

ORIGINAL ARTICLE

Zika Virus Outbreak on Yap Island, Federated States of Micronesia

Mark R. Duffy, D.V.M., M.P.H., Tai-Ho Chen, M.D.,
 W. Thane Hancock, M.D., M.P.H., Ann M. Powers, Ph.D.,
 Jacob L. Kool, M.D., Ph.D., Robert S. Lanciotti, Ph.D., Moses Pretrick, B.S.,
 Maria Marfel, B.S., Stacey Holzbauer, D.V.M., M.P.H.,
 Christine Dubray, M.D., M.P.H., Laurent Guillaumot, M.S., Anne Griggs, M.P.H.,
 Martin Bel, M.D., Amy J. Lambert, M.S., Janeen Laven, B.S., Olga Kosoy, M.S.,
 Amanda Panella, M.P.H., Brad J. Biggerstaff, Ph.D., Marc Fischer, M.D., M.P.H.,
 and Edward B. Hayes, M.D.

ABSTRACT

BACKGROUND

From the Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention (CDC), Fort Collins, CO (M.R.D., A.M.P., R.S.L., A.G., A.J.L., J.L., O.K., A.P., B.J.B., M.F., E.B.H.); the Epidemic Intelligence Service Field Assignments Branch, CDC, Atlanta (T.-H.C., S.H., C.D.); the Wa'ab Community Health Center (W.T.H., M.B.) and the Yap State Department of Health Service (M.M.) — both in Yap, Federated States of Micronesia; the Office for the South Pacific, World Health Organization, Suva, Fiji (J.L.K.); the Department of Health, Education, and Social Affairs, Pohnpei, Federated States of Micronesia (M.P.); and the Pasteur Institute, Noumea, New Caledonia (L.G.). Address reprint requests to Dr. Fischer at the Arboviral Diseases Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, 3150 Rampart Rd., Fort Collins, CO 80521, or at mfischer@cdc.gov.

N Engl J Med 2009;360:2536-43.
 Copyright © 2009 Massachusetts Medical Society.

In 2007, physicians on Yap Island reported an outbreak of illness characterized by rash, conjunctivitis, and arthralgia. Although serum from some patients had IgM antibody against dengue virus, the illness seemed clinically distinct from previously detected dengue. Subsequent testing with the use of consensus primers detected Zika virus RNA in the serum of the patients but no dengue virus or other arboviral RNA. No previous outbreaks and only 14 cases of Zika virus disease have been previously documented.

METHODS

We obtained serum samples from patients and interviewed patients for information on clinical signs and symptoms. Zika virus disease was confirmed by a finding of Zika virus RNA or a specific neutralizing antibody response to Zika virus in the serum. Patients with IgM antibody against Zika virus who had a potentially cross-reactive neutralizing-antibody response were classified as having probable Zika virus disease. We conducted a household survey to estimate the proportion of Yap residents with IgM antibody against Zika virus and to identify possible mosquito vectors of Zika virus.

RESULTS

We identified 49 confirmed and 59 probable cases of Zika virus disease. The patients resided in 9 of the 10 municipalities on Yap. Rash, fever, arthralgia, and conjunctivitis were common symptoms. No hospitalizations, hemorrhagic manifestations, or deaths due to Zika virus were reported. We estimated that 73% (95% confidence interval, 68 to 77) of Yap residents 3 years of age or older had been recently infected with Zika virus. *Aedes hensilli* was the predominant mosquito species identified.

CONCLUSIONS

This outbreak of Zika virus illness in Micronesia represents transmission of Zika virus outside Africa and Asia. Although most patients had mild illness, clinicians and public health officials should be aware of the risk of further expansion of Zika virus transmission.

ZIKA VIRUS IS A FLAVIVIRUS (FAMILY FLAVIVIRIDAE) related to West Nile, dengue, and yellow fever viruses.¹ Zika virus was isolated in 1947 from a rhesus monkey in the Zika forest near Entebbe, Uganda²; its genome was sequenced in 2006.³ There is serologic evidence of human Zika virus infection in Africa and Asia, and the virus has been isolated from humans in Uganda, Nigeria, and Senegal.²⁻¹² Zika virus is believed to be transmitted to humans by infected mosquitoes and has been isolated from *Aedes africanus*, *Aedes luteocephalus*, and *Aedes aegypti*.¹³⁻¹⁶ No outbreaks and only 14 cases of human Zika virus disease have been previously documented.^{2,12,17-19} Until this outbreak, no transmission of Zika virus had been reported outside of Africa and Asia.

