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Short Communication

Prevalence of Antibodies to Japanese Encephalitis Virus among Pigs in Bali and East Java, Indonesia, 2008

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SUMMARY: Japanese encephalitis virus (JEV) is a fatal disease in Asia. Pigs are considered to be the effective amplifying host for JEV in the peridomestic environment. Bali Island and Java Island in Indonesia provide a model to assess the effect of pigs on JEV transmission, since the pig density is nearly 100-fold higher in Bali than Java, while the geographic and climatologic environments are equivalent in these areas. We surveyed antibodies to JEV among 123 pigs in Mengwi (Bali) and 96 pigs in Tulungagung (East Java) in 2008 by the hemagglutination-inhibition (HAI) test. Overall prevalences were 49% in Bali and 6% in Java, with a significant difference between them ($P < 0.001$). Monthly infection rates estimated from age-dependent antibody prevalences were 11% in Bali and 2% in Java. In addition, 2-mercaptoethanol-sensitive antibodies were found only from Bali samples. Further, the average HAI antibody titer obtained from positive samples was significantly higher in Bali (1:52) than Java (1:10; $P < 0.001$). These results indicated that JEV transmission in nature is more active in Bali than East Java.

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus distributed throughout Asia. It causes Japanese encephalitis (JE), with an estimated 30,000 to 50,000 cases and 10,000 deaths reported every year (1). JEV exists in a transmission cycle between *Culex* mosquitoes and birds in nature. In a peridomestic environment, pigs are considered to be an effective amplifying host.

Bali Island is adjacent to Java Island in the Indonesian archipelago. Based on statistics of the pig population in Indonesia, 2008 (2), a large number of pigs (899,582 heads) existed in Bali Island (5,633 km^2), whereas only a small pig population (227,953 heads) was raised in Java Island (127,499 km^2). The majority of Balinese and Javanese are Hindu and Muslim, respectively, which probably affects the number of pigs raised in the respective islands. The densities of pigs are nearly 100-fold different at 160 and 1.79 heads/km^2 in Bali and Java, respectively. Rural areas containing rice fields and pig farms provide an almost complete environment to maintain and amplify JEV in the presence of vector mosquitoes in both Bali (3) and Java (4).

Reflecting the difference in swine populations, confirmed JE cases have been reported mainly from Bali (5,6) and only recently from Java (7,8). Therefore, pigs may act as an important amplifier in these islands. However, no antibody surveys among pigs have been published from Bali or East Java, to the best of our knowledge. The present small survey of JEV antibodies was carried out using pig sera collected in Bali and East Java.

Serum samples were collected from 123 pigs at a farm in Mengwi of Bali and 96 pigs at a farm in Tulungagung (East Java province) of Java in 2008. Samples in Bali were collected in the dry season (August), while samples in Java were collected in the rainy season (March through April). Since pigs are considered to have frequent natural exposures, the ages of subjects were limited to 1–6 months, and approximately 20 individuals were used in each age group (Table 1), except for Java samples aged 6 months (unavailable) and 1 month (the number was half that of other groups). The pigs were housed in these farms under similar environments where the farms were 3,000–5,000 m^2 in area and adjacent to rice fields. These two study sites were located in a single area designated the East Java/Bali region from agricultural and climatologic aspects (9), providing equivalent environments involved in transmission of JEV by vector mosquitoes.

Hemagglutination-inhibition (HAI) assay was performed by a micro-modification of the method of Clarke and Casals (10), with 4 hemagglutinin units of the JEV antigen (Nakayama strain; Denka Seiken, Niigata, Japan). Sera with an HAI antibody titer of 1:10 or higher were considered positive, and those with 1:20 or higher were treated with 2-mercaptoethanol (2-ME) to detect 2-ME-sensitive antibodies. When the difference between HAI antibody titers before and after treatment with 2-ME was 4-fold or greater, the sample was determined to contain IgM antibodies to JEV.

