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Outbreak of Japanese Encephalitis on the Island of Saipan, 1990

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During October 1990, an outbreak of encephalitis occurred on Saipan. Although no virus was isolated, patients seroconverted to Japanese encephalitis (JE) virus, indicating the first known occurrence of JE on US territory since 1947. Ten cases occurred among a population of 40,000. The prevalence of antibody to JE virus among 234 lifelong Saipan residents surveyed after the outbreak was 4.2%. Age, household crowding, and lack of air conditioning were risk factors for infection. The seroprevalence in pigs, which are important amplifying hosts of JE virus, was 96% (n = 52). None of 288 stored serum specimens from lifelong Saipan residents sampled in 1984 were seropositive. These data suggest that JE virus was recently introduced onto Saipan and that peridomestic factors affected the risk of human infection. Transmission of JE virus probably ended with exhaustion of the supply of susceptible amplifying hosts. Surveillance for human cases and seroconversions in pigs during 1991 revealed no evidence of ongoing JE virus transmission.

Japanese encephalitis (JE) is a mosquitoborne viral disease indigenous to rural Asia, where it causes tens of thousands of illnesses and thousands of deaths each year [1]. The disease is associated with a case-fatality rate of 20%–25% and a high rate of permanent neurologic sequelae among survivors [2, 3]. In humans, JE virus is associated with a high ratio of subclinical to clinical infection: For each case of clinical encephalitis, 25–400 individuals may be infected asymptomatically or develop only a mild viral syndrome [4–7].

Culex tritaeniorhynchus, which breeds in rice fields and other flooded areas, serves as the principal vector of the JE virus throughout much of rural eastern and southern Asia. The virus is sustained in an enzootic cycle by birds and mosquitoes. Pigs play an important role in amplifying the virus and propagating outbreaks [8, 9].

During October 1990, several patients with suspected viral encephalitis were treated at the Commonwealth Health Center (CHC) on Saipan, Commonwealth of the Northern Mariana Islands (CNMI). Tests of acute-phase serum and cerebrospinal fluid (CSF) specimens obtained from 5 patients were negative for IgM to JE virus, dengue viruses 1–4, and Murray Valley encephalitis (MVE) virus, which are antigenically similar flaviviruses found in the Pacific region. No autopsies were done in the fatal cases, and delays in transit caused the CSF and serum specimens to thaw before arrival at the Centers for Disease Control and Prevention (CDC), precluding efforts to isolate virus. In December 1990, convalescent-phase serum specimens obtained from 3 patients were found to have IgM with affinity for both JE and MVE viruses. Titers of neutralizing antibody in these specimens were fourfold higher to JE virus than to MVE virus, indicating that this outbreak was most likely an epidemic of JE.

JE had not been previously reported from Saipan and had not been reported from a US territory since an outbreak on Guam in 1947 [10]. The objectives of our investigation in February 1992 were to document the extent of the outbreak, to describe risk factors for infection with JE virus, and to determine whether this outbreak represented a new introduction of JE virus onto the island.

Background and Methods

With a population of 40,000, Saipan is the largest and most populated island in the CNMI. Because of an expanding tourist economy, the island has undergone a rapid growth in population during the last 10 years due to an influx of migrant labor from the Philippines, Korea, and China. In addition, large quantities of construction materials and machinery have been imported to Saipan from Pacific Rim countries.

Outbreak investigation. A confirmed case of JE was defined as a febrile illness (temperature >38.5°C) with one or more specified symptoms (headache, confusion, seizure, or coma) occurring in a Saipan resident between 1 September 1990 and 28 February 1991, in whom a fourfold rise in serum antibody titer to JE virus (without a similar rise in antibody to dengue viruses)

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was demonstrated by serum dilution neutralization or complement fixation (CF). A probable case was defined as a similar illness with one serum specimen demonstrating IgM or CF antibody to JE virus or with paired specimens demonstrating a fourfold rise in neutralizing antibody to JE and one or more dengue viruses. Thus, illnesses compatible with encephalitis in which antibody was present that suggested recent flaviviral infection (IgM, CF, or a fourfold change in neutralizing antibody) and showed broad affinity to JE and the dengue viruses were considered probable cases of JE in this outbreak.