In April and May 2007, physicians on Yap Island, Federated States of Micronesia, noted an outbreak of illness characterized by rash, conjunctivitis, subjective fever, arthralgia, and arthritis. Although three patients tested positive with a commercially available dengue IgM kit, the physicians had the impression that this illness was clinically distinct from dengue, which had been detected on Yap in two previous outbreaks.^{20,21} In June 2007, serum from acutely ill patients was sent to the Centers for Disease Control and Prevention (CDC) Arbovirus Diagnostic and Reference Laboratory in Fort Collins, Colorado. Ten of 71 samples (14%) were found to contain Zika virus RNA according to reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay. RT-PCR assays with the use of consensus primers for nucleic acid of other arboviruses, including dengue, chikungunya, o'nyong-nyong, Ross River, Barmah Forest, and Sindbis viruses, were all negative. We conducted an investigation to define the epidemiologic features of the outbreak and to describe the clinical manifestations of Zika virus disease.

METHODS

SETTING

The Federated States of Micronesia is an archipelago nation located northeast of Papua New Guinea. Yap State is the westernmost of the four states of the country and comprises four closely grouped islands and several outer islands. This investigation was conducted on the main group of four islands, here referred to as Yap. Yap is approximately 6 km wide by 15 km long with a population of 7391 persons (2000 census data).

CASE DEFINITION AND FINDING

We reviewed medical records and conducted prospective surveillance at the hospital and all four health centers on Yap to identify patients with suspected Zika virus disease during the period from April 1 through July 31, 2007. A patient with suspected disease had acute onset of generalized macular or papular rash, arthritis or arthralgia, or non-purulent conjunctivitis. Patients with suspected disease were asked to provide blood specimens during the acute phase (i.e., within 10 days after the onset of symptoms) and during the convalescent phase (i.e., 14 days later). We interviewed a convenience sample of these patients with the use of a standard questionnaire to collect information about demographic features, clinical signs and symptoms, and the duration and severity of the illness.