Overall, 60 (49%) of 123 pigs in Bali and 6 (6%) of 96 pigs in Java were positive for HAI antibodies, showing a significant difference between them ($P < 0.001$ by the chi-square test with the Yates' correction factor; Table 1). Comparisons in each age group also detected significant differences between Bali and Java, except for pigs aged 1 month. The antibody prevalence increased with age, except for Bali subjects aged 2 months or less, which were probably affected by maternal antibodies: the duration of maternal antibodies in most piglets is 2 months (11,12). Average monthly infection rates estimated from age-dependent antibody prevalences were 11% in Bali and 2% in Java, supposing that sterile immunity due to maternal antibodies is negligible and that these pig

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Table 1. Prevalence of antibodies to JEV among pigs in Bali and East Java, Indonesia, 2008

Study site	Age (month)	Total no. of samples	No. of samples with HAI antibody titers (reciprocal) of:[1]								Total no. of positives	% positive[2]	Monthly infection rate (%)[3]	No. of samples with IgM antibodies	% of samples with IgM antibodies
			<10	10	20	40	80	160	320	640					
Bali	1	20	14	4		2					6	30	–	0	0
	2	20	15	2			1	1		1	5	25**	–	3	15
	3	21	16		2	3					5	24*	7	3	14
	4	21	11	1	4	3	2				10	48*	11	1	5
	5	21	4	6	7	3		1			17	81***	15	5	24
	6	20	3	1	5	6	3	1	1		17	85	13	2	10
	Total[4]	123	63	14	18	17	6	3	1	1	60	49***	11	14	11
Java	1	10	10								0	0	–	0	0
	2	28	28								0	0**	–	0	0
	3	19	19								0	0*	0	0	0
	4	18	16	2							2	11*	3	0	0
	5	21	17	4							4	19***	4	0	0
	Total[4]	96	90	6							6	6***	2	0	0

[1]: When the number of samples was zero, the result is indicated as a blank.
[2]: Significant differences between Bali and Java in each age group are indicated by *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$) as determined by the chi-square test with the Yates' correction factor. Comparison was done using pigs aged 1 to 5 months.
[3]: Calculated by dividing the "% positive" by the average survival period of pigs in each age group. The average survival period was supposed to be 0.5 + the number of months used for representing the age of pigs: for instance, pigs aged 4 months were supposed to have survived 4.5 months in average. Pigs aged 3 months or older were used for calculation, since pigs aged 1 or 2 months may have maternal antibodies: the "–" indicates "not calculated".
[4]: Percentages on the "Total" line indicates averages of the results obtained in each of 1–6 months, unless otherwise specified.

populations were infected at the same frequency during 6 months. Moreover, 5–24% of pigs aged over 3 months in Bali with an average of 13% (11/83) possessed IgM antibodies, and this percentage was comparable to the monthly infection rate estimated as described above (11%).

HAI antibody titers were distributed from <1:10 to 1:640 in Bali samples, whereas the maximum antibody titer in Java samples was 1:10. The average HAI antibody titer obtained from positive samples was significantly higher in Bali (1:52) than Java (1:10; $P < 0.001$ by the Student's t test).

The significantly higher qualitative (antibody prevalence) and quantitative (antibody titer) results obtained with Bali samples compared to Java samples relate to the difference in pig density between Bali and Java. One report available on a JEV antibody survey among pigs in Indonesia indicated a prevalence of as high as approximately 90%, but the survey was done in West Java and Central Java in the early of 1970s (or before) with pig subjects of older ages (6 to 24 months old; 13). In addition, one report from Bali Island described an antibody prevalence of approximately 70%, but this was described as "unpublished data" without details (6).

The serodiagnostic method used in the present study (HAI test) detects antibodies cross-reactive to dengue viruses, which are also distributed in the present survey areas. However, vector mosquitoes that can transmit dengue viruses (*Aedes aegypti* and *Aedes albopictus*) are anthrophilic, and the rural area has low human densities, particularly around pig farms, with only low levels of dengue virus activity, if any. Thus, it is highly probable that the antibodies detected by an HAI test using JEV antigens were those against JEV, although the possibility of measuring cross-reactive dengue antibodies is not completely ruled out.

In conclusion, natural JEV activities were significantly more prevalent in Bali than Java. High percentages of pigs were infected before age 6 months in Bali, which may provide a large number of infected mosquitoes in nature. Although less active in Java, JEV did circulate and produce relatively high antibody prevalences among humans (14).

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