

Virological and Epidemiological Studies on Encephalitis in Chiang Mai Area, Thailand, in the Year of 1982

VI. Antibody survey on animals

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Abstract: Antibody prevalence against Japanese encephalitis (JE) virus among various vertebrates was measured by the hemagglutination-inhibition (HI) test. Swine, horse, mule, sheep, and dog possessed anti-JE antibodies at high rates. On the other hand, antibody prevalence among monkey, duck and sparrow was low, and chicken and lizard did not have anti-JE antibodies as far as tested. Enzyme-linked immunosorbent assay (ELISA) on swine sera revealed that almost one-third of the swine population appeared to have recently been infected with JE virus.

Key words: Antibody survey, Animals, Chiang Mai, Thailand, Encephalitis

INTRODUCTION

During our study period from July 19 to August 17, 1982, in Chiang Mai Area, (Igarashi *et al.*, 1983), serum specimens were collected from various vertebrates in order

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Table 1. Number of livestocks in Chiang Mai Province

District	Swine	Duck	Chicken	Cattle	Water buffaloes
Muang	8170	7674	65896	1765	1085
Hang-Dong	8902	11917	86916	3489	2802
San-Pa-Tong	54320	65850	184480	8970	7921
Jom-Tong	50170	4657	299646	13760	12436
Hod	14495	625	25014	7965	8511
Mae-Jam	14650	1223	24766	7895	6985
Om-Koi	6800	30	13000	4500	8600
Doi-Taw	5788	—	80227	17699	4728
Sarapee	25600	24500	99200	4972	6350
San Kamphang	14123	10131	331028	12341	10342
Doi-Saket	97000	26000	227000	6530	6980
San-Sai	33290	3740	8275	5065	6056
Mae-Rim	7114	7414	12530	4822	4462
Sa-Moeng	11752	1940	12440	7714	8556
Mae-Taeng	9526	2578	81978	4839	4718
Chieng Daw	9745	2711	92285	6178	7565
Praw	8648	7462	54138	4828	4768
Fang	10296	1412	76853	8638	9315
Mae-Ai	5400	3000	65300	3285	6985
Total	395744	181864	1840972	135255	129178

Geese: 4752, Sheep: 2425, Goat: 390, Horse: 1345, Elephant: 300 (July, 1982)

to measure antibody titers against JE virus by the HI test. The purpose is to know the antibody prevalence among these vertebrates, which might serve hosts of JE virus. Some of them may be effective amplifiers of the virus as already shown in the case of swine in Japan (Scherer *et al.*, 1959; Konno *et al.*, 1966). Since ELISA was found to be a suitable method to measure IgM-antibodies in swine sera (Bundo *et al.*, 1982), we used the method for swine sera along with the HI test in order to detect recent infections by JE virus.

MATERIALS AND METHODS

Serum specimens and the HI test: The number of livestocks in various Districts in Chiang Mai Province is shown in Table 1. Population of swine is more than the sum of cattles and water buffaloes, which effectively attract *Culex* vector of JE virus, however, do not serve as efficient amplifier of the virus. Specimens of several species of vertebrates were collected as follows: swine at a slaughter house of Chiang Mai City; horses and mules at Mae Rim Farm of Royal Thai Army Department; sheep and dogs in the campus of Chiang Mai University; monkeys at Chiang Mai Zoo; chicken, ducks, sparrows and lizards at

several markets in Chiang Mai City. Sera were separated from clotted blood and were treated with kaolin (Fischer, acid-washed), followed by absorption with goose red blood cells as described in the accompanying paper (Fujita *et al.*, 1983). The procedure of Clarke and Casals (1958) was followed in the HI test modified to microtiter system. Sucrose-acetone extracted antigen of JE virus, JaGAR-01 strain, was kindly supplied by Chemoserotherapeutic Institute, Kumamoto, Japan. Antigen of dengue virus type 1 (D1) Mochizuki strain was prepared from infected suckling mouse brains by sucrose-acetone extraction.

ELISA procedures: Indirect micromethod of Voller *et al.* (1967) was followed with modifications as described (Igarashi *et al.*, 1981; Bundo *et al.*, 1982). Formalin-inactivated purified JE vaccine concentrate was obtained from Research Foundation for Microbial Diseases of Osaka University (Takaku *et al.*, 1968), and was used as ELISA antigen to coat microplate. Anti-swine IgM (μ -chain specific) rabbit IgG, peroxidase-conjugated anti-swine IgG (heavy and light chains), and peroxidase conjugated anti-rabbit IgG goat IgG were obtained from Cappel Laboratories Pa. USA.