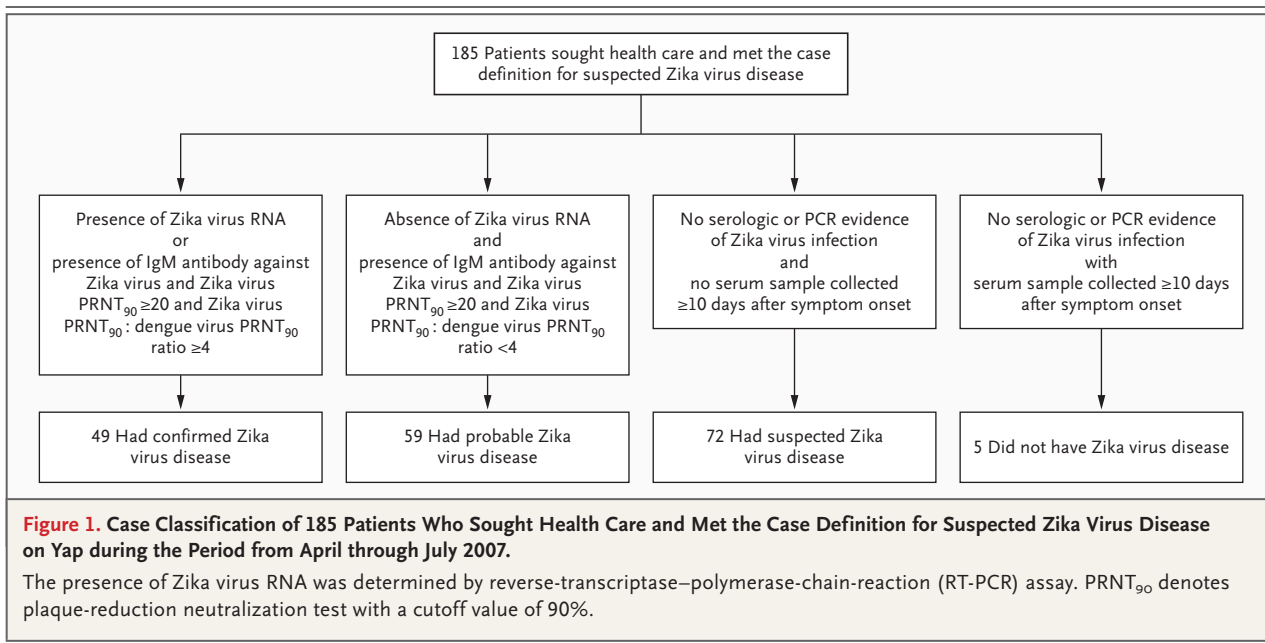
LABORATORY ANALYSIS AND CASE CLASSIFICATION

Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) for IgM antibodies against Zika virus and dengue virus.^{22,23} Titers of neutralizing antibody to Zika virus and dengue virus were determined with the use of plaque-reduction neutralization tests with a cutoff value of 90% (PRNT₉₀).²⁴ Serum samples from patients in the acute phase were tested by RT-PCR for Zika virus and dengue virus RNA.^{22,25}

We considered a patient to have confirmed Zika virus disease if Zika virus RNA was detected in the serum or if all the following findings were present: IgM antibody against Zika virus (detected by ELISA), Zika virus PRNT₉₀ titer of at least 20, and a ratio of Zika virus PRNT₉₀ titer to dengue virus PRNT₉₀ titer of at least 4. A patient was classified as having probable Zika virus disease if IgM antibody against Zika virus was detected by ELISA, Zika virus PRNT₉₀ titer was at least 20, the ratio of Zika virus PRNT₉₀ titer to dengue virus PRNT₉₀ titer was less than 4, and either no Zika virus RNA was detected by RT-PCR or the serum sample was inadequate for the performance of RT-PCR (Fig. 1).

HOUSEHOLD SURVEY

We surveyed the community to define the extent of the outbreak and determine risk factors for infection. We used simple, random, one-stage cluster sampling to select 200 (16%) of the 1276 households on Yap, and we sought to enroll all household members 3 years of age or older. We used a



standard questionnaire to collect data from consenting household residents regarding age, sex, birthplace, potential risk factors for infection, and illness since April 1, 2007. We obtained blood samples from all eligible consenting household residents. Participants in whom IgM antibody against Zika virus was detected by ELISA were considered to have evidence of recent Zika virus infection.

ENTOMOLOGIC INVESTIGATION

Water-holding containers and containers with mosquito larvae or pupae were counted at the surveyed households. Larvae and pupae were collected and identified. Adult mosquitoes were collected by light traps, gravid traps, and aspiration at representative points on Yap and were then pooled according to species, trap location, and collection date.^{26,27} Pools of immature and mature mosquitoes were tested by viral culture and by RT-PCR for Zika virus RNA for evidence of infection.

STATISTICAL ANALYSIS

The attack rates of Zika virus infection were calculated with the use of 2000 census data for the Federated States of Micronesia. Survey and surveillance data were analyzed with the use of SPSS software, version 12.0, and S-Plus software, version 8. For descriptive results, categorical variables were given as proportions and continuous variables were described by the mean or the median and range.

For population inferences from the household

survey, standard errors, confidence intervals, and P values were calculated with the sampling design taken into account and with the use of a finite population correction.²⁸ Categorical variables from the household survey were compared with the use of the Rao and Scott correction to the chi-square test. Standard calibration-weighted estimators were used to adjust for nonresponse to the household survey by calibration to the Yap 2000 census population according to 10-year age groups and sex for the eligible population.²⁹ Inferences for the entomologic survey were based on a simple random sample of households, and the score confidence interval was used for population binomial proportions.

RESULTS

CASE FINDING

We identified 185 cases of suspected Zika virus disease. Of these, 49 (26%) were confirmed and 59 (32%) were probable cases (Fig. 1). Acute-phase serum samples were collected within 10 days after the onset of illness from 45 of the 49 patients with confirmed disease (92%), and Zika virus RNA was detected in 15 of these 45 patients (33%). No dengue virus RNA was detected in any of the 137 acute-phase serum samples tested (45 of these 137 patients had confirmed Zika virus disease, 51 had probable disease, and 41 had suspected disease).

The date of symptom onset among patients

with confirmed or probable disease ranged from April 15 to July 14 (Fig. 2). The number of cases peaked in late May and subsided in early July. The median age of patients with confirmed or probable disease was 36 years (range, 1 to 76); 66 of these patients (61%) were female.