At the time of the outbreak in October 1990, physicians at CHC (the only hospital in CNMI) identified 10 suspected cases of viral encephalitis. To find additional unrecognized cases, in February 1991 we reviewed hospital discharge records for the years 1987–1990 and records of CSF examinations and emergency room visits for febrile illness with headache from the months September 1990 to February 1991. Blood and CSF specimens from these patients were tested for antibodies to JE and dengue viruses when available. Surveillance of patients evaluated at CHC for illnesses compatible with JE continued through December 1991.

Community serosurveys. To estimate the prevalence of JE virus–specific antibody in the general population, we conducted a cluster-sampled serosurvey [11] of Saipan residents during February 1991. Forty-six starting points were identified on a map within populated areas of the island. Each member of the household nearest to the designated starting point was interviewed and asked for a blood specimen. If <7 persons gave specimens and were interviewed at a given house, adjacent houses were visited until at least 7 individuals participated. Demographic information, including duration of residence on Saipan, and a history of symptoms were recorded for each participant, and information about household characteristics was obtained for each household.

To determine whether evidence of previous JE virus transmission on Saipan existed, we tested stored serum specimens from a community serosurvey conducted after an outbreak of hepatitis A in 1984 (unpublished data, CDC, Division of Viral and Rickettsial Diseases) for antibody to JE virus.

To assess rates of infection among potential amplifying hosts of JE virus, we analyzed blood samples obtained from pigs and from a small number of ducks, chickens, dogs, and goats on 28 February and 1 March 1991.

Serologic testing. Blood specimens were centrifuged to separate serum and transported at 4°C to CDC. Serum specimens from individuals with suspected encephalitis were tested for antibodies by IgM-capture ELISA [12], complement fixation [13], and serum dilution neutralization tests [14, 15] to JE and dengue viruses 1–4. Specimens from the community serosurveys and the animal serosurvey were screened at a 1:10 dilution for the presence of neutralizing antibody to JE virus. End-point neutralization titrations to JE and the dengue viruses were determined for specimens that were positive on initial screening. Participants demonstrating neutralizing antibody to JE at a titer at least fourfold higher than the titer to any dengue virus were considered to have been infected with JE virus.

Results

Outbreak investigation. Ten cases of JE were identified (3 confirmed, 7 probable), resulting in an island-wide attack rate of $25/10^5$. Three patients originally diagnosed as having possible JE did not meet the case definition, and 3 additional cases were identified among patients seen in the emergency room for febrile illness with headache. Seven patients were hospitalized, 2 of whom died. The patients' age range was 13–70 years (median, 32; table 1). Three patients had a prior history of neurologic injury (craniotomy, posttraumatic seizure disorder, cerebrovascular accident). Of the 10 case-patients, 2 were schoolchildren and 5 were unemployed or retired. Patients' homes were located in the southern and western parts of the island (figure 1); the distribution of case households appeared to reflect that of the general population. All patients had onset of illness during a 3-week period in October 1990 (table 1).

One person had an illness clinically compatible with viral encephalitis that began 2 weeks before the first documented case of JE. This patient was a Filipino migrant worker who

Table 1. Characteristics of Japanese encephalitis cases, Saipan, 1990.

Case no.	Onset date	Age, sex	Occupation	Prior brain injury	Headache	Myalgia	Meningismus	Confusion	Seizure	Coma	Hospitalized	Death	CSF white blood cells/mm³	CSF protein, mg/dL	Case type
1	10/11	70, M	Retired			+		+		+	+	+	ND	ND	Probable
2	10/15	67, M	Retired		+			+			+		15	127	Probable
3	10/19	13, F	Student		+		+	+	+		+		150	116	Confirmed
4	10/19	22, F	Office worker		+								ND	ND	Probable
5	10/20	57, F	Homemaker	+		+	+	+		+	+	+	104	118	Probable
6	10/20	30, F	Unemployed		+	+							ND	ND	Probable
7	10/22	32, F	Unknown		+								ND	ND	Probable
8	10/24	15, M	Student		+		+	+	+		+		259	59	Confirmed
9	10/25	25, M	Unemployed	+	+		+	+			+		59	133	Confirmed
10	10/29	61, F	Retired	+	+			+	?	+	+		1	77	Probable

NOTE. ND, not done.

returned to Saipan from a vacation in the Philippines 2 days before onset of symptoms and left Saipan for the Philippines before a diagnostic serum specimen could be obtained. A convalescent-phase serum specimen obtained from the patient 18 months later demonstrated broadly reactive neutralizing antibody to JE and other flaviviruses. Since recent infection with JE virus could not be documented in this patient, his illness was not considered a case of JE for the purposes of this study. None of the patients with confirmed or probable JE gave a history of travel in the 2 weeks preceding their illness.

