importance of virus accumulation and spread in the vector as quantitative determinants of a successful transmission event.

The structure of the TSWV virion is characteristic of members of the family Bunyaviridae, and the virion is spherical and composed of an outer membrane envelope derived from the host. Two glycoproteins (GPs) are embedded in the membrane and project from the surface. The GPs are designated G_N and G_C and thrips transmission of TSWV maps to the M segment, the viral RNA that encodes the GPs (Sin et al., 2005).

Due to the unique biology of the TSWV–thrips interaction, there is a narrow window of opportunity for virus acquisition during larval development that is a good target for blocking virus entry. Defining the molecular determinants of a plant virus–vector interaction enabled the development of novel virus control strategies that aim to specifically disrupt the interaction. TSWV acquisition is mediated by the molecular interaction between the virus membrane glycoprotein G_N, which serves as a viral attachment protein, and the thrips midgut. Previously, we found that an exogenously-applied soluble form of G_N (G_N-S) inhibits TSWV binding, acquisition (Whitfield et al., 2004), and transmission to a plant host (Whitfield et al., 2008). We generated transgenic tomato plants expressing a soluble form of GN and found that thrips that fed on these transgenics had significantly lower virus titers and adult transmission efficiencies than thrips fed on TSWV-infected non-transgenic tomato plants (Montero-Astúa et al., 2014). These results demonstrate that an initial reduction in virus infection of the larval insect midgut can result in a significant decrease in virus titer and transmission over the life-span of the vector.

The inhibition results with G_N-S and TSWV are supported by the results of research with *Rice ragged stunt virus*, which is a Reovirus that is transmitted in a circulative, propagative manner by rice brown planthoppers (Guoying et al., 1999; Shao et al., 2003). In those experiments, the viral spike protein inhibited virus transmission and insects that were fed a nonstructural virus protein exhibited no transmission inhibition. These results support the concept of disrupting the insect-mediated transmission of viruses via viral attachment proteins. Future work with this transmission-blocking strategy will focus on the spectrum of efficacy, that is, does TSWV G_N block other related tospoviruses and transmission by other thrips vectors. This research is important because new tospoviruses of significance to agriculture have been recently described including *Soybean vein necrosis-associated virus* (SVNaV) and a naturally-occurring interspecies reassortant between *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) (Zhou et al., 2011; Webster et al., 2011).

Potential for Disruption of Transmission of Noncirculative Viruses

Similar strategies as those described above for circulative viruses may provide reasonable methods for disrupting transmission of non-circulative viruses due to the specific nature of binding and retention documented for these viruses. The non-circulative viruses generally bind to cuticular surfaces of the insect body including the insect stylet and foregut. Many of the stylet-borne viruses are associated with specific regions of the stylet, and virions that are successfully transmitted to host plants are those that bind to the distal tip of the stylet where the food and salivary canals merge (Uzest et al., 2007; Wang et al., 1996).

Work with *Cauliflower mosaic virus* (CaMV) binding to aphid stylets has also directed attention to the presence of a specialized region of the aphid maxillary stylet termed the "acrostyle," an electron-dense area where virions of CaMV are specifically retained (Uzet et al., 2010). For the semi-persistent criniviruses, virion bind to the whitefly foregut and the minor coat protein (CPm) is the VAP (Chen et al., 2004). For Cucumber mosaic virus, the coat protein is the primary determinant of aphid transmission and helper proteins have not been identified. Specific regions of the virion including a surface loop and the quasi-threefold axis of symmetry have been shown to be essential for virus transmission by aphids (Liu et al., 2002; Bricault and Perry, 2013). Plants and bacteria have been engineered to produce viral proteins that bind to the insect stylet or foregut. Using these tools, determining if excess helper component or coat protein can compete with virions to saturate binding sites in the vector to subsequently prevent virus attachment is an exciting avenue to pursue for this category of vector-transmitted viruses.

Disruption of Other Insect-Borne Plant Pathogens

Much like with plant viruses, recent work has focused on blocking transmission of other arthropod-borne plant pathogens. The plant pathogenic bacterium, *Xylella fastidiosa*, is transmitted by hemipteran (leafhopper) vectors and is retained in the vector foregut. Unlike non-circulative plant viruses, *X. fastidiosa* cells attach to the foregut and replicate in the insect and this is termed non-circulative propagative transmission. Like plant viruses, the bacterial cells that attach to the foregut are lost during insect molts. Progress has been made toward identifying the bacterial components of the interaction, pointing to afimbrial adhesins as playing a major role in pathogen attachment to the vector (Killiny and Almeida, 2009). Complementary studies using antibodies to various bacterial cell-associated proteins and molecules confirmed the role of afimbrial adhesins (carbohydrate-binding proteins) in transmission (Killiny et al., 2012). Additionally, competition assays with lectins and carbohydrates confirmed the importance of these host carbohydrate-bacterial protein interactions in *X. fastidiosa* transmission by leafhopper vectors. Exogenous application of excess amounts of N-acetylglucosamine carbohydrates reduced pathogen transmission

indicating that this specific carbohydrate may be a vector component recognized by *X. fastidiosa*, facilitating binding of bacteria to the vector foregut (Killiny et al., 2012). Conversely, competition experiments with carbohydrate-binding proteins (lectins) also disrupted the interaction presumably by binding the carbohydrates on the foregut attachment site and blocking *X. fastidiosa* attachment. This work supports the hypothesis that pathogen retention in insect vectors is mediated by specific interactions and highlights commonalities in vector transmission of diverse types of pathogens.

Potential Use of RNAi for Disruption of Plant Virus Transmission

RNAi is an insect control approach that can also be used to directly target insect vectors and is considered to be the basis for the next generation of transgenic plants designed for insect pest control (Gordon and Waterhouse, 2007). RNAi is an attractive option for plant-feeding insects because dsRNA can be delivered via transgenic expression in plants, transiently-expressed by viral vectors (i.e., attenuated plant viruses), or exogenously-applied by soil drench or foliar sprays to plants. For insect control, silencing of an insect gene by endogenous RNAi cellular machinery is triggered by delivery of dsRNA of the same sequence to the gene transcript into the cell. Once inside, dsRNA is recognized by Dicer, an RNAse III enzyme that cleaves the RNA into short interfering RNAs (siRNAs). The siRNAs are incorporated into the RNA-induced silencing complex (RISC) which then targets the degradation of homologous transcript sequences by the activity of the endonuclease, Argonaute. In insects, systemic silencing occurs when the dsRNA signal spreads in the body of the insect by cellular uptake mechanisms that are still under investigation for various insect pest species. For example, several well-characterized insect genomes have SID orthologs, multi-spanning transmembrane proteins essential for systemic RNAi in *Caenorhabditis elegans*, while other mechanisms including endocytosis (V-ATPases) and scavenger receptor-mediated and other pattern recognition receptor-mediated uptake, that is, innate immunity, have been proposed (Huvenne and Smagghe, 2010; Winston et al., 2002; Feinberg and Hunter, 2003). Functional analysis of genes implicated in these cellular processes in insects including virus vectors remains a need. To add to the mystery, to date, there is no evidence of the presence of RNA-dependent RNA polymerase in insects and therefore amplification of the RNAi signal, a process that leads to transitive RNAi as documented for ticks, *C. elegans*, and plants (Gordon and Waterhouse, 2007).

The success of RNAi varies depending on the insect species and developmental stage targeted, dsRNA delivery method, length and concentration of dsRNA input, and gene target (Scott et al., 2013). An effective target for several insect vectors of plant viruses has been the V-ATPase gene and has been shown to reduce insect life-span and egg production (Yao et al., 2013; Khan et al., 2013). Genome and transcriptome-wide data-mining projects will likely aid in the

identification of additional RNAi gene targets unique to vector insects, thereby addressing concerns of off-target effects. In addition, the discovery of essential interactions for vector transmission may yield additional targets for pest control and reduction of virus transmission by RNAi. Targeting the vector components is challenging because of the relatively large genome sizes and ploidy of insect vectors (Hanrahan and Johnston, 2011; Jacobson et al., 2013), and vector transmission strategies among vector–virus systems are vastly different, and thus insect gene targets are likely more diverse and may vary between tissue types (i.e., receptors in guts vs. salivary glands) in the same insect vector. Despite challenges of working with non-model insects, significant progress has been made in defining vector molecules that interact with viruses.

Virus-Interacting Proteins for Circulative, Propagative Viruses

Propagative viruses have been the focus of proteomics examination of viral–vector interactions. A common theme emerging is the involvement of insect proteins associated with virus transport and dissemination in the vector. A yeast two-hybrid screen with the reovirus, *Southern rice black-streaked dwarf virus* (SRBSDV), and the planthopper vector, *Sogatella furcifera*, revealed 153 putative interactions between P7-1, the major nonstructural viral protein that induces host cell tubular structures that serve as conduits for virus movement, and the insect vector (Mar et al., 2014). Key partners identified in the screen are consistent with the role that P7-1 is thought to play in virus movement (cytoskeletal network and endomembrane system) and the potential insect host response to infection (ubiquitin proteasome and nervous system). Experiments with the tenuivirus *Rice stripe virus* (RSV) and the vector, *Laodelphax striatellus*, revealed insect proteins that also provide insight into the biology of tissue tropism in the vector (Huo et al., 2014). Using a yeast two hybrid screen, vitellogenin, the major yolk protein precursor of egg-laying animals, was identified to interact with the RSV major nucleocapsid protein, pc3. In this study, RNAi-knockdown of vitellogenin transcripts demonstrated the importance of the protein in transovarial transmission of the virus. These findings support the hypothesis that RSV directly binds and 'hijacks' the vitellogenin transport route to enter *L. striatellus* oocytes.

