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EMERGING INFECTIOUS DISEASES

Preliminary Observations on the Zika Virus Outbreak

Statement of Timothy M. Persons, Chief Scientist

Accessible Version

March 2016

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PRELIMINARY OBSERVATIONS ON THE ZIKA VIRUS OUTBREAK

GAO Highlights

Highlights of GAO-16-470T, a testimony before the Subcommittee on Oversight and Investigations, Committee on Energy and Commerce, House of Representatives.

Why GAO Did This Study

Emerging infectious diseases constitute a clear and persistent threat to the health and well-being of people and animals around the world. The Zika virus, which at present appears to be primarily transmitted to humans by infected mosquitos, can cause symptoms including fever, rash, and joint pain. A large ongoing outbreak is occurring in Brazil that started in May 2015. As of February 24, 2016, over 100 cases of U.S. travel-associated Zika virus disease cases have been reported.

Due to concerns about its potential impact, you asked GAO to present preliminary observations on the Zika virus. This statement addresses (1) the epidemiology and transmission of the Zika virus disease, including reporting on the incidence of disease and what is known about its link to microcephaly; (2) detection and testing methods; (3) methods for mosquito control; and (4) the proposed federal research agenda as it relates to the Zika virus and Zika virus disease.

To report on these questions, GAO reviewed relevant peer-reviewed scientific literature, epidemiological alerts, agency documents, and prior GAO work from 2003-2016 on related topics; consulted experts in the fields of virology, infectious diseases, and vector control, including industry representatives; and interviewed officials of the CDC and NIH.

What GAO Recommends

GAO is not making recommendations at this time.

View GAO-16-470T. For more information, contact Timothy Persons at (202) 512- 6412 or personst@gao.gov

What GAO Found

While several countries have reported outbreaks of Zika virus disease—which appear to be primarily transmitted to humans by mosquitos—unanswered questions remain regarding the epidemiology and transmission of the disease. Many factors—including a large number of asymptomatic patients and patients with mild symptoms, and a lack of a consistent international case definition of Zika virus disease—complicate understanding of the virus and may hinder responses to the current outbreak. For example, an estimated 80 percent of individuals infected with the Zika virus may not manifest clinical symptoms. As a result, incidence of the infection may be underestimated. Questions also remain regarding the strength of the association between Zika virus infection and two other conditions: microcephaly and Guillain-Barré syndrome.

A lack of validated diagnostic tests, consistent international case definitions, and trend information may also contribute to difficulty in estimating the prevalence of the virus. The United States uses two diagnostic tests for Zika, and according to the U.S. Centers for Disease Control and Prevention (CDC), while there are no commercially-available diagnostic tests for Zika, an antibody-based test for Zika virus was recently authorized for Emergency Use by the U.S. Food and Drug Administration. Diagnosing Zika virus infection is also complicated because it is difficult to differentiate it from other similar diseases, such as dengue or yellow fever. For example, a person previously infected with dengue could be falsely identified as also having been exposed to the Zika virus (and vice-versa). Moreover, the World Health Organization has acknowledged the need for a consistent case definition—that is, a set of uniform criteria to define the disease for public health surveillance and to determine who is included in the count and who is excluded. Additionally, a lack of pattern and trend data has made surveillance challenging.

Because Zika virus disease cannot yet be prevented by drugs or vaccines, vector (mosquito) control remains a critical factor in preventing and mitigating the occurrence of this disease. There are three methods for mosquito control: (1) standing water treatment, (2) insecticides, and (3) emerging technologies. Mosquito control has been achieved in some locations by methods such as reducing or chemically-treating water sources where mosquitoes breed or mature, or by insecticide dispersal. Emerging technologies, including biological control methods—such as infecting mosquitoes with bacteria— genetically-modified mosquitoes, and auto-dissemination traps, show some promise but are still in development and testing phases.

The National Institutes of Health (NIH) and the CDC have identified several high priority areas of research. Research priorities include basic research to understand viral replication, pathogenesis, and transmission, as well as the biology of the mosquito vectors; potential interactions with co-infections such as dengue and yellow fever viruses; linkages between Zika and the birth defect microcephaly; improving diagnostic tests; vaccine development; and novel vector control methods. These efforts are ambitious, and agencies may face challenges in implementing this agenda.

Chairman Murphy, Ranking Member DeGette, and Members of the Subcommittee:

I am pleased to be here today to discuss our preliminary observations on Zika virus disease. Zika virus—which at present appears to be primarily transmitted to humans by infected mosquitoes—can cause symptoms including fever, rash, conjunctivitis (red eyes), and joint and muscle pain. Although a possible link between Zika virus disease and microcephaly in newborn babies, as well as a link with Guillain-Barré syndrome in infected adults has been hypothesized, to date, the link has not been firmly established.[1] The Government Accountability Office (GAO) has issued several reports dealing with preparedness for, detection of, and response to emerging infectious diseases domestically and internationally, as well as pandemics. (See appendix 1 for a listing of these reports).

Emerging infectious diseases represent an ongoing threat to the health and livelihoods of people and animals worldwide.[2] Many advances in medical research and treatments have been made during the last century, but infectious diseases are nevertheless a leading cause of death worldwide. In addition to causing nearly one in five human deaths worldwide, infectious diseases impose a heavy societal and economic burden on individuals, families, communities, and countries.[3] Infectious diseases are a continuous threat for reasons that include: (1) emergence—at times rapid—of new infectious diseases; (2) re-emergence of previously-known infectious diseases; and (3) persistence of intractable infectious diseases. According to the National Institutes of Health (NIH), infectious diseases continue to emerge and evolve. Changes in human demographics, behavior and land use—among other factors—bring people into closer and more frequent contact with pathogens and are contributing to

[1] Microcephaly is a medical condition in which the circumference of the head is smaller than normal because the brain has not developed properly or has stopped growing. Guillain-Barré syndrome is a disorder in which the body's immune system attacks part of the peripheral nervous system, which can cause muscle weakness, and sometimes, paralysis.

[2] Emerging infectious diseases are infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range. See S. S. Morse, "Factors in the Emergence of Infectious Diseases," *Emerging Infectious Diseases*, vol. 1, no. 1, (1995).

[3] Institute of Medicine, *Emerging Viral Diseases: The One Health Connection* (Washington, D.C.: National Academies Press, 2015).

infectious disease emergence. This may involve exposure to animal or insect carriers of disease. There is also an increase in the opportunities for pathogens to jump between animal and human reservoirs. In addition to Zika, other examples of emerging infectious diseases include: Ebola virus disease, severe acute respiratory syndrome (SARS), influenza, dengue, and chikungunya, among others.

Due to concerns about the potential impact of Zika virus disease, you asked us to present our preliminary observations on several issues associated with Zika virus disease.[4] This statement addresses issues, if any, associated with (1) the epidemiology and transmission of the Zika virus disease, including reporting on the incidence of disease and what is known about its link to microcephaly; (2) detection and testing methods; (3) methods for mosquito control; and (4) the proposed federal research agenda as it relates to the Zika virus and Zika virus disease.

To report on these questions, we reviewed relevant peer-reviewed scientific literature, epidemiological alerts, agency documents, and prior GAO work from 2003-2016 on related topics such as disease surveillance and emerging infectious diseases; consulted experts in the fields of virology, infectious diseases, and vector (mosquito) control, including industry representatives; and interviewed officials of the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH).

