CROSS-SECTIONAL SEROSURVEY FOR JAPANESE ENCEPHALITIS SPECIFIC ANTIBODY FROM ANIMAL SERA IN MALAYSIA 1993

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Abstract. Serum specimens were collected from 6 species of animals living in 9 states of Malaysia including Sabah, North Borneo in 1993. Antibodies against Japanese encephalitis (JE) virus in these sera were detected by means of hemagglutination-inhibition (HI) and neutralization (NT) tests. By HI test, 702 of 2,152 (32.6%) sera showed positive results. Higher positive rates were obtained by the NT test, in which 1,787 of 1,927 (92.7%) sera had antibodies against JE virus. All serum specimens with positive HI were confirmed as positive by the NT. Swine sera showed especially higher rates of antibody positive and higher antibody titers compared with other animals. These results suggest that JE infections are widely distributed among many animals of Malaysia, and pig is the most susceptible amplifier host for JE virus.

INTRODUCTION

Japanese encephalitis (JE) virus was first isolated from the brain of a fatal human encephalitis case inTokyo in 1935. JE has been one of the serious viral infections in South and South-east Asian countries (Umenai *et al*, 1985; Igarashi, 1992). Two billion people are currently living in its endemo-epidemic regions and approximately 50,000 human JE cases are estimated to occur annually in Asia (Burke and Leake, 1988).

In Malaysia, the earliest published report of its occurrence described an outbreak among British prisoners of war during the Second World War (Cruikshank, 1951). This clinical report, however, was not confirmed by the laboratory examination on its etiological agent. Outbreaks of JE were then recognized in 1951 in Malaysia with JE virus isolation and serological examination, and became a public health concern (Peterson *et al*, 1952). The epidemics of JE in Malaysia have been occurring every year, and 9-35 cases have been confirmed by laboratory examination annually from 1977 to 1990 (Sinniah, 1989; Ministry of Health, Malaysia, 1990).

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Paterson *et al* (1952) and Pond *et al* (1954) demonstrated neutralizing antibodies against JE virus in human and equine sera in Malaysia. The latter report also demonstrated anti-JE antibodies among various animal species in addition to equines, such as pigs, cows, dogs, goats and sheep in Malaysia. These data were obtained, however, on relatively small number of specimens more than 40 years ago.

In order to assess the magnitude of JE virus circulation in Malaysia in recent years, it seemed important to examine serum antibodies in different animal species in various parts of the country. This paper describes the anti-JE antibodies examined by the hemagglutination-inhibition (HI) and neutralization (NT) tests on sera from animals in Peninsular Malaysia, as well as from pigs and birds in Sabah, North Borneo, Malaysia.

MATERIALS AND METHODS

Serum specimens

A total of 2,152 sera examined in this study was obtained from domestic animals raised in 9 States of Malaysia including Sabah, North Borneo, in 1993. The animal species consisted of 177 pigs, 576 cows, 347 buffalos, 492 sheep, 449 goats, and 111 birds. The birds comprised of 71 quails and 25 sparrows in Perak State, and 15 quails in Sabah

State. These sera were collected aseptically by Veterinary Research Institute of the Federation of Malaysia at Ipoh during the period from February to October 1993 (Table 1).

Serological tests

Hemagglutination (HA) and hemagglutination-inhibition (HI) tests were performed according to the method of Clark and Casals (1958) modified for a microsystem (Sever, 1962) using 8 units of hemagglutinating antigens. Neutralizing (NT) antibody titers were determined by an improved rapid focus reduction NT test in 96-well tissue culture plates (Okuno *et al,* 1985). The titers were expressed as 50% focus reduction units, the reciprocal of the highest serum dilution that reduced the number of foci to 50% of the control value. In both tests, a titer of more than 1:10 was considered as positive. JE virus, JaGar01 strain (Matsuyama *et al,* 1960; and Oda, 1976), was used as antigen and challenge virus throughout the study.

RESULTS

Anti-JE HI antibody positive rates in animal sera

Table 1 shows the number of animal sera collected from different locations for use in this study. Specimens were obtained from 9 of the 13 States and Kuala Lumpur territory in Malaysia. Penang is an island, and the other States are agricultural zones in Peninsular Malaysia except Sabah in North Borneo (Fig 1). These regions are relatively flat lands with less than 300 meters above the sea level, and belong to the marine tropical area with a wet climate. All serum samples were examined by the HI test, and the results were presented in Table 2. The positive rate of anti-JE HI antibodies was highest at 88.1% in pigs, then 41.5-45.0% in cows and buffalos, 13.8-17.9% in goats and sheep, and lowest at 0.9% in birds. Although the number of birds was small, only one quail out of 111 birds examined was positive. The differnce in the anti-JE HI antibody

Table 1

The number of serum specimens collected from various animals in different places in Malaysia, 1993.