In a separate study, vitellogenin was the most abundant transcript in RSV-infected planthoppers in a global gene expression analysis indicating that in addition to possibly capitalizing on the natural route of vitellogenin into oocytes, induced expression of this protein may enable more efficient transovarial passage of the virus (Zhang et al., 2010). Proteomic analysis of *F. occidentalis* in response to TSWV infection identified 26 protein spots that varied in density between the virus-infected and non-infected larval thrips (Badillo-Vargas et al., 2012). The differential proteins included nine proteins that are putatively associated with steps in the viral infection cycle of entry and exit from insect vector cells (i.e., localization to membranes and protein transport) and 14 proteins associated with

response to stimuli, including nine that are potentially involved in the defense response to TSWV infection. Virus-interacting proteins and proteins that respond to virus infection provide potential targets for disruption of transmission and/or for silencing by RNAi.

Virus-Interacting Proteins for Circulative, Nonpropagative Viruses

A variety of virus-binding and responsive proteins have been identified using a combination of protein-protein interaction discovery methods including virus overlay, yeast two-hybrid, co-immunoprecipitation, and proteomics. For luteoviruses transmitted by aphids, the development of aphid offspring from crosses between efficient and inefficient vectors, *Schizaphis graminum*, enabled the genetic mapping of barriers to transmission (gut vs. salivary gland) and provided a resource for identification of proteins associated with vector competence (Burrows et al., 2006; Yang et al., 2008; Tamborindeguy et al., 2013). Virus overlays and proteomics both revealed the importance of cyclophilins which are proposed to play a role in virus transcytosis through the insect gut and possibly salivary gland tissues. The presence and abundance of cyclophilin proteins and isoforms has been associated with vector competence in the *S. graminum* F2 genotypes of genetic crosses biotypes and field collected biotypes (Tamborindeguy et al., 2013). Other proteins identified to interact with viruses in the genera *Luteovirus* and *Polerovirus* include cytoskeletal proteins such as actin and tubulin; additional endocytic pathway proteins RACK1, GAPDH3, and luciferase-like proteins; and cuticular proteins with chitin-bind 4 domains (Burrows et al., 2006; Yang et al., 2008; Tamborindeguy et al., 2013; Seddas et al., 2004; Cilia et al., 2011). The peptide (GBP3.1) that was found to bind pea aphid guts and reduce the PEMV delivery into the insect hemocoel was also found to bind to a large aphid protein that was identified as an aminopeptidase, a candidate receptor for entry of luteoviruses into the gut of the aphid vector (Chougule et al., 2013).

Virus-Interacting Proteins for Noncirculative Viruses

Vector proteins have also been implicated in binding and transmission of non-circulative viruses. Virus overlay assays identified two types of green peach aphid (*M. persicae*) proteins that bound to the helper component protein of potyviruses. *Zucchini yellow mosaic virus* (ZYMV) helper component protein (HC-Pro) bound to cuticular proteins extracted from whole aphid bodies (Dombrovsky et al., 2010), and using a similar approach but enriching for aphid heads (the stylet is site of virus attachment) ribosomal S2 proteins bound to *Tobacco etch virus* (TEV) HC-Pro (Fernandez-Calvino et al., 2006). Because non-circulative viruses bind to specialized regions of the insect stylet or foregut, it is hypothesized that once virus binding partners in the vector are identified, strategies to saturate binding sites can be deployed to prevent viral protein binding and subsequent

transmission. Verifying virus–vector protein interactions in vivo is an essential step in documenting the validity of interactions identified under *in vitro* conditions or using heterologous systems such as yeast two-hybrid screens. The use of RNAi and advanced imaging technologies will be an important component of interaction studies and has already proven to be useful for validation of interactions identified within vectors of propagative viruses.

Closing Remarks

Our global community faces the mounting threat of newly emergent and re-emerging viruses on the world food supply. Of those viruses, 70 percent of plant-infecting viruses are transmitted from one host to another by arthropod vectors (Hogenhout et al., 2008). As the human population increases from 7 billion to a predicted 9 billion by 2050, it is crucial that plant science research aims to bolster food security by developing reliable, safe, and sustainable plant virus control strategies. The identification of unique steps in the viral infection cycle that enable the design of molecules that interfere with the infection process is a promising approach for virus disease control. The development of new biotechnology-based strategies to reduce transmission by vectors and to decrease vector populations is attractive because they target pathways in the transmission cycle. However, the long-term effectiveness of these control methods relies on their judicious use and incorporation into existing virus-control and vector-control regimes. One approach to increase the durability of the new biotech-based control strategies would be to "stack" these novel traits with traditional virus and vector resistance genes or combine multiple biotech approaches such as deploying transgenic plants that co-express a viral protein to block virus acquisition and dsRNA hairpins to target vital genes in vector populations. The integration of new technologies with traditional integrated pest management strategies (IPM) such as altering planting date and reflective mulches to reduce vector landing rates will also extend the shelf-life of biotechnological traits. This is particularly important for managing resistance to viruses as they have great potential for genetic change and have been shown to rapidly overcome single-target resistance strategies (Lafforgue et al., 2011; Lopez et al., 2011).

Other promising strategies that deserve further exploration for vectors of plant viruses include, firstly, insect transgenesis and secondly, microbial manipulation to reduce vector transmission (Alphey, 2014). Transgenic insects expressing viral dsRNA have been shown to elicit RNAi to reduce virus loads and prevent dissemination to the salivary glands (Franz et al., 2006), thus rendering these transgenics refractory to virus. This type of strategy could be applied to circulative, propagative plant viruses. An alternative transgenic approach is population suppression by introduction of a lethal gene into the population. The development of the 'release of insects carrying a dominant lethal genetic system' (RIDL) has been highly effective for mosquito vectors of human-infecting pathogens in lab

experiments (Alphey, 2014; Alphey and Alphey, 2014; Massonnet-Bruneel et al., 2013).

There are several field studies that have examined the effectiveness and persistence of the transgenic insect strategy (Harris et al., 2012; Lacroix et al., 2012). For insect vectors that reproduce sexually, the RIDL technology could provide new ways to reduce plant vector populations. Metagenomics and microbiome studies have directed attention to the influence of microbes on multiple biotic processes and ecological interactions, including virus transmission. The use of microbial manipulation to alter vector competence and/or capacity is an emerging field of study. For example, the mosquito-*Wolbachia* system has led to exciting findings and several examples of pathogen reduction and stimulation have been documented (Martinez et al., 2014; van den Hurk et al., 2012; Dodson et al., 2014). *Wolbachia* and other endosymbionts are commonly found in plant-feeding insects and the potential role of microbes in transmission by plant virus vectors warrants further exploration and manipulation (Zhang et al., 2010; Bing et al., 2014; see also Pinheiro et al., 2015). With the increase in tools available for the control of viruses and their vectors, the next phase of this research is to move discoveries from the lab to the field. With the vast array of tools available and the collaborative networks that can be developed, it appears that virologists will be up for the challenge of feeding a growing global population while keeping our environment healthy and productive.

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References

Adams, M.J., J.F. Antoniw, and J.G.L. Mullins. 2001. Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Archives of Virology* 146(6):1139–1153.

Alphey, L. 2014. Genetic control of mosquitoes. *Annual Review of Entomology* 59:205–224.

Alphey, L., and N. Alphey. 2014. Five things to know about genetically modified (GM) insects for vector control. *PLoS Pathogens* 10:e1003909.

Ammar, E., C. Tsai, A.E. Whitfield, M.G. Redinbaugh, and S.A. Hogenhout. 2009. Cellular and molecular aspects of *Rhabdovirus* interactions with insect and plant hosts. *Annual Review of Entomology* 54:447–468.

Andret-Link, P., C. Schmitt-Keichinger, G. Demangeat, V. Komar, and M. Fuchs. 2004. The specific transmission of grapevine fanleaf virus by its nematode vector *Xiphinema* index is solely determined by the viral coat protein. *Virology* 320:12–22.

Badillo-Vargas, I.E., D. Rotenberg, Y. Hiromasa, J.M. Tomich, and A.E. Whitfield. 2012. Proteomic analysis of *Frankliniella occidentalis* and differentially-expressed proteins in response to tomato spotted wilt virus infection. *Journal of Virology* 86(16):8793–8809.

Bing, X., W. Xia, J. Gui, G. Yan, X. Wang, and S. Liu. 2014. Diversity and evolution of the *Wolbachia* endosymbionts of *Bemisia* (Hemiptera: Aleyrodidae) whiteflies. *Ecology and Evolution* 4(13):2714–2737.

Blanc, S., M. Drucker, and M. Uzest. 2014. Localizing viruses in their insect vectors. *Annual Review of Phytopathology* 52:403–425.

Bonning, B.C., N. Pal, S. Liu, Z. Wang, S. Sivakumar, P.M. Dixon, G.F. King, and W.A. Miller. 2014. Toxin delivery by the coat protein of an aphid-vectored plant virus provides plant resistance to aphids. *Nature Biotechnology* 32(1):102–105.

Brault, V., J. Mutterer, D. Scheidecker, M.T. Simonis, E. Herrbach, K. Richards, and V. Ziegler-Graff. 2000. Effects of point mutations in the readthrough domain of the beet western yellows virus minor capsid protein on virus accumulation in plants and on transmission by aphids. *Journal of Virology* 74(3):1140–1148.