The work upon which this testimony is based was conducted from February through March 2016 in accordance with generally accepted government auditing standards. Those standards require that we plan and perform the audit to obtain sufficient, appropriate evidence to provide a reasonable basis for our findings and conclusions based on our audit objectives. We believe that the evidence obtained provides a reasonable basis for our findings and conclusions based on our audit objectives.

[4] Epidemiology is the study of the distribution and determinants of diseases and injuries in human populations.

Background

Zika virus is a member of the flavivirus family related to dengue virus, yellow fever virus, and West Nile virus, and its primary mode of transmission is via mosquito, most notably by the *Aedes aegypti* mosquito.[5] *Aedes albopictus* mosquitoes are a potential vector for the Zika virus. The disease was first identified in the Zika Forest in Africa in the 1940s, from which it subsequently moved eastwards over the following decades, though the Pacific Islands until it reached Brazil, where the population had no indigenous immunity against the virus. According to CDC officials with whom we spoke, the United States also has no indigenous immunity to the Zika virus.

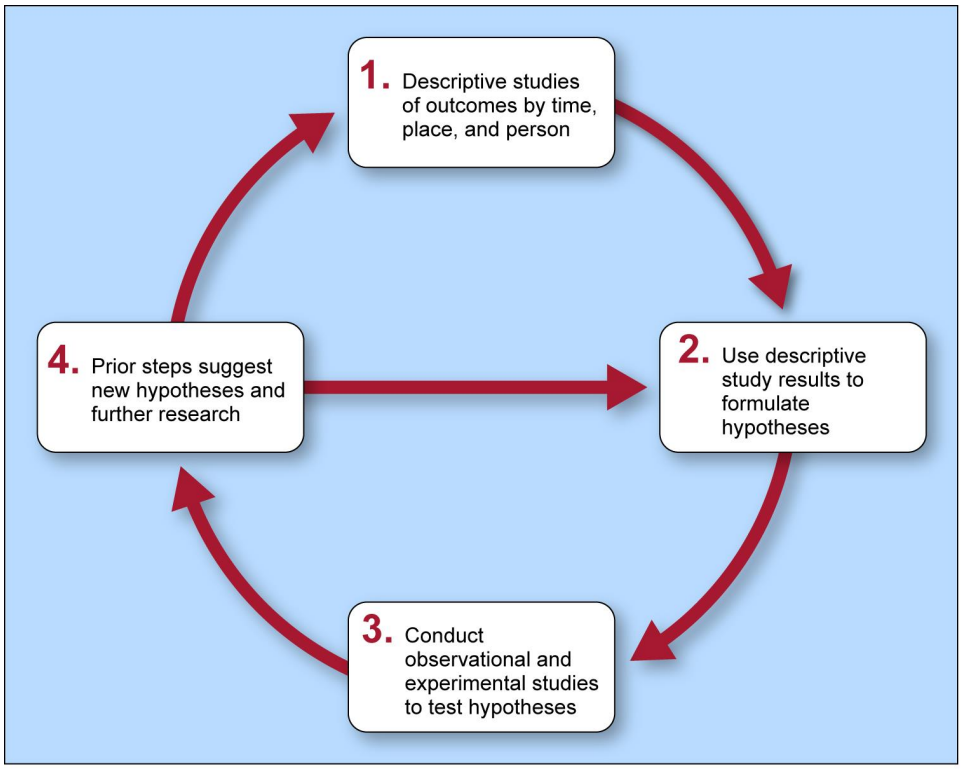
A major factor contributing to the declaration of Zika virus disease as a Public Health Emergency of International Concern by the World Health Organization (WHO) is a possible link between Zika virus and microcephaly as well as Guillain-Barré syndrome. Microcephaly—an abnormally small head due to failure of brain growth—is a concern because children with microcephaly can experience impaired cognitive development, delayed motor function and speech, seizures, and reduced life expectancy.

To better understand the spread of this disease worldwide, epidemiological studies are required. Epidemiology is concerned with the patterns of disease occurrence in human populations and of the factors that influence these patterns. The goals of epidemiologic study, and more specifically outbreak investigations, are to determine the extent and distribution of the disease in the population, the causes and factors associated with the disease and its modes of transmission, the natural history of disease, and the basis for developing preventive strategies or interventions[6] (see figure 1).

[5] Flaviviruses are positive, single-stranded, enveloped RNA viruses found in arthropods, (primarily ticks and mosquitoes), and can occasionally infect humans and other vertebrates.

[6] D.E. Lilienfeld and D. Schneider, *Lilienfeld's Foundations of Epidemiology*, 4th ed. (New York, NY: Oxford University Press, 2015).

Figure 1: Epidemiological Study Cycles



Source: GAO, based on concepts regarding the steps in conducting epidemiological studies drawn from Lilienfeld, 2015.
GAO-16-470T

U.S. Role in Global Disease Surveillance

The United States has played a significant role in improving global disease surveillance and response capacity. In the mid-1990s, recognizing the threat posed by previously unknown infectious diseases, the United States and other countries initiated a broader effort to ensure that countries can detect disease outbreaks that may constitute a Public Health Emergency of International Concern. The United States has participated in the WHO's efforts to develop and implement the International Health Regulations, currently an agreement among 196 countries, to develop and maintain global capabilities to detect and respond to disease and public health threats. The CDC has helped to define and establish the International Health Regulations and has been designated by the WHO as a key partner in helping to implement the critical capacities for detecting and responding to emerging infectious disease outbreaks.

The recent Ebola outbreak in West Africa has highlighted the importance of further improving the U.S. government's global disease surveillance efforts. To help ensure that such threats are addressed early and at their source, the "National Health Security Strategy and Implementation Plan 2015-2018" released by the U.S. Department of Health and Human Services prioritizes efforts to strengthen national capacities and capabilities globally to detect disease in a timely manner, prevent the global spread of public health threats and diseases, and respond to public health emergencies.[7] For example, CDC's Global Disease Detection and Field Epidemiology Training programs aim to strengthen laboratory systems for the rapid detection and control of emerging infectious diseases and train epidemiologists to effectively detect, investigate, and respond to health threats.

The Epidemiology and Transmission of the Zika Virus are Not Fully Understood and Accurate Information on the Incidence is Lacking

While several countries have reported outbreaks of Zika virus disease, unanswered questions remain regarding the epidemiology and the transmission of the disease. Many factors, including a large number of asymptomatic patients, mild symptoms, a lack of a consistent international case definition of Zika virus disease, as well as of microcephaly, and a lack of validated diagnostic tests complicate our understanding of the virus and may hinder our response to the current outbreak. Questions also remain regarding the strength of the association between Zika virus infection and microcephaly or Guillain-Barré syndrome.

