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Detection of Japanese Encephalitis Virus Antibody in a Pig on Yonaguni Island, Where All Pigs Were Slaughtered in 1997

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Japanese encephalitis virus (JEV) is distributed throughout Southeast Asia and South Asia (1). Pigs are seroconverted to JEV every year in Japan except for on Hokkaido, the northern island. JE patients are reported annually, although the number of the cases has been less than 10 since the 1990s. These facts indicate that JEV is still active in most areas of Japan (2). JEV is active on Okinawa Island as well; and pigs there are positive for JEV antibody (2). Although seroprevalence among pigs has been surveyed on the main Okinawa Island, the survey has not been performed on other islands of Okinawa Prefecture in the last 10 years.

Yonaguni Island belongs to Okinawa Prefecture, and is located approximately 500 km southwest of Okinawa Island and about 100 km east of Taiwan (Fig. 1). When an outbreak of foot-and-mouth disease occurred among pigs in Taiwan in 1997, all pigs on Yonaguni Island were slaughtered. It is not known whether JEV was transmitted between pigs and *Culex tritaeniorhynchus* on Yonaguni Island before 1997. Thereafter, pigs were newly introduced to the island, and there are pig farms in two areas. In order to determine JEV activity on Yonaguni Island, we surveyed JEV antibody prevalence among pigs between July 2004 and March 2006.

A total of 64 blood samples were collected from 63 pigs aged 5-8 months and one adult pig at two areas within the island (Table 1). Blood samples were centrifuged at 3,000 rpm for 10 min, and sera were collected and stored at –30°C. Serum specimens were examined for JEV antibody by hemagglutination inhibition (HI) assay (3) with 4 hemagglutinin units of the JEV antigen (JaGAr #01 strain) (Denka Seiken, Tokyo, Japan). Serum specimens were serially 2-fold diluted from 1:10 to 1:5120. Among 64 serum specimens, one was positive for JEV antibody with a titer of 1:5,120. This serum was treated with 2-mercaptoethanol to detect IgM, but IgM was not detected. Further, this serum was examined for neutralization antibody by focus reduction neutralization assay with peroxidase-anti-peroxidase staining (4). This serum was serially 4-fold diluted from 1:10 to 1:10,240. JEV, Oki128S strain (2003 JEV Okinawa isolate from a pig) was used as challenging virus. The neutralization antibody titer was 1:640.

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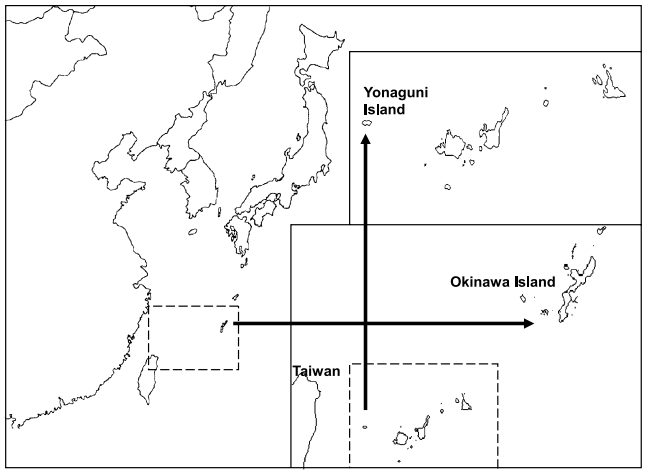

Fig. 1. Locations of Yonaguni Island, Okinawa Island and Taiwan.

Table 1. Number of blood samples collected from pigs in two areas each month

Year	Area	Month												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
2004	A							1	6		3	1		11
	B								2	2				4
2005	A		2						2	6[1)]	5	9	5	29
	B						1		1		1		2	5
2006	A	3		7										10
	B		5											5
Total	A	3	2	7				1	8	6[1)]	8	10	5	50
	B		5				1		3	2	1		2	14

[1)]: One adult pig is included.

This serum was collected from a pig slaughtered in February, 2005. The pig was raised in area A, and there was only one pig farm in this area. According to the owners of this pig farm, they introduced five pigs (2-3 months old) from Okinawa Island in December, 2001. The breeding scale was about 30 pigs during this examination period. During breeding, these pigs were not vaccinated with JEV vaccine, and they had never been off of Yonaguni Island. Moreover, we surveyed mosquito distribution on the island using CDC light traps between April 2004 and March 2006. *C. tritaeniorhynchus* and other species of mosquitoes were trapped around the pig farm throughout the year, and the number reached a peak in

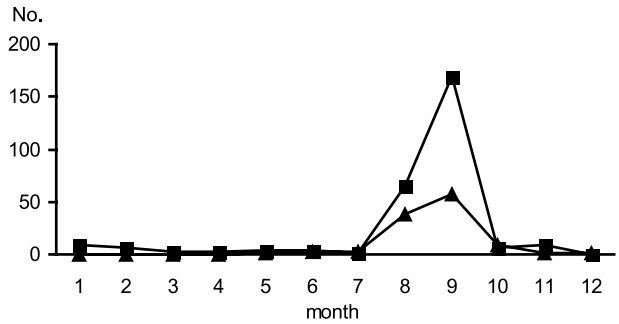

Fig. 2. Number of mosquitoes per trap set around the pig farm. Both *Culex tritaeniorhynchus* (▲) and other mosquitoes (■) reached a peak in September.

September (Fig. 2).

These facts indicate that this pig was infected with JEV on Yonaguni Island. Two JEV infection routes can be assumed. One possibility is that JEV is maintained among newly introduced pigs, or other domestic or wild animals in the island. The other possibility is that JEV was newly introduced to Yonaguni Island from outside. The JEV antibody was not detected in pigs slaughtered during September or in the one adult pig. It is possible that migratory birds carry JEV to new geographical areas (1). Birds migrate to Yonaguni Island every spring and autumn. In the present study, the JEV antibody was detected in only one pig. Therefore, it is more likely that JEV was newly introduced from outside by migratory birds, rather than JEV being maintained on Yonaguni Island. It is important to further survey JEV activity on Yonaguni Island, and to determine the JEV infection source. Further studies are needed to answer these questions.

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