Bricault, C.A., and K.L. Perry. 2013. Alteration of intersubunit acid–base pair interactions at the quasi-threefold axis of symmetry of cucumber mosaic virus disrupts aphid vector transmission. *Virology* 440(2):160–170.

Burrows, M.E., M.C. Caillaud, D.M. Smith, E.C. Benson, F.E. Gildow, and S.M. Gray. 2006. Genetic regulation of *Polerovirus* and *Luteovirus* transmission in the aphid *Schizaphis graminum*. *Phytopathology* 96(8):828–837.

Chen, A.Y.S., G.P. Walker, D. Carter, and J.C.K. Ng. 2011. A virus capsid component mediates virion retention and transmission by its insect vector. *Proceedings of the National Academy of Sciences of the United States of America* 108:16777–16782.

Chougule, N.P., H. Li, S. Liu, L.B. Linz, K.E. Narva, T. Meade, and B.C. Bonning. 2013. Retargeting of the *Bacillus thuringiensis* toxin Cyt2Aa against hemipteran insect pests. *Proceedings of the National Academy of Sciences of the United States of America* 110:465–8470.

Cilia, M., C. Tamborindeguy, T. Fish, K. Howe, T.W. Thannhauser, and S. Gray. 2011. Genetics coupled to quantitative intact proteomics links heritable aphid and endosymbiont protein expression to circulative polerovirus transmission. *Journal of Virology*, 85:2148–2166.

Dodson, B.L., G.L. Hughes, O. Paul, A.C. Matacchiero, L.D. Kramer, and J.L. Rasgon. 2014. *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. *PLoS Neglected Tropical Diseases* e29658.

Dombrovsky, A., N. Gollop, S. Chen, N. Chejanovsky, and B. Raccah. 2007. In vitro association between the helper component-proteinase of zucchini yellow mosaic virus and cuticle proteins of *Myzus persicae*. *Journal of General Virology*, 88:1602–1610.

Feinberg, E.H., and C.P. Hunter. 2003. Transport of dsRNA into cells by the transmembrane protein SID-1. *Science* 301:1545–1547.

Fernandez-Calvino, L., E. Goytia, D. Lopez-Abella, A. Giner, M. Urizarna, L. Vilaplana, and J. Jose Lopez-Moya. 2010. The helper-component protease transmission factor of tobacco etch potyvirus binds specifically to an aphid ribosomal protein homologous to the laminin receptor precursor. *Journal of General Virology* 91(11):2862–2873.

Franz, A.W.E., I. Sanchez-Vargas, Z.N. Adelman, C.D. Blair, B.J. Beaty, A.A. James, and K.E. Olson. 2006. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the United States of America* 103(11):4198–4203.

Gordon, K.H.J., and P.M. Waterhouse. 2007. RNAi for insect-proof plants. *Nature* 25(11) 1231–1232.

Govier, D.A., and B. Kassanis. 1974. Virus-induced component of plant sap needed when aphids acquire potato virus-Y from purified preparations. *Virology* 61:420–426.

Gray, S., M. Cilia, and M. Ghanim. 2014. Circulative "Nonpropagative" virus transmission: An orchestra of virus-, insect-, and plant-derived instruments. *Advances in Virus Research* 89:141–199.

Guoying, Z., L. Xiongbin, and L. Huijuan. 1999. Rice ragged stunt oryzavirus: Role of the viral spike protein in transmission by the insect vector. *Annals of Applied Biology* 135(3):573–578.

Hanrahan, S.J., and J.S. Johnston. 2011. New genome size estimates of 134 species of arthropods. *Chromosome Research* 19(6):809–823.

Harris, A.F., A.R. McKemey, D. Nimmo, Z. Curtis, I. Black, S.A. Morgan, M.N. Oviedo, R. Lacroix, N. Naish, N.I. Morrison, et al. 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nature Biotechnology* 30:828–830.

Hogenhout, S.A., E.D. Ammar, A.E. Whitfield, and M.G. Redinbaugh. 2008. Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology* 46:327–359.

Huo, Y., L. Wenwen, Z. Fujie, C. Xiaoying, L. Li, L. Qifei, Z. Yijun, W. Taiyun, F. Rongxiang, and X. Wang. 2014. Transovarial transmission of a plant virus is mediated by vitellogenin of its insect vector. *PLoS Pathogens* 10(3):e1003949.

Huvenne, H., and G. Smagghe. 2010. Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review. *Journal of Insect Physiology* 56:227–235.

Jacobson, A.L., J.S. Johnston, D. Rotenberg, A.E. Whitfield, W. Booth, E.L. Vargo, and G.G. Kennedy. 2013. Genome size and ploidy of Thysanoptera. *Insect Molecular Biology* 22(1):12–17.

Khan, A.M., M. Ashfaq, Z. Kiss, A.A. Khan, S. Mansoor, and B.W. Falk. 2013. Use of recombinant tobacco mosaic virus to achieve RNA interference in plants against the citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae). *PLoS One* 8(9):e73657.

Killiny, N., A. Rashed, and R.P.P. Almeida. 2012. Disrupting the transmission of a vector-borne plant pathogen. *Applied and Environmental Microbiology* 78(3):638–643.

Killiny, N., and R.P.P. Almeida. 2009. *Xylella fastidiosa* afimbrial adhesins mediate cell transmission to plants by leafhopper vectors. *Applied and Environmental Microbiology* 75(2):521–528.

Kritzman, A., A. Gera, B. Raccah, J.W.M. van Lent, and D. Peters. 2002. The route of tomato spotted wilt virus inside the thrips body in relation to transmission efficiency. *Archives of Virology* 147(11):2143–2156.

Lacroix, R., A.R. McKemey, N. Raduan, L.K. Wee, W.H. Ming, T.G. Ney, S.A.A. Rahidah, S. Salman, S. Subramaniam, O. Nordin, et al. 2012. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. *PLoS One* 7:e42771.

Lafforgue, G., F. Martinez, J. Sardanyes, F. de la Iglesia, Q. Niu, S. Lin, R.V. Solé, N. Chua, J.-A. Daròs, and S.F. Elena. 2011. Tempo and mode of plant RNA virus escape from RNA interference-mediated resistance. *Journal of Virology* 85:9686–9695.

Liu, S., S. Sivakumar, Z. Wang, B.C. Bonning, and W.A. Miller. 2009. The readthrough domain of pea enation mosaic virus coat protein is not essential for virus stability in the hemolymph of the pea aphid. *Archives of Virology* 154(3) 469–479.

Liu, S., S. Sivakumar, W.O. Sparks, W.A. Miller, and B.C. Bonning. 2010. A peptide that binds the pea aphid gut impedes entry of pea enation mosaic virus into the aphid hemocoel. *Virology* 401(1):107–116.

Liu, S.J., X.H. He, G. Park, C. Josefsson, and K.L. Perry. 2002. A conserved capsid protein surface domain of cucumber mosaic virus is essential for efficient aphid vector transmission. *Journal of Virology* 76(2) 9756–9762.

Lopez, C., J. Aramburu, L. Galipienso, S. Soler, F. Nuez, and L. Rubio. 2011. Evolutionary analysis of tomato Sw-5 resistance-breaking isolates of tomato spotted wilt virus. *Journal of General Virology* 92(1):210–215.

Lung, M.C.Y., and T.P. Pirone. 1974. Acquisition factor required for aphid transmission of purified cauliflower mosaic-virus. *Virology* 60:260–264.

Mar, T.T., L. Wenwen, and W. Xifeng. 2014. Proteomic analysis of interaction between P7-1 of Southern rice black-streaked dwarf virus and the insect vector reveals diverse insect proteins involved in successful transmission. *Journal of Proteomics* 102:83–97.

Martinez, J., B. Longdon, S. Bauer, Y. Chan, W.J. Miller, K. Bourtzis, L. Teixeira, and F.M. Jiggins. 2014. Symbionts commonly provide broad spectrum resistance to viruses in insects: A comparative analysis of *Wolbachia* strains. *PLoS Pathogens* 10:e1004369.

Massonnet-Bruneel, B., N. Corre-Catelin, R. Lacroix, R.S. Lees, Kim Phuc Hoang, D. Nimmo, L. Alphey, and P. Reiter. 2013. Fitness of transgenic mosquito *Aedes aegypti* males carrying a dominant lethal genetic system. *PLoS One* 8:e62711.

Montero-Astúa, M., D. Rotenberg, A. Leach-Kieffaber, B.A. Schneweis, S. Park, J.K. Park, T.L. German, and A.E. Whitfield. 2014. Disruption of vector transmission by a plant-expressed viral glycoprotein. *Molecular Plant Microbe Interactions* 27(3):296–304.

Moritz, G., S. Kumm, and L. Mound. 2004 Tospovirus transmission depends on thrips ontogeny. *Virus Research* 100(1):143–149.

Nagata, T., A.K. Inoue-Nagata, J. van Lent, R. Goldbach, and D. Peters. 2002. Factors determining vector competence and specificity for transmission of tomato spotted wilt virus. *Journal of General Virology* 83(3):663–671.

Ng, J., and B.W. Falk. 2006: Virus–vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. *Annual Review of Phytopathology* 44:183–212.

Ohki, T., F. Akita, T. Mochizuki, A. Kanda, T. Sasaya, and S. Tsuda. 2010. The protruding domain of the coat protein of melon necrotic spot virus is involved in compatibility with and transmission by the fungal vector *Olpidium bornovanus*. *Virology* 402(1):129–134.