Several Countries Have Reported Outbreak of Zika Virus Disease

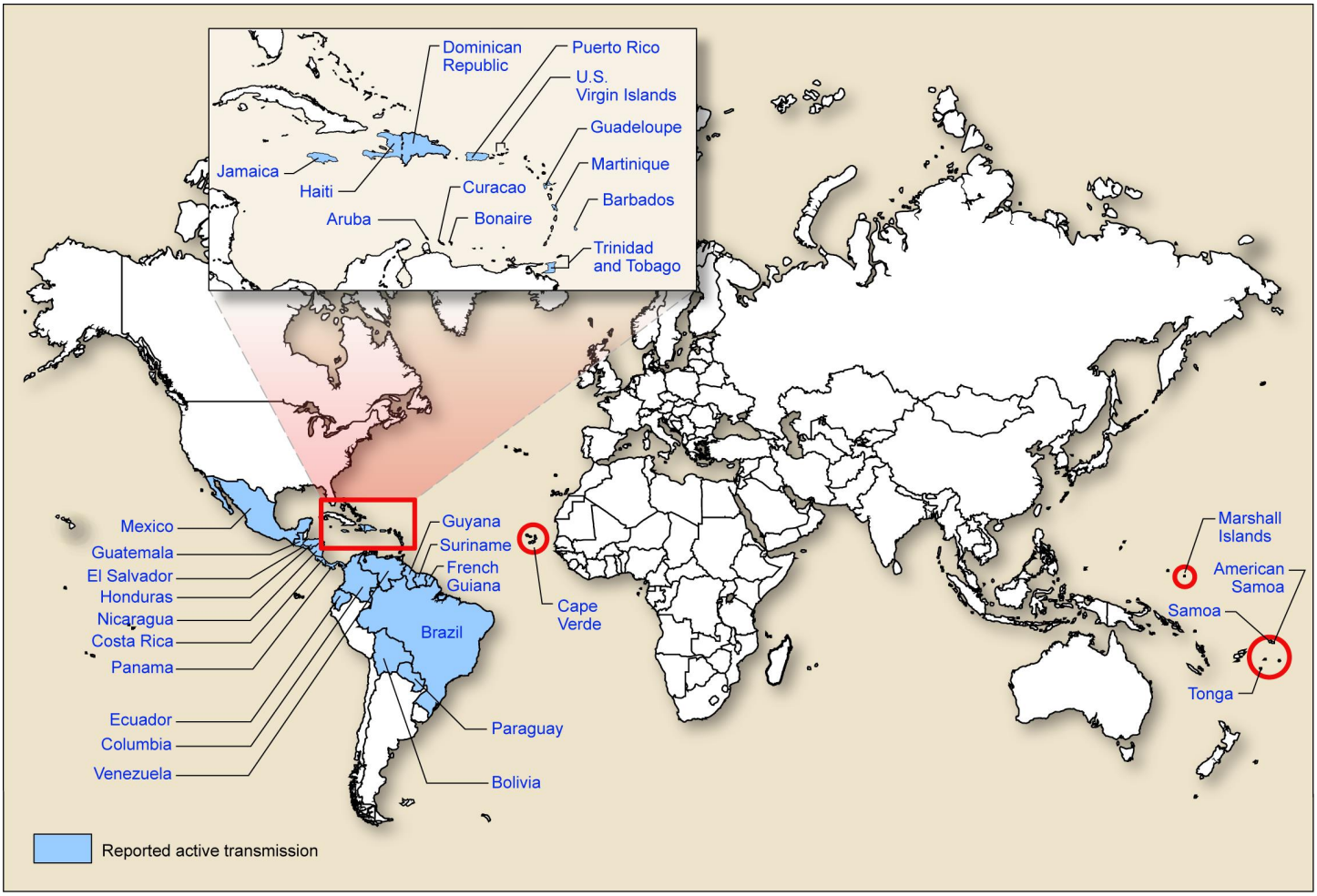
Since the 1960s, the Zika virus has been known to occur within a narrow equatorial belt from Africa to Asia. In 2007, the virus was detected in Yap Island, the first report that the virus spread outside of Africa and Indonesia to Pacific Islands.[8] In 2014, the virus spread east across the Pacific Ocean to French Polynesia, then to Easter Island. According to the WHO, the virus has continued to spread to the Americas, with the outbreak in Brazil that began in May 2015 and is ongoing. Zika has spread to Mexico, Central

[7] Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response, National Health Security Strategy and Implementation Plan for 2015-2018 (Washington, D.C.: 2015).

[8] R. S. Lanciotti, et al., "Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007," *Emerging Infectious Diseases*, vol. 14, no. 8 (2009).

America, the Caribbean, and South America, where the outbreak has reached epidemic levels (see figure 2). Recent outbreaks have also been reported in Puerto Rico, as well as the Cape Verde Islands.

Figure 2: All Countries and Territories with Active Zika Virus Transmission



Sources: U.S. Centers for Disease Control and Prevention (CDC), 2016; MapResources (map). | GAO-16-470T

Note: This map depicts local mosquito-borne transmission – infections originating in the place where they are found. This is different from travel-associated infections which originate in one place, and are found in another.

A Number of U.S. Travelers Have Been Infected with the Zika Virus

According to CDC documentation, Zika virus disease is now a nationally notifiable disease.[9] As of February 24, 2016, 107 cases of continental U.S. travel-associated Zika virus disease have been reported, according to CDC. Although CDC documentation states that Zika virus has not yet seen local mosquito-borne spread in the continental United States, some states have mosquito species potentially capable of transmitting the virus.

Incidences of mosquito-borne transmission have been reported in the Commonwealth of Puerto Rico, the U.S. Virgin Islands, and American Samoa. The first locally-acquired case of Zika virus disease in Puerto Rico was reported in December of 2015. Through late January of 2016, about 30 additional laboratory-confirmed cases were identified in Puerto Rico, including one pregnant woman. In January of 2016, the CDC issued travel guidance for travel to affected countries, including the use of enhanced precautions for all travelers, as well as the recommendation that pregnant women postpone travel to affected areas.

Questions Remain about the Transmission of the Zika Virus

According to CDC documentation, there are a few known routes of transmission of the Zika virus to and among people. These include mosquitoes, mother to child, sexual contact and blood transfusions. The Zika virus is transmitted to people primarily through the bite of infected *Aedes* species mosquitoes (primarily *Aedes aegypti* and possibly *Aedes albopictus*). These are the same mosquitoes that spread dengue and chikungunya viruses. These mosquitoes typically lay eggs in and near standing water in containers like buckets, bowls, animal dishes, flower pots, and vases. They prefer to bite people, and live both indoors and outdoors. Mosquitoes that spread dengue, chikungunya, and Zika are aggressive daytime biters, but also bite at night. Mosquitoes can become infected when they feed on a person already infected with the virus.

According to the CDC, the Zika virus is rarely transmitted from an infected mother to child, and there have been no reports of infants contracting the Zika virus through breastfeeding. It is possible but rare that an infected mother would pass the virus to a newborn at delivery. However, an infected mother can pass the Zika virus to her fetus during pregnancy.

[9]The Nationally Notifiable Diseases Surveillance System is a nationwide collaboration that enables public health at the local, state, territorial, federal, and international levels to share notifiable disease-related health information and uses this information to monitor, control, and prevent the occurrence and spread of those diseases.

According to the CDC, it is possible for the Zika virus to be spread by a man to his sexual partners. A few recent cases of Zika virus transmission were reported through sexual contact. In December 2013, during a Zika virus outbreak in French Polynesia, Zika was isolated from the semen of a patient.[10] In one known case of likely sexual transmission, the virus was spread before symptoms developed. The virus appears to be present in semen longer than in blood. Sexual transmission of the disease—acquired outside of the United States—has been reported in the United States.[11] As of February 23, 2016, the CDC and state public health departments are investigating 14 additional reports of possible sexual transmission of the virus, including several involving pregnant women.

Zika virus can also be transmitted through blood transfusion, according to U.S. Food and Drug Administration (FDA) documents. While there have been no reports to date of Zika virus entering the U.S. blood supply, the risk of blood transmission is considered high based on the most current scientific research of how Zika virus and similar viruses (i.e. flaviviruses) are spread, as well as recent reports of transfusion-associated infection outside of the United States, according to the FDA.[12] CDC reports that there have been reports of possible blood transfusion transmission cases in Brazil. During the French Polynesian outbreak in 2013, 2.8 percent of blood donors tested positive for Zika. The maximum time the virus remains in the bloodstream is unknown, but scientists estimate that it is less than 28 days. On February 16, 2016, as a safety measure against the emerging Zika virus outbreak, the FDA issued new guidance recommending that blood donors be deferred for four weeks if they have been to areas with active Zika virus transmission, potentially have been exposed to the virus, or have had a confirmed Zika virus infection.