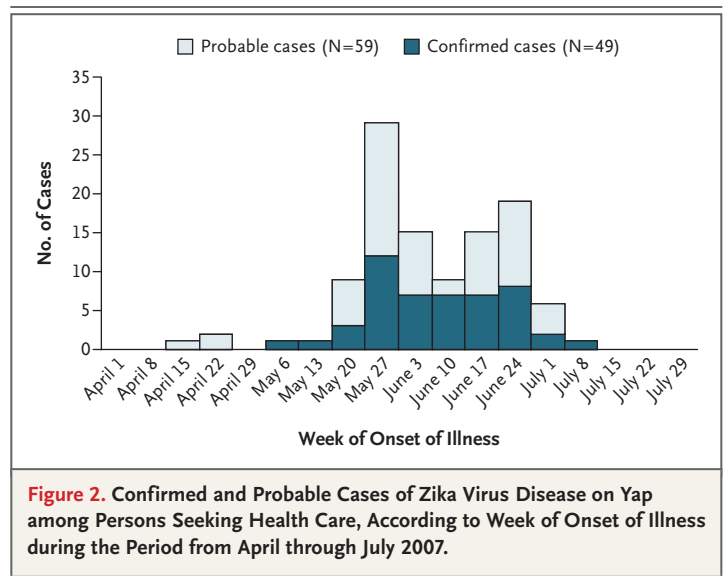
The overall attack rate for confirmed and probable Zika virus disease detected among patients presenting to health care facilities was 14.6 per 1000 Yap residents. The attack rates ranged from 3.6 per 1000 population in both the Kanifay and the Gilman municipalities to 21.5 per 1000 population in Tomil municipality (Fig. 3). The sex-specific attack rates were 17.9 per 1000 females and 11.4 per 1000 males. Cases occurred among all age groups, but the incidence of confirmed and probable Zika virus disease detected by health care surveillance was highest among persons 55 to 59 years of age (Fig. 4).

Of 49 patients with confirmed Zika virus disease, 31 (63%) provided information regarding symptoms and exposures. The age and sex distributions of these 31 patients were similar to those of the remaining 18 patients with confirmed disease. The most commonly reported symptoms were rash, fever (measured or reported), arthritis or arthralgia, and conjunctivitis (Table 1). Other symptoms included myalgia, headache, retro-orbital pain, edema, and vomiting. Twenty patients (65%) reported a subjective fever; the body temperature of 12 of these patients was measured by a health care provider, and none of the recorded temperatures were above 37.9°C. The median duration of rash was 6 days (range, 2 to 14), and that of arthralgia was 3.5 days (range, 1 to 14). No deaths, hospitalizations, or hemorrhagic complications were associated with Zika virus illness during this outbreak. None of the 31 patients who reported symptoms had traveled outside of Yap within 2 weeks before the onset of symptoms.

HOUSEHOLD SURVEY

Surveys were completed in 173 of 200 randomly selected households (86%). The 173 enrolled households had 852 residents, and the median number of residents per household was 5 (range, 1 to 18). Forty-four residents were under 3 years of age and therefore were not eligible to participate in the survey.

We obtained blood samples from 557 of the 808 eligible residents (69%). The age and sex distributions of the participants differed significantly



from those of the eligible Yap population recorded in the 2000 census ($P < 0.001$). Children from 3 through 9 years of age were underrepresented, and adults 40 years of age or older were overrepresented in the survey in comparison with the census population. Overall, males were underrepresented and females were overrepresented, although the ratio of male to female participants varied according to age group.

Among the 557 household residents who provided blood samples, 414 (74%) had IgM antibody against Zika virus, and 156 of these 414 persons (38%) reported an illness during the outbreak period that met the definition of suspected Zika virus disease. However, among the 143 participants who provided blood samples and had no detectable IgM antibody against Zika virus, 27 (19%) also reported an illness that met the definition for suspected Zika virus disease. Thus, among the survey participants who were positive for IgM antibody against Zika virus, a total of 19% (38%–19%) reported a clinical illness that was probably attributable to Zika virus infection.