Peter, K.A., D. Liang, P. Palukaitis, and S.M. Gray. 2008. Small deletions in the potato leafroll virus readthrough protein affect particle morphology, aphid transmission, virus movement and accumulation. *Journal of General Virology* 89(8):2037–2045.

Pinheiro, P., A. Kliot, M. Ghanim, and M. Cilia. 2015. Is there a role for symbiotic bacteria in plant virus transmission by insects? *Current Opinion in Insect Science* 8:69–78.

Rotenberg, D., N.K.K. Kumar, D.E. Ullman, M. Montero-Astúa, D.K. Willis, T.L. German, and A.E. Whitfield. 2009. Variation in tomato spotted wilt virus titer in *Frankliniella occidentalis* and its association with frequency of transmission. *Phytopathology* 99:404–410.

Scott, J.G, K. Michel, L.C. Bartholomay, B.D. Siegfried, W.B. Hunter, G. Smagghe, K.Y. Zhu, and A.E. Douglas. 2013. Towards the elements of successful insect RNAi. *Journal of Insect Physiology* 59(12):1212–1221.

Seddas, P., S. Boissinot, J. Strub, A. Van Dorsselaer, M. Van Regenmortel, and F. Pattus. 2004. Rack-1, GAPDH3, and actin: Proteins of *Myzus persicae* potentially involved in the transcytosis of beet western yellows virus particles in the aphid. *Virology* 325(2):399–412.

Shao, C., J. Wu, G. Zhou, G. Sun, B. Peng, J. Lei, D. Jin, S. Chen, N. Upadhyaya, P. Waterhouse, and Z. Gong. 2003. Ectopic expression of the spike protein of Rice Ragged Stunt Oryzavirus in transgenic rice plants inhibits transmission of the virus to insects. *Molecular Breeding* 11:295–301.

Sin, S., B.C. McNulty, G.G. Kennedy, and J.W. Moyer 2005. Viral genetic determinants for thrips transmission of tomato spotted wilt virus. *Proceedings of the National Academy of Sciences USA* 102:5168–5173.

Tamborindeguy, C., M.S. Bereman, S. DeBlasio, D. Igwe, D.M. Smith, F. White, M.J. MacCoss, S.M. Gray, and M. Cilia. 2013. Genomic and proteomic analysis of *Schizaphis graminum* reveals cyclophilin proteins are involved in the transmission of cereal yellow dwarf virus. *PLoS One* 8(8):e71620.

Uzest, M., D. Gargani, A. Dombrovsky, C. Cazevieille, D. Cot, and S. Blanc. 2010. The "acrostyle": A newly described anatomical structure in aphid stylets. *Arthropod Structure and Development* 39(4):221–229.

Uzest, M., D. Gargani, M. Drucker, E. Hébrard, E. Garzo, T. Candresse, A. Fereres, and S. Blanc. 2007. A protein key to plant virus transmission at the tip of the insect vector stylet. *Proceedings of the National Academy of Sciences USA* 104:17959–17964.

van den Hurk, A.F., S. Hall-Mendelin, A.T. Pyke, F.D. Frentiu, K. McElroy, A. Day, S. Higgs, and S.L. O'Neill. 2012. Impact of *Wolbachia* on infection with chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. *PLoS Neglected Tropical Diseases* 6(11):e18926.

Wang, L., X. Wei, X. Ye, H. Xu, X. Zhou, S. Liu, and X. Wang. 2014. Expression and functional characterisation of a soluble form of tomato yellow leaf curl virus coat protein. *Pest Management Sciences* 70(10):1624–1631.

Wang, R.Y., E.D. Ammar, D.W. Thornbury, J.J. Lopez-Moya, and T.P. Pirone. 1996. Loss of potyvirus transmissibility and helper-component activity correlate with non-retention of virions in aphid stylets. *Journal General Virology* 77(5):861–867.

Webster, C.G., S.R. Reitz, K.L. Perry, and S. Adkins. 2011. A natural M RNA reassortant arising from two species of plant- and insect-infecting bunyaviruses and comparison of its sequence and biological properties to parental species. *Virology* 413:216–225.

Whitfield, A.E., D.E. Ullman, and T.L. German. 2004. Expression, purification, and characterization of a soluble form of tomato spotted wilt virus glycoprotein GN. *Journal of Virology* 78(23):13197–13206.

Whitfield, A.E., D.E. Ullman, and T.L. German. 2005. Tospovirus–thrips interactions. *Annual Review of Phytopathology* 43 459–489.

Whitfield, A.E., N.K.K. Kumar, D. Rotenberg, D.E. Ullman, E.A. Wyman, C. Zietlow, D.K. Willis, and T.L. German. 2008. A soluble form of the tomato spotted wilt virus (TSWV) glycoprotein GN (GN-S) inhibits transmission of TSWV by *Frankliniella occidentalis*. *Phytopathology* 98(1):45–50.

Winston, W.M., C. Molodowitch, and C.P. Hunter. 2002: Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. *Science* 295(5565):2456–2459.

Yang, X., T.W. Thannhauser, M. Burrows, D. Cox-Foster, F.E. Gildow, and S.M. Gray. 2008. Coupling genetics and proteomics to identify aphid proteins associated with vector-specific transmission of polerovirus (Luteoviridae). *Journal of Virology* 82(1):291–299.

Yao, J., D. Rotenberg, A. Afsharifar, K. Barandoc-Alviar, and A.E. Whitfield. 2013. Development of RNAi methods for *Peregrinus maidis*, the corn planthopper. *PLoS One* 8(8):e70243.

Zhang, F., H. Guo, Z. Zheng, T. Zhou, Y. Zhou, S. Wang, R. Fang, W. Qian, and X. Chen. 2010. Parallel pyrosequencing-based transcriptome analyses of small brown planthopper (*Laodelphax striatellus*), a vector insect transmitting rice stripe virus (RSV). *BMC Genomics* 11:303.

Zhou, J., S. Kantartzi, R- Wen, M. Newman, M. Hajimorad, J. Rupe, and I. Tzanetakis. 2011. Molecular characterization of a new tospovirus infecting soybean. *Virus Genes* 43:289–295.

Appendix B

Agenda

DAY ONE: TUESDAY, SEPTEMBER 16, 2014

8:45–9:15: Registration and continental breakfast

9:15–9:30: Welcoming remarks and overview: David A. Relman, James M. Hughes, Lonnie King

SESSION I OVERVIEW OF VECTOR–HOST–ENVIRONMENTAL RELATIONSHIPS

Moderator: Mary Wilson

9:30–10:15: Emerging vector-borne diseases in the United States: What is next, and are we prepared?
Lyle Petersen, Centers for Disease Control and Prevention

10:15–11:00: The past, present, and future of vector-borne plant diseases
Rodrigo Almeida, University of California, Berkeley

11:00–11:45: Changing patterns of vector-borne diseases in animals domestically and globally
William Karesh, EcoHealth Alliance

11:45–12:30: DISCUSSION

12:30–1:15: LUNCH

SESSION II THE CHANGING LANDSCAPE FOR VECTOR-BORNE DISEASES

Moderator: James M. Hughes

1:15–1:45: Arbovirus evolution, vector competence, and virulence models: Changing patterns of infection
Rebecca Rico-Hesse, Baylor College of Medicine

1:45–2:15: Vector-borne disease emergence and spread in the European Union
Jan Semenza, European Centre for Disease Control and Prevention

2:15–2:45: Arbovirus disease surveillance capacity in the United States
James Hadler, Yale University

2:45–3:15: BREAK

3:15–3:45: Recent introductions and spread of dengue and chikungunya in the Caribbean and the Americas
Hal Margolis, Centers for Disease Control and Prevention

3:45–4:15: The changing epidemiology and geographic spread of leishmaniasis and Chagas disease
James Maguire, Harvard Medical School

4:15–4:45: Changing paradigms for tick-borne diseases in the Americas
Christopher Paddock, Centers for Disease Control and Prevention

4:45–5:15: Blood donation screening for vector-borne diseases
Susan Stramer, American Red Cross

5:15–6:00: DISCUSSION

6:00: ADJOURNMENT

DAY TWO: WEDNESDAY, SEPTEMBER 17, 2014

8:30–9:00: Registration and continental breakfast

9:00–9:15: Welcome and summary of day one: David Relman

SESSION III KEY FACTORS AND DRIVERS—CLIMATE, TRAVEL, LAND USE, TRANSPORTATION, AND TRADE

Moderator: Lonnie King

9:15–9:45: Recent weather extremes and impacts on agricultural production and vector-borne disease outbreak patterns
Ken Linthicum, U.S. Department of Agriculture

9:45–10:15: Globalization, land use, global warming, and the invasion of West Nile virus
Marm Kilpatrick, University of California, Santa Cruz

10:15–10:45: BREAK

10:45–11:15: The impact of environmental factors on mosquito-parasite interactions
Matt Thomas, Pennsylvania State University

11:15–11:45: Dengue, chikungunya, and malaria surveillance and response in Latin America and the Caribbean: The role of the Pan American Health Organization
Luis Gerardo Castellanos, Pan American Health Organization

11:45–12:30: DISCUSSION

12:30–1:15: LUNCH

SESSION IV NOVEL APPROACHES AND INTERVENTION STRATEGIES FOR VECTOR-BORNE DISEASE CONTROL

Moderator: Gerald Keusch

1:15–1:45: Why did Gorgas succeed? (And why have we failed?)
Paul Reiter, Institute Pasteur