While scientific studies have identified Zika viral components in saliva and urine, they did not report disease transmission from those bodily fluids.

[10] D. Musso et al., "Potential Sexual Transmission of Zika Virus," *Emerging Infectious Diseases*, vol. 21, no. 2 (2015).

[11] Zika virus components have been detected in semen for up to 62 days post initial infection, but the duration of viable Zika virus transmission via semen is not known.

[12] U.S. Food and Drug Administration, "FDA Issues Recommendations to Reduce the Risk for Zika Virus Blood Transmissions in the United States," Released February 16, 2016, Accessed February 25, 2016, http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm486359.htm

Questions Remain Regarding the Link between Zika Virus Infection and Neurological and Auto-Immune Disorders

It is not currently known if infection with the Zika virus causes, facilitates, or is otherwise associated with the development of certain neurologic and auto-immune conditions. Although strongly suspected, a report suggests the causal relation between in-utero exposure to Zika and microcephaly is yet to be established.[13] Scientific literature has identified several possible linkages, including the presence of Zika virus in fetal brain tissue, as well as evidence of the virus crossing the placental barrier, suggesting a causal effect is plausible, but not yet proven. For example, a fetal autopsy identified an abnormally small brain, as well physical markers of developmental delays, along with the Zika virus in the brain. The mother in the case reported an illness with a fever and rash at the end of the first trimester of pregnancy while she was living in Brazil.[14]

A retrospective analysis was reported to the WHO in 2015-2016 of a previous outbreak of Zika virus disease in 2013-2014 in French Polynesia, also established elevated numbers of neurological disorders for that outbreak.

The potential association of Guillain-Barré syndrome and Zika virus disease was suspected prior to the recent Brazilian Zika disease outbreak. According to the European Center for Disease Control, the 2013 to 2014 French Polynesian outbreak of Zika virus disease was reportedly the largest documented outbreak at that time. According to the European Center for Disease Control, over 8,000 suspected cases of Zika Virus infection had been reported by February 2014.[15] Notably, there were nearly 40 cases of Guillain-Barré syndrome reported, with all cases following disease episodes compatible with Zika virus infection. Historically, there had been 10 or fewer cases of Guillain-Barré syndrome in Polynesia annually. The European Center for Disease Control stated that further investigations could be conducted to establish the relationship between neurological and auto-immune complications and Zika virus infection.

[13] C. G. Victoria, “Microcephaly in Brazil: how to interpret reported numbers?” *The Lancet*, vol. 387, no. 10019, (2016).

[14] J. Mlakar, et al., “Zika Virus Associated with Microcephaly,” *New England Journal of Medicine*, (2016).

[15] European Centre for Disease Prevention and Control. *Rapid risk assessment: Zika virus infection outbreak, French Polynesia.* (Stockholm: February 2014).

Mild Symptoms and Asymptomatic Cases May Lead to Underestimation

Researchers have reported that an estimated 80 percent of the individuals infected with the Zika virus are asymptomatic, that is, they have the virus but do not manifest clinical symptoms. Since diagnosis of suspected Zika virus disease is often based on clinical symptoms, and in light of the fact that clinical symptoms are usually non-existent or mild, experts told us that many individuals who are infected with the Zika virus may not seek medical care, and thus are not counted as a case, resulting in significant underestimation of the true incidence of infection.

Lack of Consistent International Case Definitions May Lead to Inaccurate Count

An accurate count of the number of Zika virus disease cases requires a consistent case definition, or set of uniform criteria to define the disease for public health surveillance and to determine who is included in the count and who is excluded. However, establishing a definition is problematic for Zika virus disease. If Zika cases are diagnosed based on serology data, then the incidence count may include people who have been infected with the virus, but do not show clinical symptoms.[16] On the other hand, if cases are defined by clinical symptoms only, with no serology testing, then the incidence could be higher or lower than that count obtained from serology testing only, because people who present with clinical symptoms may or may not actually test positive for Zika virus.

According to the WHO Zika Response Strategy, there is currently a need to establish a uniform case definition for Zika virus disease, as well as historical rates, or baselines, for associated conditions. The Council of State and Territorial Epidemiologists currently has a case definition for Zika virus disease—under arboviral diseases—with two tiers: a "probable case" definition based on clinical signs and symptoms as well as the presence of certain anti-Zika antibodies, and a "confirmed case" definition for laboratory-confirmed cases based on laboratory analysis.[17] However, because other countries may be using different testing protocols, it is unclear whether their results would be consistent with the CDC case definitions, complicating epidemiological analysis. Engaging international cooperation to establish uniform case definitions and baselines for diagnosing Zika virus disease and microcephaly can facilitate discovery of modes of

[16] Serology refers to the diagnostic identification of antibodies in the serum of blood.

[17] The Council of State and Territorial Epidemiologists is an organization of member states and territories representing public health epidemiologists. Every year, their position statements are used to update the case definitions of nationally notifiable diseases, and the organization encourages state health departments to report cases of these selected diseases to CDC's National Notifiable Diseases Surveillance System.

transmission and causal links between Zika virus disease and microcephaly or Guillain-Barré syndrome.

Lack of a Case Definition of Microcephaly May Complicate Linkage with Zika Virus Disease

When the WHO declared a Public Health Emergency of International Concern on February 1, 2016, it acknowledged that there was no international standard surveillance case definition for microcephaly.[18] Problems with changing case definitions, lack of sufficient information on underlying causes and brain pathology, and lack of baseline data make it difficult to accurately determine the level of increase of microcephaly in Brazil, and how much is due to the Zika virus.[19]

Some researchers offered several possible explanations for the observed increase in microcephaly cases, other than actual increase of cases as a result of Zika virus infection. First, because of the recent attention, newborn babies with visible cranial deformities are likely to be fast-tracked for in-depth examination. This temporal increase in suspected cases of microcephaly could also be distorted given both raised awareness with more children than usual being measured and reported, and the changing definition of microcephaly over time. Although there is evidence of an increased number of cases of microcephaly in Brazil, these authors demonstrated that the number of suspected cases relied on a screening test that had very low specificity and therefore overestimated the actual number of cases.

[18] In a comment published in The Lancet, Victora, C.G. et al., reported that "Before 2015, the annual numbers of reported cases of microcephaly in Brazil were consistently below 200. Between mid-2015 and Jan 30, 2016, 4783 suspected cases of microcephaly were reported, including newborn and fetal losses. Of these, 1103 cases have completed clinical, laboratory, and imaging examinations, and 404 (36.2%) were classified as confirmed cases of microcephaly. Among the confirmed cases, brain abnormalities were detected by imaging in 387 babies and Zika virus was detected in 17 babies, including in two fetal losses. The remaining 709 cases were discarded and 3670 suspected cases of microcephaly remain under investigation."

[19] D. L. Heymann, et al., "Zika virus and microcephaly: why is this situation a PHEIC?" *The Lancet*, vol. 387, no. 10020, (2016).

Lack of Validated Diagnostics Tests May Mask the Incidence of Zika Virus Disease

According to the CDC, there are currently two Zika diagnostic tests available in the United States: reverse transcription polymerase chain reaction (RT-PCR) and Immunoglobulin M (IgM) followed by the Plaque Reduction Neutralization Test (PRNT) test.[20] The current RT-PCR test can detect infection only during the period of illness when the virus is present. According to an NIH official, the PRNT diagnostic test is the most specific for antibody detection,[21] but is cumbersome and not suitable for screening a large number of individuals.