We used the household survey data to calculate population estimates after accounting for sampling design and nonresponse; these population estimates differ slightly from the crude survey results. We estimated that 5005 of the 6892 Yap residents who were 3 years of age or older (95% confidence interval [CI], 4702 to 5308) were infected with Zika virus during the outbreak, an infection rate of 73% (95% CI, 68 to 77). An estimated 919 residents (95% CI, 480 to 1357), or 18%

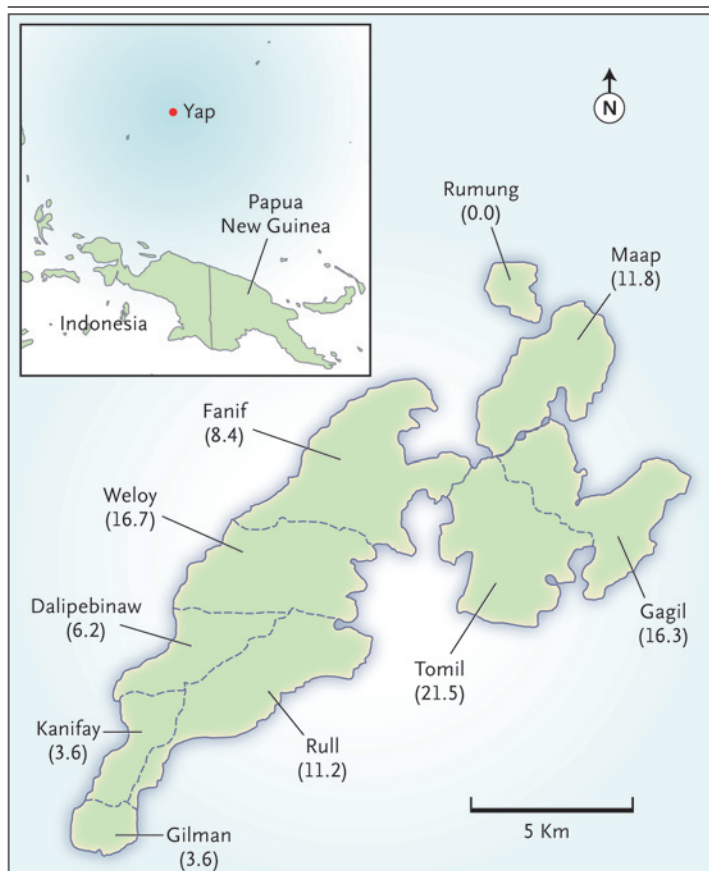


Figure 3. Attack Rates for Confirmed and Probable Zika Virus Disease per 1000 Population According to Municipality on Yap during the Period from April through July 2007.

of those infected (95% CI, 10 to 27), had a clinical illness that was probably attributable to Zika virus. Therefore, the estimated ratio of the number of residents with illness attributable to Zika virus to the number of residents who were either asymptomatic or who had illness that could not be attributed to Zika virus was 919:4086, or 1:4.4 (95% CI, 1:4.3 to 1:4.6).

Male participants were more likely than female participants to have IgM antibody against Zika virus (77% [95% CI, 72 to 83] vs. 68% [95% CI, 62 to 74]; relative risk, 1.1 [95% CI, 1.0 to 1.2]). The seroprevalence of IgM antibody against Zika virus did not vary significantly across age groups ($P=0.10$). We found no behavioral or environmental risk factors for Zika virus infection. People who reported an illness consistent with suspected Zika virus infection were significantly more likely to have IgM antibody against Zika virus than those who did not report such illness ($P<0.001$).

ENTOMOLOGIC INVESTIGATION

Of the 1366 water-holding containers identified during the household survey, 587 (43% [95% CI, 40 to 46]) were infested with mosquito larvae or pupae; infested containers were found at 148 of the 170 households surveyed (87% [95% CI, 81 to 91]). A total of 12 mosquito species belonging to four genera were identified; 9 species were identified by examination of larvae, and an additional 3 species were collected as adults. *Aedes hensilli* was the predominant species identified and was present in 489 of the water-holding containers (36% [95% CI, 33 to 38]). No other species was present in more than 3% of the containers. No virus or viral nucleic acid could be detected in any mosquito pool.