Places (State)	Pig	Cow	Buffalo	Goat	Sheep	Bird	Total
Kedah		96		96	96		288
Penang	95						95
Perak		96	96	96		96*	384
Selangor		96		96	59		251
Johor		96	60	70	96		322
Pahang	52	96	31	91	96		366
Trengganu			50		84		134
Kelantan		96	110		12		218
Sabah	30				49	15**	94
Total	177	576	347	449	492	111	2,152

* Consisted of 71 quails and 25 sparrows

** Quails

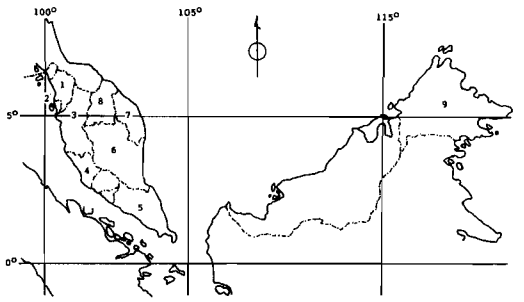

Fig 1– Map of Malaysia in which nine studied areas are indicated: 1 Kedah, 2 Penang, 3 Perak, 4 Selangor, 5 Johor, 6 Pahang, 7 Trengganu, 8 Kelantan, 9 Sabah.

Table 2

Positive rate of anti-JE HI antibodies in animal sera collected in different places in Malaysia, 1993.

Places (State)	Pig	Cow	Buffalo	Goat	Sheep	Bird	Total
Kedah		11.5*		6.3	1.0		6.3
Penang	100						100
Perak		75.0	57.3	19.8		1.0	38.3
Selangor		65.6		6.3	10.2		29.9
Johor		28.1	63.3	25.7	32.3		35.7
Pahang	76.9	47.9	32.3	14.3	33.3		38.5
Trengganu			28.0		15.5		20.1
Kelantan		20.8	35.4		16.7		30.0
Sabah	70.0				6.1	0	25.5
Total	88.1	41.5	45.0	13.8	17.9	0.9	32.6

* Percentage

positive rate was statistically significant ($p < 0.01$) by chi-square test between pigs to cows and buffalos as well as between cows to goats and sheep.

Distribution of anti-JE HI antibody titers in animal sera

Table 3 shows the distribution of HI antibody titers of the sera obtained in 9 locations. Many of the pig sera from Penang and a small number of cow and buffalo sera from Perak and Johor possessed high titers (1:160 - 1:640). Whereas the sheep sera from Kedah, Kelantan and Sabah showed low titers, most of which were distributed between <1:10 - 1:10. A single antibody positive avian serum (a quail) from Perak possessed a low titer at 1:10. It was noticed that all animal sera except a fraction of pigs, cows, buffalos and sheep possessed low titered HI antibodies between 1:10 - 1:40 which comprised 86.6% of a total HI positive sera.

Distribution of anti-JE NT antibody titers in animal sera

A total of 1,927 animal sera randomly selected from the HI antibody positive and negative specimens were examined by the NT test. The distribution of NT antibody titer in 6 species of animal sera was tabulated in Table 4. It was shown that 85.8% of pig sera possessed NT titers of 1:550 or greater, so were the 56.0 - 51.4% in cow and buffalo sera. While the titers of 5.4 - 25.4% goat and sheep sera were distributed in lower range of 1:10 - 1:550≤. However, most of birds possessed low NT titers, and 44.6% of them were negative.

Correlation between anti-JE HI and NT antibody titers

The anti-JE antibody titers were examined both by the HI and NT tests for a total 1,927 serum specimens, and the results were summarized in Table 5. Anti-JE HI antibody positive rates among different animals were 156 of 177 (88.1%) in pigs, 238 of 547 (43.5%) in cows, 143 of 307 (46.6%) in buffalos, 62 of 447 (13.9%) in goats, 84 of 375 (22.4%) in sheep, and 1 of 74 (1.4%) in birds. By the NT test, on the other hand, larger number of the specimens were anti-JE antibody positive: 176 (99.4%) of pigs, 539 (98.5%) of cows, 301 (98.0%) of buffalos, 392 (87.7%) of goats, 338 (90.1%) of sheep, and 41 (55.4%) of birds. As mentioned above, all HI positive specimens were also positive by the NT, while some HI negative specimens showed positive NT. These results are consistent with previous report that the NT is more sensitive than the HI to detect anti-JE antibodies (Oda, 1971).