When detecting antibodies, diagnosing cases of Zika virus disease and differentiating it from other diseases caused by other flaviviruses, such as dengue or yellow fever, is difficult if someone has been infected by another flavivirus. Some tests for Zika virus antibodies suffer from cross-reactivity with antibodies to similar viruses, such as from dengue virus disease, meaning that tests using these antibodies for detection are not specific for the Zika virus. For example, a person previously infected with another flavivirus such as dengue could be falsely identified as also having been exposed to the Zika virus (and vice-versa). In addition, new outbreaks like Zika may not have known patterns or trends, making effective surveillance challenging. For example, after the onset of illness, Zika virus remains in the blood for about 5-7 days, according to the CDC. After this period, called viremia, diagnosis of Zika virus disease at this time relies on detection of antibodies against the Zika virus. Since antibodies in the blood may persist longer than the virus, a positive result for antibodies against the Zika virus indicates only that the patient was previously exposed to the Zika virus. Thus, the window for detecting the

[20] Reverse transcription polymerase chain reaction (RT-PCR) is a technique to detect or quantify RNA, according to a scientific journal. It uses a reverse transcriptase enzyme to convert RNA to DNA, followed by PCR to amplify the DNA. An immunoglobulin test measures the level of certain immunoglobulins, or antibodies, in the blood. CDC defines antibodies as proteins found in the blood that are produced in response to foreign substances (e.g. bacteria or viruses) invading the body. Antibodies protect the body from disease by binding to these organisms and destroying them.

The Plaque Reduction and Neutralization Test (PRNT) measures the amount of the neutralizing antibodies in the serum of the infected individual and determines the level of protective antibodies this individual has towards the infecting virus. This test is based on how viruses and antibodies interact, resulting in inactivation of virus – when the correct antibody is present - such that it is no longer able to infect and replicate in living cells.

[21] Specificity is the percentage of persons who do not have a given condition who are identified by the assay as negative for the condition.

actual virus is small. It is not clear whether an antibody test could determine how long ago the patient was exposed.

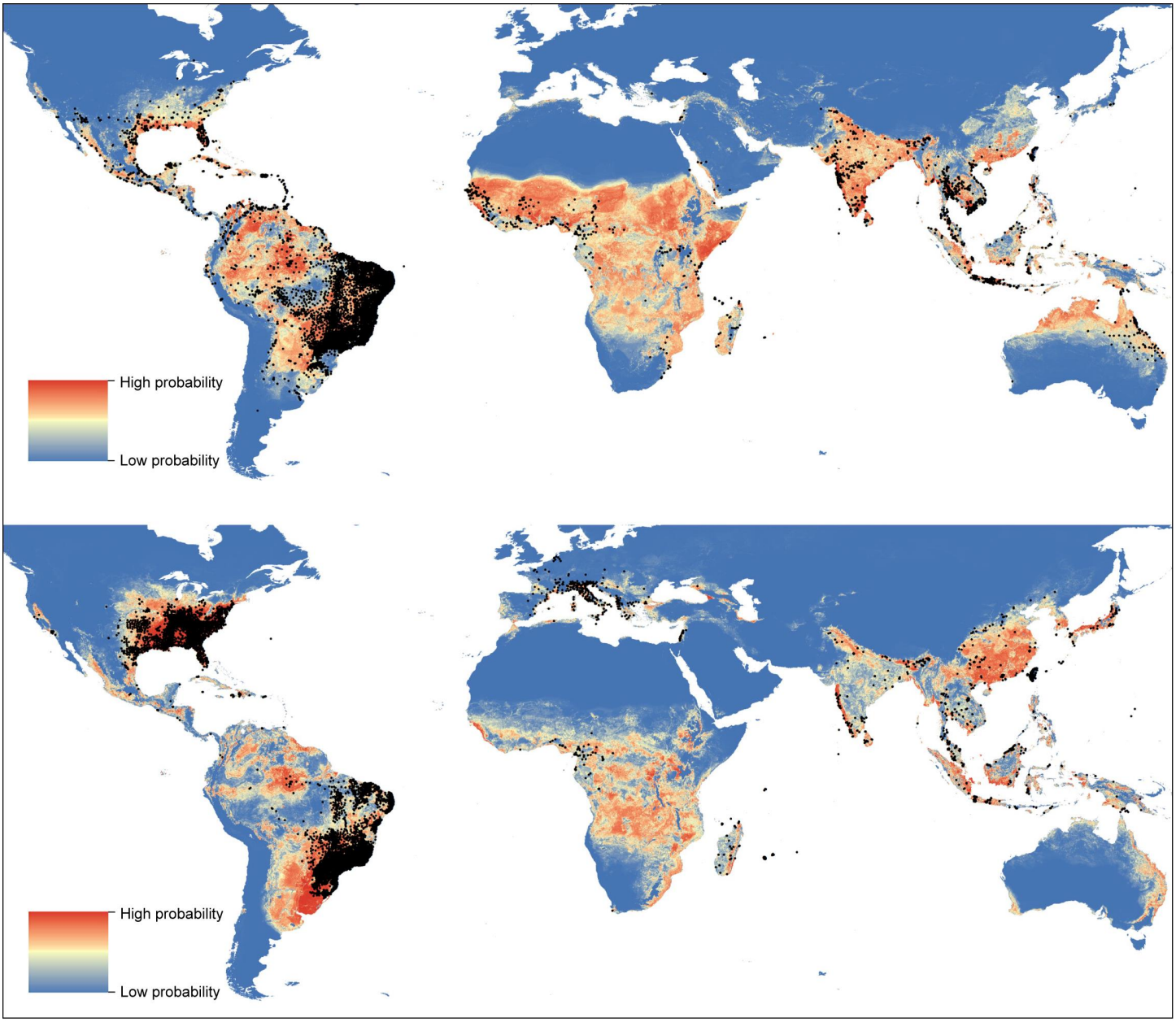
According to the CDC, while there are no commercially-available diagnostic tests for Zika, an antibody-based test for the Zika virus (Zika MAC-ELISA) was recently authorized for Emergency Use by the FDA. One of the main limitations of this test, among others, is its inability to differentiate between infection with Zika and other closely related flaviviruses such as dengue. This test in its current form may confuse the practitioners because of its lack of specificity. Since closely related flaviviruses such as dengue may also be present in Zika outbreak countries, the utilization of this assay could wrongly identify non-Zika-virus associated infections, thus putting extra burden on the laboratory and health care systems, and distort the epidemiological analyses.

Adding to the limitations of these diagnostic systems are limited numbers of facilities able to perform definitive confirmatory testing, particularly in the developing world. The WHO is undertaking an analysis of diagnostics under development, developing target product profiles, facilitating the preparation and characterization of reference reagents, and setting up an Emergency Use Assessment and Listing mechanism for priority Zika diagnostics.

Various Methods of Mosquito Control Exist and Novel Approaches Are Underway

Because Zika virus disease cannot yet be prevented by drugs or vaccines, vector (mosquito) control remains a critical factor in mitigating risks associated with this disease. The *Aedes aegypti* and *Aedes albopictus* mosquitoes are present around the world, as well as in the United States. Figure 3 shows a predicted distribution and intensity of the Aedes aegypti and Aedes albopictus mosquitoes, respectively, and indicates that the southeastern United States, particularly the Gulf Coast states, could be at risk of exposure in the near future. Figure 4 shows the approximate distribution of *Aedes aegypti* and *Aedes albopictus* mosquitoes in the United States in more detail.