DISCUSSION

In this Zika virus outbreak, approximately three quarters of Yap residents were infected with Zika virus, and we estimated that more than 900 people had illness attributable to Zika virus infection. Zika virus infection was widespread across all geographic areas of Yap and caused relatively mild illness lasting several days. There were no deaths or hospitalizations attributed to Zika virus. We were unable to detect Zika virus in any mosquito samples, and therefore we cannot determine with certainty the vector of transmission. On the basis of the relative abundance of *Aedes hensilli* and previous evidence that this species was the most likely vector of dengue virus transmission on Yap, it is plausible that *Aedes hensilli* was a vector of Zika virus transmission in this outbreak.²¹

The clinical signs and symptoms of Zika virus infection were consistent with those described in a previous report of one male patient but different from those described in a previous case series.^{2,20} In that case series, rash or conjunctivitis was not reported and arthralgia was noted in only one of seven patients. Fever was reported in all seven patients, but the study included only patients who were hospitalized with febrile illness.¹⁹

The detection of Zika virus RNA in the serum of acutely ill patients and the absence of nucleic acid of other arboviruses provide convincing evidence that the outbreak was caused by Zika virus. Although the transmission of dengue virus is common in Micronesia, none of the 137 patients for whom acute-phase specimens were available had evidence of dengue virus RNA in their serum.^{21,22}

All 108 patients with confirmed or probable Zika virus disease had IgM antibody against Zika virus and neutralizing antibodies. ELISA for IgM is a relatively sensitive and specific assay for detecting arboviral infections.²³ Although the ELISA for IgM antibody against Zika virus may cross-react with IgM against other flaviviruses, such as dengue virus or yellow fever virus, it is not likely to cross-react with IgM against alphaviruses such as chikungunya or Ross River viruses. PRNTs effectively discriminate among different primary flavivirus infections, but patients who have secondary infections (those who have been previously vaccinated against or exposed to another flavivirus) may have indeterminate PRNT results.^{30,31} The patients with confirmed Zika virus disease had titers of neutralizing antibodies against Zika virus that were at least four times as high as their titers of neutralizing antibodies against dengue virus, a finding that provides strong evidence of primary Zika virus infection. The patients with probable Zika virus disease also had neutralizing antibodies against Zika virus, but the titers were less than four times as high as the titers of neutralizing antibodies against dengue virus. Although the results from these patients do not definitively confirm Zika virus infection, they are consistent with Zika virus infection after a previous dengue virus infection.

The attack rates of Zika virus disease detected by surveillance were higher among females than males and among older persons than younger persons. In contrast, the prevalence of IgM antibody against Zika virus detected by the survey was higher in male participants (perhaps because of the possibility of their greater exposure to mosquitoes) and was relatively evenly distributed across age groups. These discrepancies may be because of differences in health care-seeking behavior for this relatively mild illness. The estimated ratio of symptomatic to asymptomatic patients with Zika virus infection in this outbreak is similar to that described for West Nile virus infection.^{32,33}

We think it unlikely that Zika virus circulated unrecognized on Yap before this outbreak. The compact clustering of cases in May and June and the high seroprevalence of IgM antibody against Zika virus are consistent with an acute outbreak of Zika virus illness in a population without previous immunity to Zika virus. Although precise estimates of the persistence of IgM antibody against Zika virus are not available, IgM antibodies to

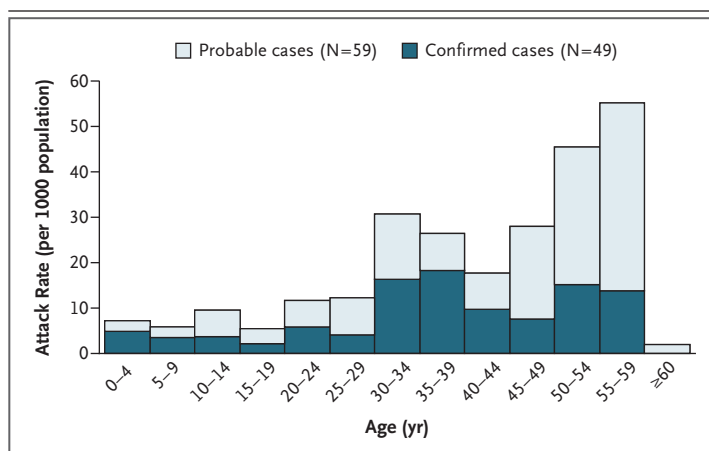


Figure 4. Attack Rates for Confirmed and Probable Zika Virus Disease on Yap According to Age Group during the Period from April through July 2007.

Table 1. Clinical Characteristics of 31 Patients with Confirmed Zika Virus Disease on Yap Island during the Period from April through July 2007.