1991 community serosurvey. In total, 410 persons in 51 households were surveyed. Serum specimens from 94 (22.9%) of the 410 participants demonstrated neutralizing antibody to JE virus; 176 of the serosurvey participants (42.9%) had immigrated to Saipan from the Philippines, Korea, and other Asian countries where JE or dengue viruses (or both) are known to circulate and might have been previously infected with one or more of these flaviviruses. The prevalence of neutralizing antibody to JE virus (with or without antibody to dengue viruses) in these immigrants to Saipan was 47% (83/176). Eight immigrants (4.5%) had neutralizing antibody to JE virus at a titer at least fourfold higher than the titer to any dengue virus.

Because previous infections with other flaviviruses can induce antibody that cross-reacts with JE virus, we used only results for residents who had not resided off of Saipan for ≥1 years to estimate human infections with JE virus occurring on the island. Of 234 lifelong Saipan residents, 10 (4.3%; 95% confidence interval, 1.6%–6.9%) had JE virus–specific antibody. None of these 10 seropositive subjects had neutralizing antibody to a dengue virus. One case-patient identified through follow-up of an emergency room visit for febrile illness with headache was coincidentally a participant in the community serosurvey and was included both as a case and as a seropositive survey participant. The remaining 9 seropositive individuals in the survey reported no illness occurring during September–November 1991.

Seropositive subjects ranged in age from 6 to 40 years (mean, 23.4); participants >15 years old were more likely to be seropositive than were children (table 2). In univariate analysis, Carolinian (minority) ethnicity and lower income were associated with seropositivity. Outdoor occupation also appeared to be a risk factor for infection, but relatively few lifelong Saipan residents sampled were outdoor workers. Lack of employment was not a risk factor for infection, but 6 of the 10 seropositive persons were retired, unemployed, or in school.

Seven of 51 households sampled had at least 1 seropositive individual. Three of these 7 households had ≥2 seropositive household members. This apparent clustering of infections in households was of borderline statistical significance ($P \approx .06$, Poisson distribution). Lack of air conditioning and household crowding were household factors associated with

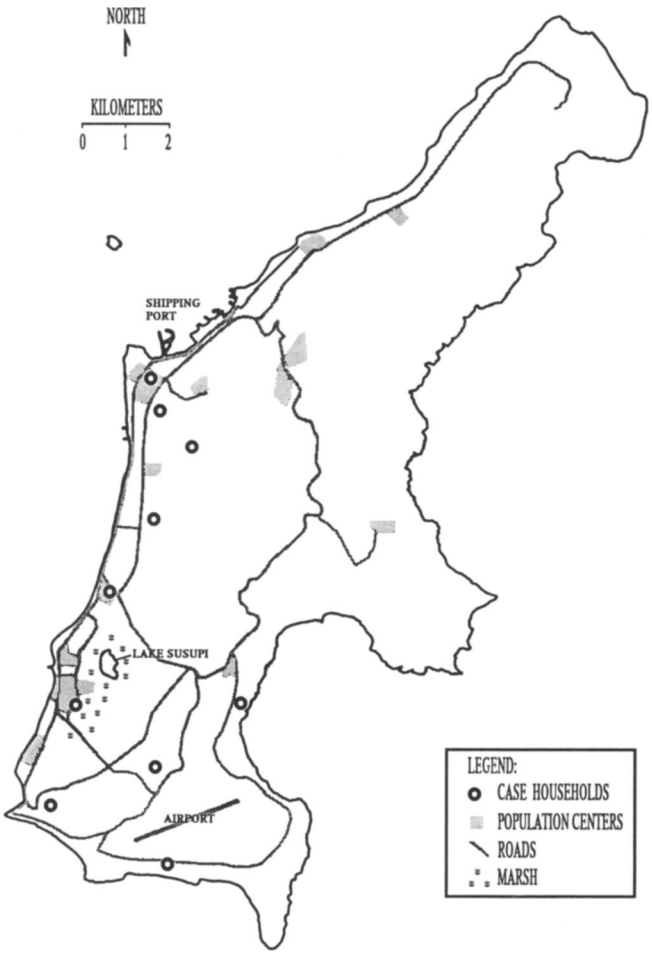

Figure 1. Locations of patient households on Saipan.

risk of infection. Household factors not associated with infection included the presence of pigs or other animals on the premises, a lack of adequate screens in windows and doors, and the presence of water in containers or on the ground (stagnant pools or marshy areas).