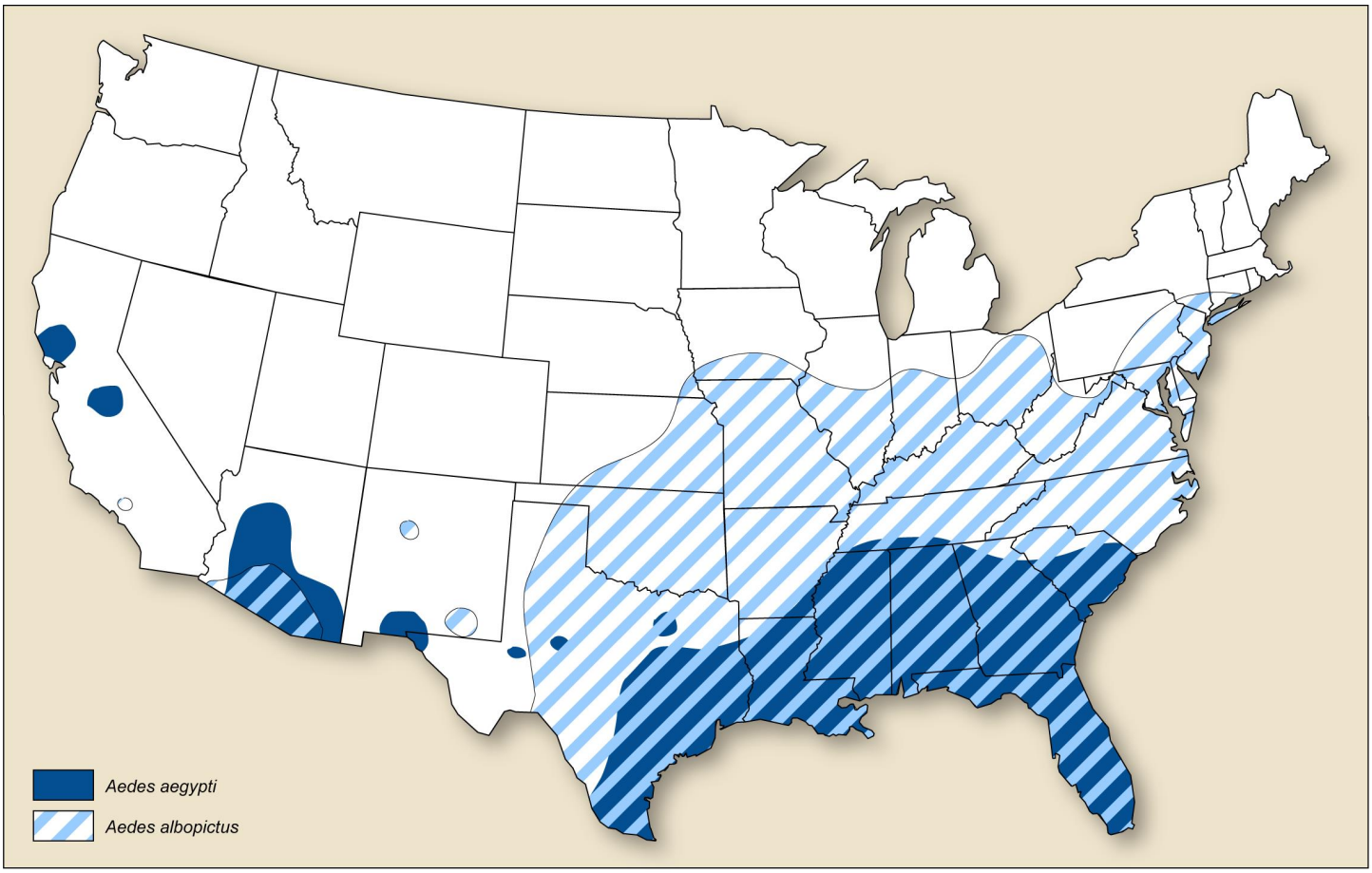
Figure 3: The actual and predicted distribution of two species of mosquito that can transmit Zika virus: *Aedes aegypti* (top) and *Aedes albopictus* (bottom)



Sources: Moritz UG Kraemer, University of Oxford, http://dx.doi.org/10.7554/eLife.08347; MapResources (map). | GAO-16-470T

Note: Black dots represent a measured occurrence of each mosquito species and colored shading represent an estimated or projected distribution of each mosquito on a spectrum from low probability (blue) to high probability (red).

Figure 4: Approximate distribution of *Aedes aegypti* and *Aedes albopictus* mosquitoes in the United States



Sources: U.S. Centers for Disease Control and Prevention (CDC); Map Resources (map). | GAO-16-470T

Note: Maps were developed by CDC using currently available information (as of January 2016). Mosquito populations may be detected in areas not shaded on this map, and may not be consistently found in all shaded areas.

There are both large scale and personal methods for mosquito control. We provide a brief preliminary overview of some population-scale control methods identified in the literature, agency documents, and interviews with industry officials and academicians, which include three potentially-overlapping categories: (1) standing water treatment, (2) insecticides, and (3) emerging technologies. For some of these emerging techniques, their effectiveness remains to be demonstrated, but they have the potential to be additional tools in mosquito control. Personal methods, such as use of repellents, nets or long-sleeved clothing and staying indoors in air-conditioned locations are out the scope of this report.

Standing Water Treatment

Standing water treatment for mosquito control can be achieved by the physical reduction of bodies of water or by treating the water with chemicals that kill mosquito larvae or interfere with their development. According to CDC documentation, these treatments include use of certain bacteria or insecticides that mimic mosquito hormones, which prevents mosquitoes from maturing or kills them as larvae. Another method involves coating water with a thin film of oil to suffocate immature larvae.

Insecticide Dispersal

According to CDC documentation, insecticide dispersal relies on various techniques such as space spraying by trucks or aircraft, or residual spraying, which entails coating surfaces with insecticide. Spraying is one method currently used in the United States. CDC documentation and scientific literature have established that in the long-term, the effectiveness of spraying may be diluted due to insecticide resistance, concerns over environmental exposure, and questionable efficacy of externally-delivered wide-area fogging or spraying. Additionally, the WHO notes that reactive space spraying during emergencies has a low impact unless integrated with other control strategies. Researchers are developing new chemicals that are more targeted towards mosquitoes, and are attempting to alleviate human toxicity issues. [22]

The use of insecticides as control methods effectively reduced mosquito-borne diseases, including malaria and yellow fever, in most of the world in the 1940s-1960s. The WHO determined that maintaining vector control after a disease subsides is complicated by dwindling resources. Indeed, by the 1980s-1990s, many dangerous vector-borne diseases re-emerged or spread to new regions.

Additionally, the spread of some diseases, such as dengue virus disease, can be attributed to a combination of mosquito, viral, and human factors. To address the resulting complexities of such disease transmissions, the WHO uses “Integrated Vector Management,” which leverages multiple control methods based on surveillance and evaluation of involved insects and disease epidemiology.

[22] A. B. Nuss et al., “Dopamine Receptor Antagonists as New Mode-of-Action Insecticide Leads for Control of *Aedes* and *Culex* Mosquito Vectors,” *PLOS Neglected Tropical Diseases*, vol. 9, no. 3, (2015).