Sign or Symptom	No. of Patients (%)
Macular or papular rash	28 (90)
Fever*	20 (65)
Arthritis or arthralgia	20 (65)
Nonpurulent conjunctivitis	17 (55)
Myalgia	15 (48)
Headache	14 (45)
Retro-orbital pain	12 (39)
Edema	6 (19)
Vomiting	3 (10)

* Cases of measured and subjective fever are included.

dengue virus generally do not persist longer than 90 days.³³ IgM antibodies to West Nile virus typically persist for about 5 months.³⁴⁻³⁶ There were no reports of widespread disease outbreaks on Yap in the 2 years before this outbreak. These results support the conclusion that this outbreak resulted from a recent introduction of Zika virus.

Zika virus might have been introduced to Yap by a viremic nonhuman primate (monkeys live on nearby Palau but not on Yap), but there were no reports of any importation or recent arrival of nonhuman primates. No other nonprimate vertebrates or birds are known to be reservoirs of Zika virus; unless evidence of such a reservoir is discovered, introduction of Zika virus through an infected

nonprimate vertebrate seems unlikely. It is more likely that Zika virus was introduced by an infected mosquito or a viremic human. We did not find any recently ill residents who had traveled outside of Yap, but the virus could have been imported by a person with undetected infection. Serologic evidence of Zika virus infection in humans has been reported in the Philippines, and travel between Yap and the Philippines is common.

The accessibility of air travel and the abundance of mosquito vectors of flavivirus in the Pacific region raise concern for the spread of Zika virus to other islands in Oceania and even to the Americas. The potential for such spread is illustrated by the following anecdote. A medical volunteer visited Yap from June 17 to June 29, 2007, and had onset of symptoms meeting the case definition of suspected Zika virus disease on July 7, 2007, after her return to the United States. She had IgM antibody

against Zika virus and neutralizing antibody in her serum on July 20, indicating she had been infected with Zika virus on Yap and was probably viremic after arrival in the United States. The emergence of Zika virus as an important human pathogen on Yap in 2007 underscores the ease with which exotic pathogens are transported between continents and the need for clinical vigilance and strong epidemiologic and laboratory surveillance systems to detect the spread of infectious diseases.

No potential conflict of interest relevant to this article was reported.

The views expressed are those of the authors and do not necessarily represent the views of the Department of Health and Human Services.

We thank the physicians and staff at the Wa'ab Community Health Centers, the Yap State Department of Health Service, the Federated States of Micronesia Department of Health, Education, and Social Affairs, the CDC Arboviral Diseases Branch Diagnostic and Reference Laboratory, and the CDC Global Disease Detection Program for their support and assistance with this investigation.