1:45–2:15: Towards the diagnosis and prognosis of emerging vector-borne diseases
Barry Beaty, Colorado State University

2:15–2:45: Malaria eradication strategies at the Gates Foundation
Alan Magill, The Bill & Melinda Gates Foundation

2:45–3:00: BREAK

3:00–3:30: Development and evaluation of transgenic insects for use in the control of insect-borne disease
Luke Alphey, Pirbright Institute

3:30–4:00: Exploiting the specificity of virus–vector interactions for new disease control strategies
Anna Whitfield, Kansas State University

4:00–4:30: Dengue, Japanese encephalitis, West Nile, chikungunya, and yellow fever: Challenges for the development and use of vaccines
Thomas Monath, Harvard Medical School

4:30–5:15: DISCUSSION

5:15–5:30: CLOSING REMARKS AND ADJOURNMENT

Appendix C

Acronyms

AFHSC	Armed Forces Health Surveillance Center
APHIS	Animal and Plant Health Inspection Service
APHL	Association of Public Health Laboratories
BTV	bluetongue virus
CDC	Centers for Disease Control and Prevention
CHIKV	chikungunya virus
DDSS	Dengue Decision Support System
DDT	dichlorodiphenyltrichloroethane
DENV	dengue virus
DF	dengue fever
DHF	dengue hemorrhagic fever
DSS	dengue shock syndrome
DTP	dengue transmission potential
DV	dengue virus
ECDC	European Center for Disease Control
EIS	Epidemic Intelligence Service
ENSO	El Niño–Southern Oscillation
HAT	human African trypanosomiasis
HFRS	hemorrhagic fever with renal syndrome

IOM	Institute of Medicine
ITM	insecticide-treated material
ITN	insecticide-treated bed net
IVCC	Innovative Vector Control Consortium
JEV	Japanese encephalitis virus
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NMCP	National Malaria Control Programme
OIE	Office International des Epizooties
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
PD	Pierce's disease of grapevines
RIDL	release of insects carrying dominant lethal
RNA	ribonucleic acid
RNAi	RNA interference
rRT-PCR	real-time reverse transcription-polymerase chain reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SLE	St. Louis encephalitis
SLEV	St. Louis encephalitis virus
SNV	Sin Nombre virus
SOI	Southern Oscillation Index
SST	sea surface temperature
subsp.	subspecies
TBE	tick-borne encephalitis
VBD	vector-borne disease
VEE	Venezuelan equine encephalitis
VEEV	Venezuelan equine encephalitis virus
VP	viral structural protein
WHO	World Health Organization
WNV	West Nile virus
YF	yellow fever
YFV	yellow fever virus

Appendix D

Glossary

Agent (of disease): Factor such as a microorganism whose presence is essential for the occurrence of a disease.

***Anopheles*:** A genus of mosquitoes that includes all mosquitoes that transmit malaria to humans.

Anopheline: Any of various mosquitoes of the genus *Anopheles*, which can carry the malaria parasite and transmit the disease to humans.

Anthroponotic: Transmission from human to human and potentially from human to animal.

Antibiotic: Class of substances that can kill or inhibit the growth of some groups of microorganisms. Used in this report to refer to chemicals active against bacteria. Originally antibiotics were derived from natural sources (e.g., penicillin from molds), but many currently used antibiotics are semisynthetic and modified with additions of man-made chemical components. See *Antimicrobials*.

Antibiotic resistance: Property of bacteria that confers the capacity to inactivate or exclude antibiotics or a mechanism that blocks the inhibitory or killing effects of antibiotics.

Antimicrobials: Class of substances that can destroy or inhibit the growth of pathogenic groups of microorganisms, including bacteria, viruses, parasites, and fungi.

Arboviral diseases: Shortened form of arthropod-borne virus. Any of a group of viruses that are transmitted to man and animals by mosquitoes, ticks, and sand flies; they include such agents as yellow fever and eastern, western, and Venezuelan equine encephalitis viruses.

Arthropod: As used in this report, refers to insects and ticks, many of which are medically important as vectors of infectious diseases.

Arthropod-borne: Capable of being transmitted by insect and tick (arthropod) vectors.

Asymptomatic: Presenting no symptoms of disease.

Bacteria: Microscopic, single-celled organisms that have some biochemical and structural features different from those of animal and plant cells.

Chagas disease: A potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi*. Predominantly found in Latin America, *T. cruzi* is commonly transmitted to humans and other mammals by an insect vector.

Climate: Average meteorological conditions over a specified time period, usually at least a month, resulting from interactions among the atmosphere, oceans, and land surface. Climate variations occur over a wide range of spatial and temporal scales.

Climate change: A change of climate attributed directly or indirectly to human activity that alters the composition of the global atmosphere and is in addition to natural climate variability observed over comparable time periods.

Climate extremes: Used to represent weather extremes (see definition below), but viewed over seasons (e.g., droughts), or longer periods.

Climate variability: Refers to variations or deviations from the mean state of the climate or temporal variations of the atmosphere–ocean system around a mean state measure over a long period of time. Typically, this term is used for time scales longer than those associated with synoptic weather events (i.e., months to millennia and longer). The term *natural climate variability* is further used to identify climate variations that are not attributable to or influenced by any activity related to humans. However it is recognized that such internal or natural variability could be affected by external factors driving climate change such as changes in the atmospheric concentration of greenhouse gases. The El Niño–Southern Oscillation (ENSO) phenomenon is a good example of the variability in the coupled oceanic and atmosphere system that is a central factor in short-term

climate variability and the interannual time scale (http://www.cpc.noaa.gov/products/analysis_monitoring/ensostuff/prelude_to_ensofaq.shtml; http://www.ncdc.noaa.gov/paleo/outreach/coral/coralenso.html; http://www.sws.uiuc.edu/atmos/statecli/Climate_change/glossary.htm [accessed March 29, 2016]).

Communicable disease: An infectious disease transmissible (as from person to person) by direct contact with an infected individual or the individual's discharges or by indirect means (as by a vector).

Dengue/dengue hemorrhagic fever (DHF): A vector-borne viral disease, dengue is transmitted between people by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, which are found throughout the world. Dengue fever (DF) is caused by any of four closely related viruses, or serotypes, dengue 1–4. Infection with one serotype does not protect against the others, and sequential infections put people at greater risk for DHF and dengue shock syndrome (DSS).

Disease: As used in this report, refers to a situation in which infection has elicited signs and symptoms in the infected individual; the infection has become clinically apparent.

Ecosystem: Mutually interrelated communities of species and abiotic components, existing as a system with specific interactions and exchange of matter, energy, and information.

El Niño: A warming of the surface waters of the tropical Pacific that occurs every 3 to 5 years, temporarily affecting weather worldwide.

Elimination: Cessation of transmission in a country, continent, or other limited geographic area; complete prevention of a clinical presentation of disease.

Emerging infection: Either a newly recognized, clinically distinct infectious disease or a known infectious disease whose reported incidence is increasing in a given place or among a specific population.

Emerging infections: Any infectious disease that has come to medical attention within the last 2 decades or for which there is a threat that its prevalence will increase in the near future (IOM, 1992). Many times, such diseases exist in nature as zoonoses and emerge as human pathogens only when humans come into contact with a formerly isolated animal population, such as monkeys in a rain forest that are no longer isolated because of deforestation. Drug-resistant organisms could also be included as the cause of emerging infections since they exist because of human influence. Some recent examples of agents responsible

for emerging infections include human immunodeficiency virus, Ebola virus, multidrug resistant *Mycobacterium tuberculosis,* and influenza A (H1N1).

Encephalitis: An acute inflammatory disease of the brain due to direct viral invasion or to hypersensitivity initiated by a virus or other foreign protein.

Endemic: Present in a community or common among a group of people; said of a disease prevailing continually in a region.

Enzootic: A disease of low morbidity that is constantly present in an animal community.

Epidemic: Appearance of an abnormally high number of cases of infection in a given population.

Epidemiology: Study of the distribution and determinants of health-related states or events in specified populations. Epidemiology is the basic quantitative science of public health.

Epizootic: A disease of high morbidity that is only occasionally present in an animal community.

Eradication: Reducing the incidence of a disease to zero worldwide, such that further control measures are unnecessary; total interruption of transmission.

Extreme weather: Refers to weather phenomena that are at the extremes of the historical distribution and are rare for a particular place and/or time, especially severe or unseasonal weather. Such extremes include severe thunderstorms, severe snowstorms, ice storms, blizzards, flooding, hurricanes, high winds, and heat waves. For example, although flooding is common in the United States, the impacts of flooding are not consistent from year to year through time. Many years of small floods with little impact may be followed by a single large flood with a sizable loss (e.g., the June 2008 flooding in the Midwestern United States) (http://www.greenhouse.gov.au/impacts/resources/glossary.html; http://en.wikipedia.org/wiki/Extreme_weather; http://www.sws.uiuc.edu/atmos/statecli/General/Illinois-climate-narrative.htmn [accessed March 29, 2016]).

Extrinsic incubation period: Time required for the development of a disease agent in a vector from the time of uptake of the agent to the time the vector is infective.

Globalization: The increased interconnectedness and interdependence of peoples and countries is generally understood to include two interrelated elements: the

opening of borders to increasingly fast flows of goods, services, finance, people, and ideas across international borders; and the changes in institutional and policy regimes at the international and national levels that facilitate or promote such flows (http://www.who.int/trade/glossary/story043/en/index.html [accessed March 29, 2016]).