DISCUSSION

Although the first clinical description of JE in Malaysia was reported in 1951 (Cruikshank, 1951), human sera obtained from inhabitants of Malaysia during the period 1948 through 1950 were shown to

Table 3

Distribution of anti-JE HI antibody titers in animal sera collected in different places in Malaysia, 1993.

Place (State)	Animal	HI titers								HI-positive rate (%)
		<10	10	20	40	80	160	320	640≤	
	Cow	85*	6	4	1					11.3
Kedah	Goat	90	2	1	3					6.3
	Sheep	95	1							1.0
Penang	Pig		5	2	24	9	36	19		100
	Cow	24	18	29	18	4		1	2	75.0
Perak	Buffalo	41	41	12	2					57.3
	Goat	77	13	4	2					19.8
	Bird	95	1							1.0
	Cow	33	27	28	8					65.6
Selan-	Goat	90	2	2	2					6.3
gor	Sheep	53	3	2	1					10.2
	Cow	69	8	14	5					28.1
Johor	Buffalo	22	13	17	6		1	1		63.3
	Goat	52	6	8	4					25.7
	Sheep	65	21	9	1					32.3
	Pig	12	1	3	21	13	2			76.9
	Cow	50	31	12	3					47.9
Pahang	Buffalo	21	6	4						32.3
	Goat	78	9	3	1					14.3
	Sheep	64	22	10						33.3
Treng-	Buffalo	36	7	3	4					28.0
ganu	Sheep	71	4	5	2	2				15.5
	Cow	76	16	3	1					20.8
Kelan-	Buffalo	71	19	16	4					35.5
tan	Sheep	10	2							16.7
	Pig	9	4	5	8	4				70.0
Sabah	Sheep	46	3							6.1
	Bird	15								0
Total		1,450	291	196	121	32	39	21	2	32.6

* Number of case

contain significant amounts of neutralizing antibodies against JE virus (Paterson *et al*, 1952). These results indicate that JE virus has long been prevalent in the human populations of Malaysia. Pond *et al* (1954) demonstrated that the NT antibody positive rates among inhabitants in Malaysia were 74.0% in indigenous peoples in Peninsular Malaysia, 5.0% in non-Asian people with less than 3 years living in Peninsular Malaysia, and 67.0% in indigenous people in Borneo, respectively.

They also examined anti-JE NT antibody posi-

Table 4

Distribution of anti-JE NT antibody titers in animal sera in Malaysia, 1993.

Animal	NT titers								Total
	<10	10	20	40	80	160	320	550≤	
Pig	1*	1	-	3	1	5	14	152	177
	0.6**	0.6		1.7	0.6	2.8	7.9	85.8	
Cow	8	7	19	24	51	59	72	307	547
	1.5	1.3	3.5	4.4	9.3	10.8	13.2	56.0	
Buffalo	6	5	14	21	31	37	35	158	307
	2.0	1.6	4.6	6.8	10.1	12.1	11.4	51.4	
Goat	55	24	36	38	48	66	66	114	447
	12.3	5.4	8.1	8.5	10.7	14.8	14.8	25.4	
Sheep	37	46	52	50	44	37	39	79	375
	9.9	12.3	13.9	13.3	11.7	9.9	10.4	18.6	
Bird	33	13	15	5	7	1	-	-	74
	44.6	17.6	20.2	6.8	9.5	1.3			
Total	140	96	136	141	182	205	226	801	1,927
	7.3	5.0	7.1	7.3	9.4	10.6	11.7	41.6	

* Number of case.

** Percentage of cases to the total specimens tested in each animal.

tive rate among several animal species and reported that the rate was high among pigs, cows, and equines, while lower rate was found among sheep and goats. In contrast, clinically apparent human JE cases were not clearly recognized at that time. From 1970 onward, the disease has been monitored by the Institute for Medical Research, Kuala Lumpur (Sinniah, 1989), and the reported number of JE cases was in the range of 37 - 92 per year with 9 - 35 laboratory confirmed cases annually. These results indicate that JE virus infection is widely distributed among many species of animals in Malaysia. Since both antibody positive rates and antibody titers were significantly higher among pigs than any other animal species, pigs could be regarded as the most important amplifier host for JE virus in Malaysia, as in the case of other Asian countries (Scherer *et al,* 1959). On the other hand, avian species of quails and sparrows examined in this study do not play a significant role in JE virus transmission as deduced from their low antibody positive rates and titers.