REFERENCES

1. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus *Flavivirus*. *J Virol* 1998;72:73-83.
2. Simpson DI. Zika virus infection in man. *Trans R Soc Trop Med Hyg* 1964;58:335-8.
3. Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 2007;152:687-96.
4. Pond WL. Arthropod-borne virus antibodies in sera from residents of south-east Asia. *Trans R Soc Trop Med Hyg* 1963;57:364-71.
5. Smithburn KC. Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo. *Am J Hyg* 1954;59:157-63.
6. Jan C, Languillat G, Renaudet J, Robin Y. A serological survey of arboviruses in Gabon. *Bull Soc Pathol Exot Filiales* 1978;71:140-6. (In French.)
7. Adekolu-John EO, Fagbami AH. Arthropod-borne virus antibodies in sera of residents of Kainji Lake Basin, Nigeria 1980. *Trans R Soc Trop Med Hyg* 1983;77:149-51.
8. Darwish MA, Hoogstraal H, Roberts TJ, Ahmed IP, Omar F. A sero-epidemiological survey for certain arboviruses (Togaviridae) in Pakistan. *Trans R Soc Trop Med Hyg* 1983;77:442-5.
9. Olson JG, Ksiazek TG, Gubler DJ, et al. A survey for arboviral antibodies in sera of humans and animals in Lombok, Republic of Indonesia. *Ann Trop Med Parasitol* 1983;77:131-7.
10. Monlun E, Zeller H, Le Guenno B, et al. Surveillance of the circulation of arbovirus of medical interest in the region of eastern Senegal. *Bull Soc Pathol Exot* 1993;86:21-8. (In French.)
11. Fagbami A. Epidemiological investigations on arbovirus infections at Igbo-Ora, Nigeria. *Trop Geogr Med* 1977;29:187-91.
12. Moore DL, Causey OR, Carey DE, et al. Arthropod-borne viral infections of man in Nigeria, 1964-1970. *Ann Trop Med Parasitol* 1975;69:49-64.
13. Gubler D, Kuno G, Markoff L. Flaviviruses. In: Knipe D, Howley P, eds. *Fields virology*. 4th ed. Philadelphia: Lippincott-Raven, 2001:1152.
14. Dick GW. Zika virus. II. Pathogenicity and physical properties. *Trans R Soc Trop Med Hyg* 1952;46:521-34.
15. Lee VH, Moore DL. Vectors of the 1969 yellow fever epidemic on the Jos Plateau, Nigeria. *Bull World Health Organ* 1972;46:669-73.
16. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am J Trop Med Hyg* 1969;18:411-5.
17. Fagbami AH. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. *J Hyg (Lond)* 1979;83:213-9.
18. Filipe AR, Martins CM, Rocha H. Laboratory infection with Zika virus after vaccination against yellow fever. *Arch Gesamte Virusforsch* 1973;43:315-9.
19. Olson JG, Ksiazek TG, Suhandiman, Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg* 1981;75:389-93.
20. Durand MA, Bel M, Ruwey I, Marfel M, Yug L, Ngaden V. An outbreak of dengue fever in Yap State. *Pac Health Dialog* 2005;12:99-102.
21. Savage HM, Fritz CL, Rutstein D, Yolwa A, Vorndam V, Gubler DJ. Epidemic of dengue-4 virus in Yap State, Federated States of Micronesia, and implication of *Aedes hensilli* as an epidemic vector. *Am J Trop Med Hyg* 1998;58:519-24.
22. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008;14:1232-9.
23. Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;38:1823-6.
24. Calisher CH, Karabatsos N, Dalrymple JM, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *J Gen Virol* 1989;70:37-43.
25. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* 1992;30:545-51.
26. Nasci RS, Gottfried KL, Burkhalter KL, et al. Comparison of vero cell plaque assay, TaqMan reverse transcriptase polymerase chain reaction RNA assay, and VecTest antigen assay for detection of West Nile virus in field-collected mosquitoes. *J Am Mosq Control Assoc* 2002;18:294-300.
27. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya

- and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol* 2000;81:471-9.
28. Särndal C, Swensson B, Wretman J. Model assisted survey sampling. New York: Springer-Verlag, 1992.
29. Särndal C-E, Lundström S. Estimation in surveys with nonresponse. West Sussex, United Kingdom: John Wiley, 2005.
30. Halstead SB, Rojanasuphot S, Sangkawibha N. Original antigenic sin in dengue. *Am J Trop Med Hyg* 1983;32:154-6.
31. Inouye S, Matsuno S, Tsurukubo Y. "Original antigenic sin" phenomenon in experimental flavivirus infections of guinea pigs: studies by enzyme-linked immunosorbent assay. *Microbiol Immunol* 1984; 28:569-74.
32. Mostashari F, Bunning ML, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet* 2001;358:261-4.
33. Nogueira RM, Miagostovich MP, Cavalcanti SM, Marzochi KB, Schatzmayr HG. Levels of IgM antibodies against dengue virus in Rio de Janeiro, Brazil. *Res Virol* 1992;143:423-7.
34. Roehrig JT, Nash D, Maldin B, et al. Persistence of virus-reactive serum immunoglobulin M antibody in confirmed West Nile virus encephalitis cases. *Emerg Infect Dis* 2003;9:376-9.
35. Prince HE, Tobler LH, Lapé-Nixon M, Foster GA, Stramer SL, Busch MP. Development and persistence of West Nile virus-specific immunoglobulin M (IgM), IgA, and IgG in viremic blood donors. *J Clin Microbiol* 2005;43:4316-20.
36. Busch MP, Kleinman SH, Tobler LH, et al. Virus and antibody dynamics in acute West Nile virus infection. *J Infect Dis* 2008;198:984-93.

Copyright © 2009 Massachusetts Medical Society.

POSTING PRESENTATIONS AT MEDICAL MEETINGS ON THE INTERNET

Posting an audio recording of an oral presentation at a medical meeting on the Internet, with selected slides from the presentation, will not be considered prior publication. This will allow students and physicians who are unable to attend the meeting to hear the presentation and view the slides. If there are any questions about this policy, authors should feel free to call the *Journal's* Editorial Offices.