Herd immunity: A reduction in the probability of infection that is held to apply to susceptible members of a population in which a significant proportion of the individuals are immune because the chance of coming in contact with an infected individual is less.

Host (disease): Person or other living animal that affords subsistence or lodgment to an infectious agent under natural conditions.

Human African trypanosomiasis (HAT): HAT is a protozoan parasitic disease of people and animals, caused by *Trypanosoma brucei* and transmitted by the tsetse fly. The disease is endemic in some regions of sub-Saharan Africa, covering about 36 countries and 60 million people.

Incidence: Number of cases of a disease commencing, or of persons falling ill, during a given period of time in a specified population. Incidence rate is the number of new cases of a specific disease diagnosed or reported during a defined interval of time divided by the number of all persons in a defined population during the same time.

Infection: The invasion of the body or a part of the body by a pathogenic agent, such as a microorganism or virus. Under certain conditions the agent develops or multiplies, the results of which may produce injurious effects. Infection should not be confused with disease.

Intermediate host: A host that is normally used by a parasite in the course of its life cycle and in which it may multiply asexually but not sexually.

Kinetoplastid: A group of flagellated protozoa characterized by the presence of one or two flagella in the cell body and a kinetoplast within the mitochondrion. As human parasites, kinetoplastids are associated with Chagas disease, HAT, and leishmaniasis.

La Niña: Cooler-than-normal sea surface temperatures in the central and eastern tropical Pacific ocean that impact global weather patterns. La Niña conditions recur every few years and can persist for as long as 2 years.

Microbe: A microorganism or biologic agent that can replicate in humans (including bacteria, viruses, protozoa, fungi, and prions).

Mitigation: Initiatives that reduce the risk from natural and man-made hazards. With respect to climate change, mitigation usually refers to actions taken to reduce the emissions or enhance the sinks of greenhouse gases.

Morbidity: Diseased condition or state.

Mortality: The quality or state of being mortal; the number of deaths in a given time or place; the proportion of deaths to population.

Outbreak: Localized occurrence as opposed to a generalized epidemic.

Pandemic: Epidemic occurring over a wide geographic area and affecting an exceptionally high proportion of the population.

Parasite: An organism living in, with, or on another organism.

Pathogen: Organism capable of causing disease.

Pathogenic: Capable of causing disease.

Polymerase chain reaction (PCR): A laboratory technique used to make multiple copies of a segment of DNA. PCR is very precise and can be used to amplify, or copy, a specific DNA target from a mixture of DNA molecules.

Prevalence: Proportion of persons in a population currently affected by a particular disease. Prevalence rate is the number of cases of a specific disease at a particular time divided by the population at that time living in the same region.

Protozoa and protozoan parasites: Protozoa are microscopic, unicellular organisms that can be free living or parasitic in nature. They are able to multiply in humans, which contributes to their survival and also permits serious infections to develop from just a single organism. Transmission of protozoa that live in a human intestine to another human typically occurs through a fecal–oral route (e.g., contaminated food or water or person-to-person contact). Protozoa that live in the blood or tissue of humans are transmitted to other humans by an arthropod vector (for example, through the bite of a mosquito or sand fly).

Reservoir: Any person, animal, arthropod, plant, soil, or substance (or combination of these) in which an infectious agent normally lives and multiplies, on

which it depends primarily for survival, and in which it reproduces itself in such manner that it can be transmitted to a susceptible vector.

Rickettsial disease: Infection caused by a variety of obligate intracellular, Gram-negative bacteria that are usually transmitted by ectoparasites such as fleas, lice, mites, and ticks.

Rift Valley fever: Rift Valley fever is a viral zoonosis that primarily affects animals but also has the capacity to infect humans. Infection can cause severe disease in both animals and humans. The disease also results in significant economic losses due to death and abortion among RVF-infected livestock. The virus was first identified in 1931 after an epidemic struck sheep on a farm in the Rift Valley of Kenya. Since then, outbreaks have been reported in sub-Saharan and North Africa. In 1997–1998, a major outbreak occurred in Kenya, Somalia, and Tanzania, and in September 2000, cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent and raising concerns that it could extend to other parts of Asia and Europe (http://www.who.int/mediacentre/factsheets/fs207/en/ [accessed March 29, 2016]).

RNA interference (RNAi): RNAi is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules.

rRT-PCR: A real-time polymerase chain reaction is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously detect or quantify a targeted DNA molecule.

***Salmonella*:** A genus of bacteria that cause typhoid fever, food poisoning, and enteric fever from food poisoning.

Species barrier: Difficulty or impossibility for an infectious agent to pass from one species to another (due to differences between species).

Subclinical infection: An infection where the patient does not have any apparent symptoms (also known as an asymptomatic infection).

Syndrome: A group or recognizable pattern of symptoms or abnormalities that indicate a particular trait or disease.

Transmission: Process by which a pathogen passes from a source of infection to a new host.

Universal precautions: The use of gloves, protective garments, and masks when handling potentially infectious or contaminated materials.

Vaccine: A preparation of living, attenuated, or killed bacteria or viruses, fractions thereof, or synthesized or recombinant antigens identical or similar to those found in the disease-causing organisms that are administered to raise immunity to a particular microorganism.

Vector: A carrier—especially an arthropod—that transfers an infective agent from one host (which can include itself) to another.

Vector-borne: Transmitted from one host to another by a vector.

Vector-borne disease: (1) *Mechanical*: this includes simple mechanical carriage by a crawling or flying insect through soiling of its feet or proboscis or by passage of organisms through its gastrointestinal tract. This does not require multiplication or development of the organism. (2) *Biological*: propagation (multiplication), cyclic development, or a combination of these (cyclopropagative) is required before the arthropod can transmit the infective form of the agent to humans. An incubation period (extrinsic) is required following infection before the arthropod becomes infective. The infectious agent may be passed vertically to succeeding generations (transovarian transmission); transstadial transmission indicates its passage from one stage of the life cycle to another, as nymph to adult. Transmission may be by injection of salivary gland fluid during biting, or by regurgitation or deposition on the skin of feces or other material capable of penetrating the bite wound or an area of trauma from scratching or rubbing. This transmission is by an infected nonvertebrate host and not simple mechanical carriage by a vector or vehicle. However, an arthropod in either role is termed a vector.

Viremia: The presence of virus in the blood of a host.

Virulence: The ability of any infectious agent to produce disease. The virulence of a microorganism (such as a bacterium or virus) is a measure of the severity of the disease it is capable of causing.

Xylem: The vascular tissue in plants that conducts water and dissolved nutrients upward from the root and also helps to form the woody element in the stem.

Zika virus: ZIKV is a member of the Flaviviridae virus family and the *Flavivirus* genus. In humans, it causes a disease known as Zika fever. It is related to dengue, yellow fever, and West Nile and Japanese encephalitis, viruses that are also members of the virus family Flaviviridae.

Zoonoses: Microbes that are naturally transmitted between animals and humans that cause disease in human populations but can be perpetuated solely in nonhuman host animals (e.g., influenza, rabies).

Zoonotic infection: Infection that causes disease in human populations but can be perpetuated solely in nonhuman host animals (e.g., bubonic plague); may be enzootic.

Appendix E

Speaker Biographies

Rodrigo Almeida, Ph.D., is an associate professor in ecology of emerging infectious diseases at the University of California, Berkeley. His research focuses on insect-borne plant pathogens, addressing questions on what allows these organisms to be successful in causing disease, how they interact with vectors and host plants, and how they spread in time and space. An ultimate goal of his interdisciplinary research is to generate information that will assist in the development of practices that can reduce the impact of emerging diseases. He is a Fulbright and Marie Curie fellow, and received the American Phytopathological Society's Early Career Award in 2012, among other awards.

Luke Alphey, Ph.D., is a leader in the emerging field of genetic pest management, focusing particularly on mosquitoes. He is a nonexecutive director of Oxitec Ltd, a spin-out company from Oxford University that he cofounded in 2002; he was the research director from 2002 to 2014. Oxitec aims to control insect pests by use of engineered sterile males of the pest insect species (RIDL males). Oxitec successfully conducted the world's first outdoor experiments with a genetically modified insect in the United States in 2006, and in 2010 showed that a wild mosquito population could be suppressed by this genetics-based method. Dr. Alphey's earlier career focused on basic science, using *Drosophila* as a model system, latterly at Oxford University. After 11 years at Oxitec he moved to The Pirbright Institute in February 2014. Alphey was selected as a Technology Pioneer of the World Economic Forum in 2008 and BBSRC Innovator of the Year 2014.

Barry Beaty, Ph.D., is a professor of microbiology, immunology, and pathology at the University of Colorado. His current research efforts have involved

understanding the epidemiology of vector-borne diseases; arbovirus maintenance in nature and transmission to humans; development of rapid, clinically relevant diagnostic tests for improved arbovirus surveillance, prevention, and control strategies; and for improving patient care. For the past 20 years, he has investigated dengue virus epidemiology and molecular epidemiology in the Yucatan, dengue molecular determinants of severe disease, dengue virus–*Aedes aegypti* associations and interactions, differential diagnosis of *Flavivirus* infections in Mexico (e.g., differential diagnosis of dengue and West Nile virus [WNV] infections), and development of rapid clinically relevant tests for *Flavivirus* surveillance (e.g., a blocking ELISA test for WNV infections, which is now widely used in Latin America). Current research efforts include development of (1) a metabolomics-based LC-MS/MS approach for identification of small molecular biomarkers in acute phase serum, urine, and saliva for diagnosis of dengue virus infections and for prognosis of severe disease outcomes (dengue hemorrhagic fever and shock syndrome; (2) molecular mosquitocides (a novel RNAi-nanoparticle, target-specific approach) for control of insecticide-resistant mosquito vectors; and (3) novel *casa segura*-based approaches for protecting the domicile from hematophagous arthropods and pathogen transmission.