As shown in Tables 2 and 3, anti-JE NT antibody positive rates and titers were higher than those by the HI test. The higher sensitivity of the NT test than the HI test was reflected in the results in Table 5, in which 1,103 of 1,927 sera tested (57.2%) were antibody positive by the NT test but not by the HI test. The percentage of test sera which turned out to be positive by the NT but negative HI was not uniform among different animal species. This percentage was remarkably lower for pigs (11.3%) compared with other animal species: cows (55.0%), buffalos (51.5%), goats (73.8%), sheep (67.7%), and birds (54.1%). The low percentage among pigs is apparently resulting from their high susceptibility to JE virus infection accompanied by higher antibody titer, which could mostly be detected by the HI test.

It is apparent that the NT is more reliable than the HI in places where multiple flaviviruses coexist. It has been well-documented that pigs are the most efficient amplifier vertebrate host for JE virus

Table 5

Correlation between HI and NT antibodies against JE virus.

Pig

Test		NT +	NT -	Total
HI	+	156*	0	156** (88.1)
	-	20	1	21 (11.9)
Total		176 (99.4)	1 (0.6)	177

Cow

Test		NT +	NT -	Total
HI	+	238	0	238 (43.5)
	-	301	8	309 (56.5)
Total		539 (98.5)	8 (1.5)	547

Buffalo

Test		NT +	NT -	Total
HI	+	143	0	143 (46.6)
	-	158	6	164 (53.4)
Total		301 (98.0)	6 (2.0)	307

Goat

Test		NT +	NT -	Total
HI	+	62	0	62 (13.9)
	-	330	55	385 (86.1)
Total		392 (87.7)	55 (12.3)	447

Sheep

Test		NT +	NT -	Total
HI	+	84	0	84 (22.4)
	-	254	37	291 (77.6)
Total		338 (90.1)	37 (9.9)	375

Bird***

Test		NT +	NT -	Total
HI	+	1	0	1 (1.4)
	-	40	33	73 (98.6)
Total		41 (55.4)	33 (44.6)	74

Total

Test		NT +	NT -	Total
HI	+	684 (35.5)	0	684 (35.5)
	-	1,103 (57.2)	140 (7.3)	1,243 (64.5)
Total		1,787 (92.7)	140 (7.3)	1,927

* Number of case

** Figures in parentheses indicates percentage of cases to the total specimens tested in each animal

*** Quail

transmission in nature because of their high infection rate, large population size, high turnover rate, and preferential hosts for vector feeding (Oda, 1968). In early summer in Japan, JE virus has been isolated from vector mosquitos and swine blood. Thereafter, the infection rate of mosquitos and animals reach a peak level, as a result of virus amplification by pig-mosquito cycle. Following the peak of mosquito infection, outbreaks of human JE cases occur by bites of infective mosquitos (Oda *et al,* 1969, 1978). Although the pig population may not be high in Malaysia, a Moslem society, the frequency of JE infection among other animals, *ie* cows, buffalos, goats and sheep, showed sufficiently high levels. The present data and the results of JE virus isolation from the mosquitos indicate that infection of domestic animals by JE virus occurs throughout the year and all over the country (Vythilingam, 1994). As a typical arbovirus, JE virus is maintained in nature by alternative growth in vertebrate hosts and arthropod vectors. Malaysia possesses favorable climatic conditions for JE virus transmission with sufficiently high temperature and rainfall.

Recently, Tsuchie *et al* (1994) and Vythilingam *et al* (1994) have reported genotyping of JE viruses isolated from *Culex* and *Aedes* mosquitos in Peninsular Malaysia in 1992. These strains currently circulating in Peninsular Malaysia belong to the genotypic group which exists in the epidemic regions in northern Asia or southern Asia in temperate climates such as Japan, China, Taiwan, Philippines, SriLanka, India and Nepal.

Although JE is endemic with relatively small numbers of reported cases in tropical area (Fukunaga *et al,* 1974), in tropical countries like Malaysia JE virus can exist all year round and could serve as a source to supply the virus to other epidemic regions where susceptible amplifiers and vector mosquitos are present. Further investigations are needed to clarify seasonal prevalence of JE virus infection monitored by antibody assay on slaughtered pig blood and virus isolation from vector mosquitos.

ACKNOWLEDGEMENTS

The authors wish to thank Director of the Institute for Medical Research for his permission to publish this paper. This study was supported by the Institute for Medical Research and Japan International Cooperation Agency Research Project on Tropical Diseases in Malaysia.

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