

RESEARCH REPORT

THREE JAPANESE ENCEPHALITIS CASES IN OKINAWA, JAPAN, 1991

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Abstract. Since 1974, no Japanese encephalitis (JE) case had been reported on Okinawa island in either Okinawan people or US servicemen. In 1991, three US marines stationed on Okinawa island developed encephalitis symptoms. Neutralization (N) test and IgM-capture ELISA were carried out on the serial samples of serum and cerebrospinal fluid (CSF) taken from the patients. In each patient N test on both serum and CSF samples gave a significant rise in JE antibody titer in the comparison between the acute and convalescent phases, indicating that all the cases were infected with JE virus. The IgM-capture ELISA also showed a significant rise of antibody titer of the serum and CSF samples in the convalescent phase in patients 2 and 3, while in patient 1 a significant rise in IgM antibody was observed in the serum sample, but not in the CSF sample. None of the patients had been administered JE vaccine. This report underscores the importance of JE vaccination.

Okinawa island is situated in the southernmost prefecture of Japan, located in subtropical zone. Japanese encephalitis (JE) had been a great threat before the 1970s on the island due to its high mortality and severe sequelae. According to a prefectural report, during the two largest epidemics in 1962 and 1963 about 100 JE cases were reported with more than 20 fatalities each year. The last JE case was reported in 1973 on the island, but the JE virus continued to be isolated from the main amplifier swine and the vector mosquito *Culex tritaeniorhynchus*. These observations along with the prevalence of JE antibody in swine sera indicate the persistence of JE virus circulation on Okinawa island (Hayashi *et al*, 1976; Ura, 1976a,b; Tadano *et al*, 1994).

June to October 1991, three US marines stationed on Okinawa island were clinically diagnosed with viral encephalitis. All three patients were stationed on Okinawa for at least six weeks before their onset of symptoms. Furthermore, the three patients had not left the island for any travel nor had they received the JE vaccine. Table 1 shows clinical findings. All patients displayed abrupt onset of high fever (greater than 39°C) with severe headache and emesis. Patient 1 showed severe neurological symptoms such as seizure, incontinence,

disorientation, and eventually developed semi-vegetative state of sequelae. Patient 3 had less severe symptoms than patients 1 and 2 and displayed no sequelae. Patient 2 showed moderate clinical symptoms and moderate sequelae.

Serum and cerebrospinal fluid (CSF) specimens were taken during the acute and convalescent phases from the three patients. The virus isolation was carried out by inoculating the acute phase CSF specimens onto a mosquito cell line (C6/36 cells) with negative results. A 50% focus-reduction neutralization (N) test on BHK-21 cells in 96-well microplate using the peroxidase anti-peroxidase staining method (Okuno *et al*, 1985) was carried out to measure N antibody against JE virus (Nakayama strain). The N titers in the convalescent sera were significantly elevated greater than four fold increase in all three patients. N titers of CSF likewise were elevated in the convalescent phase (Table 2). So, all three patients were serologically confirmed with JE virus infection which occurred on Okinawa island. The IgG-class and IgM-class antibodies were assayed by IgG ELISA (Igarashi *et al*, 1981) and IgM-capture ELISA (Bundo and Igarashi, 1985). Anti-JE IgG ELISA titers were significantly elevated in both serum and CSF with all three patients and the anti-JE IgM ELISA titers also showed a rise in the con-

Table 1
Clinical findings.

	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Male
Age	20	35	28
Date of admission	June 1991	Sep 1991	Oct 1991
Fever	40.9°C	40.0°C	39.0°C
Headache	+	+	++
Emesis nausea	+	+	+
Diarrhea	-	+	-
Neck stiffness	+	+	-
Seizure	+	-	-
Incontinence	+	-	-
Disorientation	++	++	++
Sequelae	semi-vegetative state	disorientation to place time and purpose	none

Table 2
Antibody titers against JE and YF.

		Days of illness	Anti-JE			Anti-YF
			N-test	IgG ELISA	IgM ELISA	N-test
Pt 1	Serum A *	4	22	500	100	170
	C *	10	300	2,000	400	400
	CSF A	1	<10	<10	<10	<10
	C	15	40	34	<10	44
Pt 2	Serum A	5	50	80	<50	150
	C	14	35,000	>25,600	>25,600	1,400
	CSF A	7	125	240	40	<10
	C	14	1,100	6,400	700	61
Pt 3	Serum A	4	140	2,000	200	1,800
	C	11	3,000	51,200	7,000	62,000
	CSF A	3	<10	100	30	<10
	C	11	400	4,500	1,000	3,900

*A = acute-phase; C = convalescent-phase

valescent phase sera. Anti-JE IgM of CSF was detected in patients 2 and 3 but not patient 1 (Table 2). Out of the three patients, patient 1 developed the most severe manifestations and sequelae with the lowest antibody response.

All three patients had a prior history of taking 17D yellow fever (YF) live vaccine. In a previous study an evaluation of dengue 2 live vaccine demonstrated that vaccinees with immunity to YF showed a significant rise in N antibody to YF after vaccination (Bancroft *et al*, 1984). The N antibody

titer against YF virus (17D vaccine strain) in the three JE patients was also measured (Table 2). A significant rise in YF antibody was shown in both the serum and CSF samples in the convalescent phase titers of patient 2 and 3, while patient 1 only demonstrated a rise in the CSF convalescent phase. Serological cross-reactivity in the sequential flavivirus infections, including these three JE cases were reported and discussed in a previous paper (Makino *et al*, 1994). In the present study, it was evident that vaccination with 17D YF vaccine does not induce a cross-protective immunity against JE infection.

The three patients were participating in a military exercise located in the central part of Okinawa before their symptoms developed. This particular training area is surrounded by 4 pig farms and several standing water areas, such as rice paddy fields, rush fields, and taro fields. Most of the above areas contained larvae of the mosquito *Culex tritaeniorhynchus*. The co-location of this area provides the ideal environmental background for the facilitation of JE virus circulation. So, the distinct possibility exists that all three patients may have acquired JE infection during one of their field exercises with the surrounding environmental circumstances.

The administration of JE vaccine to the children of Okinawa commenced in 1973; this program was started much earlier in other prefectures in Japan. During this entire period, no vaccination procedures were in place for US servicemen or their family members, but shortly following the JE outbreak, a vaccination program was initiated for US servicemen and their family members. It is this vaccination program along with education and mosquito control measures that have controlled the disease in this population. Since the last three patients, no other US servicemen or family member have been diagnosed with JE.

It should be noted that all three cases of JE occurred before a vaccination program was administered to US personnel. Our results strongly suggest that JE surveillance must be continued and that immunization with JE vaccine is not only safe but also a necessary tool to control this disease. Even though there have been no other reported cases, JE virus is actively transmitted on the island and the vaccination program is the most effective measure we have against this potentially debilitating and fatal disease at present.

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