Luis Gerardo Castellanos, M.D., Ph.D., M.P.H., was born in the Republic of Guatemala where he started his university studies to receive a degree in medicine from the University of San Carlos of Guatemala. Later he worked as a professor at the medical school of the same university until 1990 when he began his graduate studies in the United States. In 1991 he received the title of Master of Public Health from the University of Puerto Rico, and in 1994 he also received his Ph.D. in epidemiology from the School of Public Health at the University of South Carolina. Between 1994 and 1996, Dr. Castellanos completed the Epidemic Intelligence Service (EIS) training program in field epidemiology of the Centers for Disease Control and Prevention (CDC), with particular focus on outbreak investigation, prevention, and control. In 1997, he joined the Pan American Health Organization (PAHO) as a consultant for disease prevention and control, serving in Honduras, Brazil, Mexico and the Mexico–United States Border Office, based in El Paso, Texas. Since 2011, Dr. Castellanos has assumed the role of senior advisor and chief of the Neglected Tropical and Vector-borne Diseases Unit at PAHO headquarters in Washington, DC. During his career Dr. Castellanos has published scientific articles, and assisted many countries in the Americas, both in routine training and research activities, as well as technical support in the investigation, prevention, and control of outbreaks, emergencies, and natural disasters.

James Hadler, M.D., M.P.H, is currently clinical professor of epidemiology and public health at the Yale University School of Public Health and a consultant to the New York City Department of Health and to the Council of State and Territorial Epidemiologists (CSTE). Recently, he was the lead consultant for CSTE

for a national arbovirus surveillance capacity assessment in 2013, a member of the CDC's Infectious Disease Board of Scientific Counselors, his term ending several months ago, and an original member of CDC's Biosurveillance Advisory Subcommittee (term ending 2012). Dr. Hadler was the state epidemiologist and director of the Infectious Diseases Division at the Connecticut Department of Public Health for nearly 25 years before leaving full-time state service in 2008. As part of his responsibilities, he was involved in development of the Connecticut response to a wide range of emerging infectious disease issues, including HIV, tuberculosis, and Lyme disease in the 1980s; West Nile virus in 1999; anthrax in 2001; and SARS in 2003. He also was the principal investigator for the Connecticut Emerging Infections Program from 1995–2007 and responsible for public health preparedness activities relating to infectious diseases. He has an M.D. from Columbia (1972) and an M.P.H. from Yale (1982).

William Karesh, D.V.M., is the executive vice president for Health and Policy for EcoHealth Alliance. He is also the president of the World Organisation for Animal Health (OIE) Working Group on Wildlife Diseases and chairs the International Union for the Conservation of Nature SSC Wildlife Health Specialist Group. From 2009, he has served as the technical director for the U.S. Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program. Mr. Karesh has pioneered initiatives focusing attention and resources on solving problems created by the interactions among wildlife, people, and their animals. He coined the term "One Health" and created the "One World–One Health" initiative to encourage linkages among public health, agriculture, and environmental health agencies and organizations around the world. He has lead programs and projects in more than 60 countries, covering terrain from Argentina to Zambia. Mr. Karesh is internationally recognized as an authority on the subject of animal and human health linkages and wildlife. He has published more than 160 scientific papers and numerous book chapters, and written for broader audience publications such as *Foreign Affairs* and *The Huffington Post*.

A. Marm Kilpatrick, Ph.D., is assistant professor in ecology and evolutionary biology at the University of California, Santa Cruz. He has authored more than 60 publications on many aspects of disease ecology including papers in *Science*, *Nature*, *PNAS*, *Lancet*, *PLoS Biology*, and *PLoS Pathogens*. His work focuses on the drivers of pathogen transmission, including land use, host community composition, climate, the spread of pathogens to new regions, and the effects of disease on animal populations.

Kenneth J. Linthicum, Ph.D., is presently the director of the Center for Medical, Agricultural and Veterinary Entomology, U.S. Department of Agriculture-Agricultural Research Service in Gainesville, Florida. He received his B.A., M.A., and Ph.D. degrees in zoology/biology from the University of California, Los

Angeles. He retired from the U.S. Army in 2001. Since 2004 he has directed a major Agricultural Research Service facility, consisting of four research units, employing 60 scientists and 150 support personnel. His scientific interests include vector and disease control, systematics, arbovirology, malaria, rickettsial diseases, and applications of geographic information systems and remote sensing to disease surveillance and epidemiology. His research findings have been published in 203 papers in the national and international scientific literature, and presented in more than 341 papers given at national and international scientific meetings. He was the recipient of the John I. Davidson Award for Practical Papers by the American Society for Photogrammetric Engineering and Remote Sensing, the 2010 Federal Laboratory Consortium Lab Director of Year award, a 2013 Finalist for the Samuel J Heyman Service to America Awards National Security and International Affairs Medal, and is president-elect of the American Mosquito Control Association.

Alan Magill, M.D., is director of the malaria program at The Bill & Melinda Gates Foundation in Seattle, Washington. Magill is board certified in internal medicine and infectious diseases. He is a professor of medicine at the University of Washington in Seattle, Washington, and has dual appointments as associate professor of medicine and associate professor of preventive medicine and biometrics at the Uniformed Services University of the Health Sciences in Bethesda, Maryland. His primary research interests have been in malaria and leishmaniasis. His focus has been on new product development in vaccines, drugs, and diagnostics. Previous positions include program manager (2009–2012) at the Defense Advanced Research Projects Agency where he developed and enabled a plant-based vaccine production capability. He retired after 27 years active duty service in the U.S. Army in 2010. He was formerly the director of the Division of Experimental Therapeutics and the science director at the Walter Reed Army Institute of Research in Washington, DC. Magill was previously the head of parasitology at the Naval Medical Research Center Detachment in Lima, Peru, and the head of clinical research for the Malaria Vaccine Development Unit of the U.S. National Institutes of Health. He is a faculty member for the Gorgas Course in Clinical Tropical Medicine in Lima, Peru, and a sought-after speaker on travel and tropical medicine-related topics. He participates in numerous national and international advisory committees and workshops. He is the current president of the American Society of Tropical Medicine and Hygiene and a past president of their Clinical Group and a past president of the International Society of Travel Medicine. He is the lead editor of the 9th edition of *Hunter's Tropical Medicine*, the premier clinical textbook of tropical medicine. He is also a medical editor of the *CDC Health Information for International Travel* (the yellow book) for 2010, 2012, 2014, and 2016. He has authored more than 75 peer-reviewed publications, 135 abstracts, and 13 book chapters. He is a master of the American College of Physicians, a fellow of the Infectious Diseases Society of America, and a fellow of the American Society of Tropical Medicine and Hygiene.

James Maguire, M.D., M.P.H., is professor of medicine at Harvard Medical School and senior physician at Brigham and Women's Hospital in Boston. He is an infectious disease specialist who has conducted epidemiological and clinical research on parasitic diseases, primarily Chagas disease in Brazil, leishmaniasis in Brazil and Bangladesh, and malaria in Latin America and Thailand. He was clinical director of the Division of Infectious Disease at Brigham and Women's Hospital and on the faculty of Harvard's Schools of Medicine and Public Health until 2001, the chief of the Parasitic Diseases Branch at CDC until 2005, and later head of the Division of International Health at the University of Maryland School of Medicine before returning to Boston in 2008.

Harold S. Margolis, M.D., is chief of the Dengue Branch at the Centers for Disease Control and Prevention (CDC) in San Juan, Puerto Rico. He is a graduate of the University of Arizona, College of Medicine, and completed a pediatric residency at the University of Colorado, Denver. In 1975, he joined CDC as an EIS officer and subsequently held several leadership positions, including director of the Division of Viral Hepatitis. In 2004, he became director of the Pediatric Dengue Vaccine Initiative (PDVI), a program located at the International Vaccine Institute in Seoul, Korea, and funded by The Bill & Melinda Gates Foundation. While at PDVI, the program advanced five dengue vaccines into clinical trials, evaluated the performance of commercially available dengue diagnostic tests, established potential vaccine trial sites, and established regional public health networks to support introduction of dengue vaccines. Margolis is a fellow of the American Academy of Pediatrics and Infectious Diseases Society of America. He is the author or coauthor of over 200 peer-reviewed publications.