RESULTS

Table 2 shows the results of the HI titration on several vertebrate sera as measured by JE and D1 antigens. Taking the HI titer of 10 as the positive limit, the antibody positive rate as measured by JE antigen is high in swine (96%), horse (100%), mule (100%), sheep (93%), however, the rate is low in monkey (10%), duck (17%), sparrow (3%), and none in chicken and lizard. These antibody prevalence decreased when HI titers were measured by D1 antigen to the following values: horse 20%, mule 31%, sheep 57%, dog 80%, monkey 10%, duck 13%, respectively. High antibody positive rates

Table 2. HI test on various vertebrates collected in Chiang Mai, 1982

Species of vertebrate	HI titer against JE antigen									HI titer against D1						Total number	
	<10	10	20	40	80	160	320	640	≥ 1280	<10	10	20	40	80	160		
Swine	4				2	10	23	43	18	not tested						100	
Horse				1	5	15	20	6	3	40		6	4			50	
Mule				1	3	16	23	5	2	33	1	2	9	4	1	50	
Sheep	1		2	5	3	2	1			6	2	3	1	1	1	14	
Dog	1		1	2	7	4				3		2	7	3		15	
Monkey	19			2						20			2			21	
Duck	20	3		1						21	2	1				24	
Sparrow	91	3									not tested						94
Chicken	25										not tested						25
Lizard	34										not tested						34

Table 3. ELISA against JE antigen in swine sera, Chiang Mai, 1982

Total number	IgG-ELISA titer				IgM-ELISA titer				
	800	1600	3200	6400	50	100	200	400	800
101	12	11	31	47	11	58	26	5	1
			89				32		
			(88%)				(32%)		

among horses and mules were reasonable, since they are susceptible to JE virus (Clarke and Casals, 1965), and their titers were also high like swine which is the most important amplifier of JE virus. High antibody positive rates and high titer of HI antibodies among sheep and dogs were remarkable.

Table 3 shows the result of IgG- and IgM-ELISA on swine sera. All the swine sera possessed IgG-ELISA titer over 800, which is more than the positive limit of 400 as obtained in the previous studies in Japan (Bundo *et al.*, 1982). almost one-third (32%) of the swine population possessed significant levels of IgM-ELISA titer of over 200 indicating that they were recently infected with JE virus.

DISCUSSION

Johnsen *et al.* (1974) showed that the prevalence of JE virus HI antibodies was high in dogs, bovines, swine, and also in small group of horses and that the geometrical mean titer in swine was higher than in other animals, except horses. Our result is essentially similar to their report and also the data reported by Yamada *et al.* (1971). The latter authors showed that all the slaughtered swine sera collected in June and September 1970 possessed HI antibodies against JE virus, and also that HI antibodies sensitive to 2-mercaptoethanol treatment were found in April and May, indicating infections by JE virus. Antibody prevalence among swine as reported by Johnsen *et al.* (1974) was 89% in July 1970. Since one-third of swine sera collected in our study appeared to have been infected by JE virus recently and *Culex* species of mosquitoes were shown to have been attracted by swine (Mori *et al.*, 1983), *Culex*-swine cycle should be considered as active in Chiang Mai area. Although high antibody positive rate was found for horses, mules, sheep, and dogs, implication of these animals as efficient amplifier of JE virus is rather dubious, because of the small number of population and lower turnover rate. Gould *et al.* (1974) showed that dogs were not frequently bitten by *Culex* mosquitoes. Lower antibody positive rate as measured by D1 antigen, compared with JE antigen, indicated that these animals were not infected with dengue viruses, except monkeys.

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1982年タイ国チェンマイ地区における脳炎のウイルス学的疫学的調査 VI. 動物における抗体価調査

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種々の脊椎動物血清中の日本脳炎 (JE) ウイルスに対する抗体保有状況を血球凝集抑制反応を用いて調査した。ブタ, ウマ, ラバ, ヒツジ, イヌは高率に JE に対する抗体を保有しているのに対して, サル, アヒル, スズメの抗体保有率は低く, ニワトリとトカゲは調査した限り抗体は陰性であった。ブタ血清について免疫酵素測定法を行なった結果, 採血したブタの約3分の1は JE ウイルスの新鮮感染を受けたと考えられる。

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