In a multivariate logistic regression analysis (not shown), lack of air conditioning, household crowding, and age >15 years remained significant risk factors for seropositivity to JE, whereas outdoor occupation, income, and ethnicity were not significant after adjustment for these other factors.

1984 community serosurvey. To determine whether JE virus circulated on Saipan before 1990, stored serum specimens from a hepatitis survey done in 1984 were tested for JE antibodies. None of the 288 specimens from lifelong Saipan residents surveyed at that time was positive (estimated upper 95% confidence limit, 0.68%).

Animal serosurvey. About one-third of families in areas surveyed kept 1–3 pigs in pens a short distance from their homes. In addition, several pig farms on the island kept up to 60 pigs. A review of quarantine logs showed no record of recent importation of pigs from any country where JE oc-

Table 2. Risk factors for asymptomatic Japanese encephalitis virus infection among lifelong residents of Saipan.

Factor	No. (%) positive	Total tested	Relative risk (95% CI)
Age			
>15 years	8 (7)	111	4.4 (1.0–20.4)
≤15 years	2 (2)	123	
Sex			
Male	5 (5)	107	1.2 (0.4–4.0)
Female	5 (4)	127	
Ethnicity			
Chamorro (majority)	5 (3)	168*	NA
Carolinian (minority)	5 (10)	48	
Other	0	18	
Employed outdoors			
Yes	2 (17)	12	4.4 (1.1–18.7)
No	8 (4)	213	
Air conditioning			
No	9 (8)	118	8.9 (1.1–68.7)
Yes	1 (1)	116	
Household crowding†			
1–2.99	2 (2)	118*	NA
3–4.99	4 (4)	94	
5–6.99	4 (18)	22	
Household income			
<US $10,000	7 (8)	93	3.5 (1.0–12.2)
≥US $10,000	3 (2)	141	
Adequate screens			
No	5 (6)	83	1.8 (0.5–5.9)
Yes	5 (3)	146	
Open water containers			
Yes	7 (5)	152	1.3 (0.3–4.7)
No	3 (4)	82	
Surface water or marsh			
Yes	5 (4)	137	0.7 (0.2–2.4)
No	5 (5)	97	
Pigs on premises			
Yes	4 (4)	95	1.0 (0.3–3.4)
No	6 (4)	139	

NOTE. CI, confidence interval; NA, not applicable.

* $P \leq .05$, 2×3 Fisher's exact test.

† Crowding index = no. of persons in household/number of rooms used for sleeping.

curs. All fowl imported to Saipan (ducks, chickens, fighting cocks, and exotic birds) were imported from the United States.

On 28 February and 1 March 1991, serum specimens were obtained from 70 domestic animals at 14 sites in the southern half of the island. Fifty (96%) of 52 pigs and 7 of 7 ducks tested were positive for JE antibody. In small samples of chickens, dogs, and goats, the seroprevalence rates were 0 (0/5), 83% (5/6), and 67% (2/3), respectively.

Follow-up studies, 1991. No further cases of JE were identified during 15 months of surveillance following this outbreak. A sample of 20 seronegative pigs from various locations on the island that had been born after the outbreak were tested for seroconversion to JE virus biweekly during the peak rainy season (October and November 1991). None of these pigs showed evidence of infection with JE virus. In addition, specimens from 36 ducks, 49 chickens, and 14 pigeons collected at several sites on Saipan in September 1991 demonstrated no antibody to JE virus.