Emerging Technologies

Emerging technologies include (1) use of biological control methods, (2) genetically-modified mosquitoes, and (3) auto-dissemination traps. Based on scientific literature, these technologies show some promise in studies to overcome issues associated with use of insecticides, such as insecticide resistance.[23] Many of these methods have been tested in smaller-scale controlled field trials internationally. We have not done an independent, comprehensive evaluation of these technologies, due to time limitations.

Biological Control Methods

Biological control methods include introducing natural predators of mosquitoes or their eggs and using bacteria to prevent disease transmission to humans. For example, certain small crustaceans and certain fish eat mosquito larvae. The suitability of this approach for the Zika virus is uncertain because the primary mosquito species identified for Zika transmission are the *Aedes aegypti* and *Aedes albopictus*, which can breed in very small volumes of water, such as those found in tin cans or in plates under potted plants.

Scientific literature has identified another approach—using bacteria to reduce disease transmission from mosquitoes to humans. This bacteria, called *Wolbachia*, can be transferred from mosquitoes to their eggs, thereby propagating this effect to future generations. This tactic has been demonstrated in laboratory environments and is undergoing field trials internationally, particularly in areas affected by the dengue virus.

Genetically Modified Mosquitoes

Control of the disease-transmitting mosquito population using genetically modified mosquitoes can be potentially achieved in different ways.[24] Some genetically-modified mosquitoes are engineered with a "lethal" gene that constantly makes a protein that kills the mosquito larvae. According to one company creating these mosquitoes, the use of genetically modified mosquitoes allows for population control by introducing male genetically-modified mosquitoes that transfer this lethal gene to the female mosquito's eggs. As a result, mosquito larvae with the inherited lethal gene die. The company claims it has achieved a 90 percent reduction in mosquito populations using its method of releasing the modified male mosquitoes 1-3 times weekly over a period of months. The modified mosquitoes do

[23] N. L. Achee, et al., "A Critical Assessment of Vector Control for Dengue Prevention," *PLOS Neglected Tropical Diseases,* vol. 9, no. 5, (2015).

[24] L. Alphey, "Genetic control of mosquitoes," *Annual Review of Entomology*, vol. 59 (2013).

not persist in the environment, as released mosquitoes generally die out on their own. Given the estimated 200-meter range of a male mosquito during its lifetime, scalability may be challenging. Another genetically-modified mosquito method incorporates viral resistance into mosquitoes, with the goal of replacing existing populations of mosquitoes with one less capable of disease transmission, rather than reducing the number of mosquitoes. Scientific literature indicates there may be public opposition to release of genetically-modified mosquitoes for either procedure due to uncertainties about their effect on people and the environment, or other unknown consequences.

Auto-dissemination Traps

Mosquito control by auto-dissemination traps functions similarly to insecticides, but relies on containers coated with similar chemicals. After a mosquito lands on these containers, they are contaminated with the chemicals and subsequently transfer them to the location where they lay eggs, which may result in larval death. In the case of the *Aedes aegypti* mosquito, WHO documentation indicates these locations tend to be small containers, so auto-dissemination is particularly suited to these mosquitoes. Auto-dissemination traps have shown 42-98 percent decreases in *Aedes aegypti* mosquito population in field trials.

Federal Priority Research Agenda is Ambitious and Implementation of Some Research May be Challenging

NIH and CDC have identified areas of high priority for research related to the Zika virus. In addition, the Administration's $1.9 billion emergency funding request to combat the spread of the Zika virus is intended to provide resources to both NIH and CDC to this end. If approved, the NIH would receive $130 million to support vaccine development and other work related to Zika and other mosquito-borne diseases such as chikungunya. The CDC would receive $828 million, including $225 million for grants and technical assistance, in part to expand mosquito or vector control. The FDA would receive $10 million, in part to support the approval of new diagnostic tests and evaluation of treatment efficacy.

NIH has identified areas of high priority including:

- Basic research to understand viral replication, pathogenesis, and transmission, as well as the biology of the mosquito vectors; potential interactions with co-infections such as dengue and yellow fever viruses; animal models of Zika virus infection; and novel vector control methods; and

- Pursuing Zika virus research to develop sensitive, specific, and rapid clinical diagnostic tests; drug treatments for Zika virus as well as

broad spectrum therapeutics that treat multiple flaviviral infection; and effective vaccines and vaccination strategies.

On February 5th, 2016, several NIH institutes issued a notice to researchers indicating NIH' s interest in supporting research to understand transmission of the Zika virus, optimal screening and management in pregnancy, and the mechanisms by which the Zika virus affects the developing nervous system, including potential links to microcephaly. This notice was followed by a Funding Opportunity Announcement issued on February 19th, 2016, to create an expedited mechanism for funding exploratory and developmental research projects on these topics.[25]

The CDC has identified priority areas of research focus on Zika including:

- Determining the link between Zika virus infections and the birth defect microcephaly and measuring changes in incidence rates of the birth defect over time;
- Improving diagnostics for the Zika virus, including advanced methods to refine tests and support advanced developments for vector control; and
- Enhancing international capacity for virus surveillance, expanding laboratory testing, and health care provider testing in countries at highest risk of Zika virus outbreaks.

These research activities are intended to supplement other activities for response, readiness and surveillance.

The priority research areas identified by NIH and CDC are ambitious and agencies may face some challenges in implementing this agenda, including:

- Given that there are few known cases in the United States, NIH and CDC may have to rely on the cooperation of other countries with

[25] NIH also provided information about additional priority research areas: "Notice of NIAID's Interest to Highlight High-Priority Zika virus (ZIKV) Research Areas" and "NHLBI Announces High Priority Interest in Zika Virus-related Blood Supply Safety and Transfusion Research".

sufficient number of cases in order to carry on the proposed research. However, data from other countries may be different due to different definitions of Zika virus disease and microcephaly.

- Demonstrating the link between the Zika virus and microcephaly may depend not only on the presence of the virus, but also on environmental and nutritional factors.[26] In addition, shifting case definitions and a lack of baseline data makes it difficult to determine the increase, if any, in microcephaly and how much can be attributed to the Zika virus.

- The presence of a high percentage of asymptomatic cases makes it difficult to conduct epidemiological studies, both in identifying exposed and unexposed individuals for case control studies.[27]

- Prior infection or co-infection with another virus such as dengue may complicate any analyses.

NIH officials told us that prior work on similar viruses has allowed them to make rapid progress on both a DNA-based vaccine (based on prior work on West Nile Virus), and a live attenuated virus vaccine modeled after the dengue virus vaccine that is currently in phase 3 clinical trials in Brazil. NIH officials told us that prior "platform" work on similar viruses has expedited response to the Zika outbreak, and in the development of diagnostic tests and vaccines.

With regard to the dengue vaccine, NIH provided us with the following timeline:

[26] U.S. Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report, *Interim Guidelines for Pregnant Women During a Zika Virus Outbreak — United States, 2016*, (Washington, D.C.: January 22 2016).

[27] According to scientific literature, a case-control study is designed to help determine if an exposure (in this case, a viral infection) is associated with an outcome, such as a disease or condition (such as microcephaly or Guillain-Barré syndrome). This is done by identifying the cases (a group known to have the outcome) and the controls (a group known to be free of the outcome). By looking back in time to learn which subjects in each group had the exposure(s), a researcher can compare the frequency of the exposure in the case group to the control group and hypothesize a link between the exposure and condition. From: S. Lewallen and P. Courtright, "Epidemiology in practice: case-control studies," *Community Eye Health,* vol. 11, no. 28, (1998).