Thomas P. Monath, M.D., is a consultant to the biotechnology industry. He is chief medical officer of Hookipa BioTech AG and chief technical officer of PaxVax Inc, where he is engaged in development of new vaccines. His expertise and experience cut across discovery research, process and analytical development, manufacturing, preclinical and clinical development, and regulatory affairs. Monath is also a venture partner at Kleiner Perkins Caufield & Byers and is a director of Sentinext plc, Rapid Micro Biosystems Inc, and US Biologics Inc. Between 1992 and 2012 he was adjunct professor, Harvard School of Public Health. Between 1992 and 2006, Monath was chief scientific officer and an executive director, Acambis Inc. (a publicly traded biopharmaceutical company recently acquired by Sanofi Pasteur) where he pioneered the development of ChimeriVax vaccines against dengue, West Nile, and Japanese encephalitis; vaccines against yellow fever, *Clostridium difficile*, and *Helicobacter pylori*; as well as a cell-based smallpox vaccine. Monath received his undergraduate degree and M.D. from Harvard and trained in internal medicine at the Peter Bent Brigham Hospital, Boston. Col. Monath retired from the U.S. Army in 1992 after 24 years in the uniformed services (Army and U.S. Public Health Service). Between 1973

and 1988, he was director, Division of Vector-Borne Viral Diseases, CDC, Fort Collins, Colorado, and from 1989 to 1992 chief, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases. He has worked overseas in Nigeria, Sierra Leone, Cameroun, Argentina, Ecuador, and elsewhere doing field research on arboviruses and hemorrhagic fevers. In 1972, he discovered the rodent reservoir of Lassa fever virus. He received the Nathanial A. Young Award (1984), the Richard M. Taylor Award (1996), and the Walter Reed Medal (2002) from the American Society of Tropical Medicine and Hygiene and was president of that society (2004–2005). From 1998 to 2000, Monath was senior science advisor to the director of the Central Intelligence Agency. He has been a leader in the One Health initiative.

Christopher Paddock, M.D., M.P.H.T.M., is a rickettsiologist and pathologist at the CDC in Atlanta, Georgia. Paddock received his B.S. and M.S. degrees in entomology at the University of California, Davis, in 1981 and 1986, respectively, and his M.D. and M.P.H.T. M. degrees at Tulane University in New Orleans, Louisiana, in 1990. He completed his residency in anatomic pathology and laboratory medicine at the University of California, San Francisco, in 1995. His employment with CDC began in 1996, as medical officer in the Viral and Rickettsial Zoonoses Branch, where he worked until taking a position as staff pathologist with the Infectious Disease Pathology Branch from 2003 to 2014. He now serves as the team lead for the Reference Diagnostics and Microbiology Activity in the Rickettsial Zoonoses Branch at CDC. He has authored or coauthored approximately 160 scientific publications and 20 book chapters. His research interests include clinical, diagnostic, and epidemiologic aspects of rickettsial diseases, primarily newly recognized spotted fever group rickettsioses.

Lyle R. Petersen, M.D., M.P.H., has served as the director of the Division of Vector-borne Diseases, Centers for Disease Control and Prevention, since 2004. Petersen began his training at the University of California, San Diego, where he received an undergraduate degree in biology. He then studied medicine at the University of California, San Francisco, where he was awarded a Regent's Scholarship. After medical school, Petersen completed his internship and residency in internal medicine at Stanford University. He then joined Tulane University's tropical medicine research efforts in Cali, Colombia before starting CDC's EIS applied epidemiology training program in 1985. After his EIS training at the Connecticut State Health Department, he joined the CDC's Division of HIV/AIDS where he worked until 1995. During that time, he completed CDC's Preventive Medicine Residency Program, received an M.P.H. degree from Emory University, and served in several posts, including chief of the HIV Seroepidemiology Branch. From 1996 to February 2000, Dr. Petersen guided Germany's efforts in creating a new national infectious disease epidemiology program at the Robert Koch Institute in Berlin. From 2000 to 2003, he served as

the deputy director for science of the Division of Vector-Borne Diseases. He is the author of more than 175 scientific publications. Dr. Petersen has been the recipient of several scientific awards including the Charles B. Shepard Science Award, the Alexander D. Langmuir Award, James H. Nakano Citation, and twice the HHS Secretary's Award for Distinguished Service. Dr. Petersen's current research focuses on the epidemiology of arboviral and bacterial vector-borne zoonoses.

Paul Reiter, Ph.D., has worked for his entire career on the natural history, biology, and control of mosquitoes and the epidemiology of the diseases they transmit. Dr. Reiter spent 22 years with the Centers for Disease Control and Prevention, including several years working on Saint Louis encephalitis in Memphis, Tennessee, 14 years on dengue in Puerto Rico, and 2 years on West Nile virus at the Harvard School of Public Health. He has participated in a number of epidemic investigations, including yellow fever, dengue, chikungunya, and Ebola hemorrhagic fever. In 2003, Dr. Reiter moved to the Institute Pasteur, Paris, to launch a new unit of Insects and Infectious Diseases. His research remains field-orientated with special attention to West Nile virus and chikungunya in Europe. He has also been a lead player in the debate on global warming and vector-borne disease and his two decades of efforts as a "skeptic" have been exonerated in the latest Assessment Report of the Intergovernmental Panel on Climate Change.

Rebecca R. Rico-Hesse, Ph.D., M.P.H., is a professor in the Department of Molecular Virology and Microbiology, in the section of Pediatric Tropical Medicine, and in the National School of Tropical Medicine at Baylor College of Medicine, Houston, Texas. Prior to this, she was a scientist at the Texas Biomedical Research Institute, San Antonio, and assistant/associate professor at Yale University School of Medicine. She received her doctoral degree from Cornell University in 1985 and trained as a Postdoctoral Fellow at the CDC. After being raised in a small city in northern Mexico, she was inspired to become a virologist after seeing the impact of rabies virus on animals and humans in the area and the extensive effects of an epidemic of Venezuelan equine encephalitis virus on equids in Mexico and Texas. She did her theses research on equine encephalitis viruses, and worked on these, dengue, and other viral hemorrhagic fever viruses at the Yale Arbovirus Research Unit and in the BSL4 laboratory at TBRI. Her current research focuses on dengue virus transmission and pathogenesis in a mouse model of disease that mimics human infection, in "humanized" mice that can be infected by mosquito bites.

Jan C. Semenza, Ph.D., M.P.H., is the head of the Health Determinants Programme at the European Centre for Disease Prevention and Control, where he directs the work on environmental and social determinants of infectious diseases. He is particularly interested in early warning systems for emerging infectious disease threats. He was an epidemic intelligence service officer at the CDC in

1995, when he led the CDC response to the heat wave in Chicago for which he received a Certificate of Commendation. As part of his work with the regional offices of World Health Organization (WHO) including EURO, PAHO, and EMRO, he provided technical and scientific advice to the countries within their region, particularly on polio and measles eradication. He conducted public health projects in Uzbekistan, Sudan, Egypt, Denmark, Brazil, and Haiti through CDC, WHO, USAID, and nongovernmental organizations. Semenza was a faculty member at the University of California (UC), Berkeley, UC Irvine, Oregon Health and Science University, and at Portland State University where he taught in the Oregon Masters Program of Public Health. His research has been published in high-impact journals such as *Cell*, *New England Journal of Medicine*, *Lancet ID*, *Science*, *Nature Climate Change*, and in several books.

Susan Stramer, Ph.D., is the executive scientific officer at American Red Cross (ARC) and assistant laboratory director, National Testing Laboratories. Prior to joining ARC, Dr. Stramer worked for the Diagnostics Division of Abbott Laboratories. She also was a principal investigator for the ARC investigational new drug application for nucleic acid amplification testing and numerous other studies related to infectious disease testing. Dr. Stramer was the president of AABB in 2012–2013 and previously chaired or served on numerous committees of the AABB and serves on the editorial board of the journal *Transfusion*. She serves on advisory committees for blood centers internationally and diagnostic test kit manufacturers. She received numerous American Red Cross awards including the President's Award. Along with collaborators, she also received the Centers for Disease Control and Prevention's Charles C. Shepard Science Award, and was nominated twice more for the same award. She also received the Herbert Perkins Scientific Lecture Award. Dr. Stramer has authored or coauthored more than 250 peer-reviewed articles and abstracts. She received her B.S. and M.S. degrees in biological sciences from Northern Illinois University and her doctorate in bacteriology from the University of Wisconsin-Madison. Dr. Stramer also was a postdoctoral research fellow at the hepatitis branch, CDC.

Matt Thomas, Ph.D., obtained his Ph.D. at the University of Southampton in the United Kingdom. From 1991 to end of 2002 he worked as a postdoc and then research fellow at the Centre for Population Biology at Silwood Park, Imperial College London. He then took up a position as a senior lecturer and then reader in Population Biology and Biological Control at Imperial College. At the end of 2005 he joined CSIRO Entomology in Australia as a senior principal research scientist. In 2008 he moved to the United States as professor of entomology at Pennsylvania State University. He has interests in various aspects of the ecology and evolution of pests and diseases, with practical experience in a range of systems in both temperate and tropical settings. His current research focuses on the ecology and control of mosquito vectors.

Anna E. Whitfield, Ph.D., is an associate professor in the Department of Plant Pathology at Kansas State University (KSU). Her research emphasis is the biology of plant–virus–vector interactions, and the long-term goal of her research is to develop biologically based strategies for controlling viruses and arthropod vectors in agricultural croplands and greenhouses. She specializes in negative-sense RNA viruses that are transmitted in a propagative manner by arthropod vectors. Her research aims are to (1) identify insect genes that are important for virus infection of the arthropod vectors using a functional genomics-based approach, (2) develop a better understanding of virus entry and the role of viral glycoproteins in this process, and (3) characterize ecological plant–virus–vector interactions at the molecular and field level. Recent work led by Dr. Whitfield has focused on expression of viral glycoproteins in plants as a method to prevent virus transmission. Other work focuses on using RNA interference (RNAi) as a control strategy and a functional genomics tool for arthropod vectors of plant pathogens. Dr. Whitfield was awarded an NSF-CAREER grant to study the molecular mechanisms of *Rhabdovirus*–vector interactions. She teaches graduate courses in plant virology and plant–virus–vector interactions, and in 2014, she was awarded the KSU College of Agriculture Award for Excellence in Graduate Teaching.