Discussion

Although the etiology of this discrete outbreak of encephalitis was not proved by isolation of a virus from clinical specimens, the epidemiologic and serologic evidence indicates that the most likely agent was JE virus. This represents the first recorded occurrence of JE on Saipan and the first outbreak of JE in a US territory since 1947. The outbreak began and ended within 3 weeks. An island-wide serosurvey done after the outbreak revealed a relatively low seroprevalence in humans, and testing of serum specimens from a 1984 serosurvey did not reveal evidence of previous JE infections on Saipan. A high seroprevalence after the outbreak was observed in pigs, which are important amplifying hosts of JE virus. *C. tritaeniorhynchus* mosquitoes have recently been found in abundance on Saipan, and this species is suspected to have been the mosquito vector there, as it is throughout most JE-endemic areas of Asia [16, 17].

The absence of antibody to JE virus among participants in the 1984 community serosurvey and the lack of JE cases occurring before October 1990 suggest that this outbreak followed an introduction of JE virus to Saipan rather than an intensification of previously undetected viral transmission. The low seroprevalence in the community following this outbreak is also consistent with recent introduction of JE virus, since seroprevalence in areas with annual epidemics is generally higher and approaches 100% in adults [18, 19].

Possible routes of introduction of JE virus onto Saipan include importation of a viremic animal, introduction by a viremic migratory bird, importation of infected mosquitoes or mosquito eggs, or introduction by a viremic human. Importation of a viremic animal is unlikely, because domestic animals known to be important sources of JE virus were not imported to Saipan. Introduction by a viremic migratory bird is possible, as several species of birds that migrate from JE-endemic countries have been reported in the CNMI [20]. Of these, the black-crowned night heron (*Nycticorax nycticorax nycticorax*), the plumed egret (*Egretta intermedia intermedia*), and the cattle egret (*Ardeola ibis*) can develop viremia at levels capable of infecting mosquitoes [21–23]. We found no specific evidence of mosquitoes or eggs being inadvertently imported to Saipan by air or by sea.

The hypothesis that JE was introduced to Saipan by a viremic person was raised by the occurrence of clinically diagnosed encephalitis in a man only 2 days after his arrival from the Philippines and 10 days before onset of the first JE case. Because the minimum incubation period of JE virus in mosquitoes is 6–7 days [24] and in humans is thought to be at least 5 days, transmission from this man to the first case-patient by a mosquito—without an intermediate amplifying

host—would probably be required if he were the source of JE virus. Viremia with JE in humans has been documented occasionally [25–27], but it is not known whether it occurs at levels capable of infecting mosquitoes. We consider it unlikely that this patient's illness played a role in this outbreak.

Transmission of JE on Saipan probably ended because there was a limited number of susceptible amplifying hosts on the island. This is suggested by the very high prevalence of antibody in pigs, which act as important amplifying hosts of JE virus in Asia and are the preferred hosts of *C. tritaeniorhynchus* [9]. Studies of JE on Taiwan [28] and in Japan [29], areas known to have annual transmission of JE virus, have demonstrated that seroconversions in pigs in a given locality occur quickly and completely and precede outbreaks of human cases by ~2 weeks. Annual epidemics in these areas are thought to be facilitated by a high birth rate in pigs, which produces a pool of susceptible amplifying hosts, and annual reintroduction of JE virus via infected migratory birds or mosquitoes [30] or from local foci where JE virus may survive the winter [31].

The results of the serosurvey suggest that infections with JE virus occurred primarily at or near the homes of affected persons. Two of the strongest risk factors found were household factors: lack of air conditioning and household crowding. Because *C. tritaeniorhynchus* mosquitoes infrequently seek meals inside buildings [32], and adequately screened windows and doors were not associated with protection from infection, it is possible that individuals were infected primarily outside their homes. For example, members of households that are crowded or lack air conditioning might spend more time outdoors during the evening hours of peak mosquito feeding.

Three of the 10 case-patients had a clear history of a structural brain abnormality preceding JE. Although the prevalence of neurologic injury in Saipan residents of similar age without JE is not known, this observation raises the hypothesis that antecedent brain injury may be a risk factor for progression from viral infection to encephalitis. This hypothesis is supported by autopsy studies that have found a disproportionate prevalence of pathologic evidence of cerebral cysticercosis in patients with fatal JE [33, 34].

Evidence presented here suggests that transmission of JE virus did not occur on Saipan in 1991. If the virus has not become established in an enzootic cycle on Saipan, then the risk of future cases will depend on the likelihood of reintroduction of JE virus to the island. However, the introduction of JE virus to the islands of Micronesia appears to be a very rare event, having been reported only on Guam in 1947 and in the outbreak described here.

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