Pre-clinical Development	Began 1999
Phase I Clinical Trial	2000 - 2012
Phase II Clinical Trial	2012 - present
Phase III Clinical Trial	2015 - Present

It has taken more than 16 years, and trials are not yet complete. NIH officials told us that given their past experience with the development of vaccine for dengue fever, a vaccine for Zika could be ready for use in an emergency situation in three to four years, in the best case scenario. However, when we asked NIH about this estimate, NIH stated that the National Institute of Allergy and Infectious Diseases plans to begin a Phase I clinical trial within this calendar year. If a candidate vaccine shows promise in Phase I testing, additional clinical testing could begin by 2017 in countries where the disease is found, if the outbreak is still ongoing. The progress of these additional tests, and whether they can contribute to successful licensure, depends on a number of factors, including scientific and technical progress, as well as the size of any ongoing Zika outbreaks during clinical testing. For this reason, it is difficult to provide an exact estimate for the time it will take to develop a Zika vaccine from preclinical studies through clinical testing and licensure.

Zika virus disease poses new challenges to vaccine development and testing. This disease has specific and important implications for pregnant women. There are substantial knowledge gaps in current understanding of Zika, irrespective of the affected population. Since Zika virus disease is associated with, and may cause, adverse fetal outcomes, pregnant women are at particular risk and may benefit from measures such as vaccines. There are several current scientific and structural barriers to developing and testing vaccines for pregnant women.[28] Overcoming these barriers may extend timeframes for vaccine testing and approval. The information we have from NIH and our prior work suggests that development of a Zika virus vaccine may take longer than anticipated by NIH.

Summary

Though technological and scientific advances have improved outbreak detection and response in recent decades, GAO's past work has demonstrated that significant challenges remain in addressing emerging

[28] B. O. Saad and R. H. Beigi, "Pregnancy in the Time of Zika: Addressing Barriers for Developing Vaccines and Other Measures for Pregnant Women," *The Journal of the American Medical Association*, (2016).

infectious diseases, including a lack of basic public health surveillance and response capabilities in many regions, which can delay and compromise effective detection, diagnosis, and treatment (including vaccine development and therapeutics).

Most of the recent infectious diseases have origins in other parts of the world and have thrived elsewhere long before entering the United States. GAO's past work on the swine enteric coronavirus disease virus in pigs showed the lack of basic preparedness for an emerging animal disease. This, and observations on the Zika virus, point to a persistent and urgent need for a proactive, agile, integrated, and coordinated set of programs concerning research and development, including epidemiological studies, mosquito control, testing capabilities, modeling and simulation, and vaccine development, manufacturing, deployment, biosurveillance, risk management, laboratory capacity expansion, and pandemic mitigation.

Chairman Murphy, Ranking Member DeGette, and Members of the Subcommittee, this concludes my prepared statement. I would be happy to respond to any questions you may have.

For questions about this statement, please contact Timothy M. Persons at (202) 512-6412 or personst@gao.gov. Contact points for our Offices of Congressional Relations and Public Affairs may be found on the last page of this statement. Individuals making key contributions to this statement include Sushil Sharma (Assistant Director), Kiera Reifschneider (Analyst in Charge), and Hayden Huang.

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Appendix I: Related GAO Work

Biosurveillance: Ongoing Challenges and Future Considerations for DHS Biosurveillance Efforts. GAO-16-413T. Washington, D.C.: February 11, 2016.

Air Travel and Communicable Diseases: Comprehensive Federal Plan Needed for U.S. Aviation System's Preparedness. GAO-16-127. Washington, D.C.: December 16, 2015.

Emerging Animal Diseases: Actions Needed to Better Position USDA to Address Future Risks. GAO-16-132. Washington, D.C.: December 15, 2015.

Climate Change: HHS Could Take Further Steps to Enhance Understanding of Public Health Risks. GAO-16-122. Washington, D.C.: October 5, 2015.

Biosurveillance: Challenges and Options for the National Biosurveillance Integration Center. GAO-15-793. Washington, D.C.: September 24, 2015.

Biosurveillance: Additional Planning, Oversight, and Coordination Needed to Enhance National Capability. GAO-15-664T. Washington, D.C.: July 8, 2015.

Federal Veterinarians: Efforts Needed to Improve Workforce Planning. GAO-15-495. Washington, D.C.: May 26, 2015.

Biological Defense: DOD Has Strengthened Coordination on Medical Countermeasures but Can Improve Its Process for Threat Prioritization. GAO-14-442. Washington, D.C.: May 15, 2014.

National Preparedness: HHS Has Funded Flexible Manufacturing Activities for Medical Countermeasures, but It Is Too Soon to Assess Their Effect. GAO-14-329. Washington, D.C.: March 31, 2014.

National Preparedness: HHS Is Monitoring the Progress of Its Medical Countermeasure Efforts but Has Not Provided Previously Recommended Spending Estimates. GAO-14-90. Washington, D.C.: December 27, 2013.

Homeland Security: An Overall Strategy Is Needed to Strengthen Disease Surveillance in Livestock and Poultry. GAO-13-424. Washington, D.C.: May 21, 2013.

National Preparedness: Efforts to Address the Medical Needs of Children in a Chemical, Biological, Radiological, or Nuclear Incident. GAO-13-438. Washington, D.C.: April 30, 2013.

Influenza: Progress Made in Responding to Seasonal and Pandemic Outbreaks. GAO-13-374T. Washington, D.C.: February 13, 2013.

Homeland Security: Agriculture Inspection Program Has Made Some Improvements, but Management Challenges Persist. GAO-12-885. Washington, D.C.: September 27, 2012.

Biosurveillance: Nonfederal Capabilities Should Be Considered in Creating a National Biosurveillance Strategy. GAO-12-55. Washington, D.C.: October 31, 2011.

National Preparedness: Improvements Needed for Acquiring Medical Countermeasures to Threats from Terrorism and Other Sources. GAO-12-121. Washington, D.C: October 26, 2011.

Homeland Security: Challenges for the Food and Agriculture Sector in Responding to Potential Terrorist Attacks and Natural Disasters. GAO-11-946T. Washington, D.C.: September 13, 2011.

Homeland Security: Actions Needed to Improve Response to Potential Terrorist Attacks and Natural Disasters Affecting Food and Agriculture. GAO-11-652. Washington, D.C.: August 19, 2011.

Influenza Vaccine: Federal Investments in Alternative Technologies and Challenges to Development and Licensure. GAO-11-435. Washington, D.C.: June 27, 2011.

Influenza Pandemic: Lessons from the H1N1 Pandemic Should Be Incorporated into Future Planning. GAO-11-632. Washington, D.C: June 27, 2011.

Live Animal Imports: Agencies Need Better Collaboration to Reduce the Risk of Animal-Related Diseases. GAO-11-9. Washington, D.C.: November 8, 2010.

Biosurveillance: Efforts to Develop a National Biosurveillance Capability Need a National Strategy and a Designated Leader. GAO-10-645. Washington, D.C.: June 30, 2010.

Veterinarian Workforce: The Federal Government Lacks a Comprehensive Understanding of Its Capacity to Protect Animal and Public Health. GAO-09-424T. Washington, D.C.: February 26, 2009.

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Influenza Pandemic: Efforts Underway to Address Constraints on Using Antivirals and Vaccines to Forestall a Pandemic. GAO-08-92. Washington, D.C.: December 21, 2007.

Influenza Vaccine: Issues Related to Production, Distribution, and Public Health Messages. GAO-08-27. Washington, D.C: October 31, 2007.

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Influenza Vaccine: Shortages in 2004-05 Season Underscore Need for Better Preparation. GAO-05-984. Washington, D.C.: September 30, 2005.