A Serological Survey of Encephalomyocarditis Virus Infection in Pigs in Japan

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ABSTRACT. A total of 1502 pig sera collected between 1990 and 1991 at 65 farms in 22 prefectures were subjected to neutralization test to encephalomyocarditis (EMC) virus. Of them, three hundred eighty seven pigs (25.8%) from the 55 farms (84.6%) had antibody against EMC virus. The positive rates were rather high in Ehime (53.1%), Tottori (49.1%), Shimane (44.4%) and Nagasaki (38.7%). The positive rate and geometric mean titer of antibody tended to increase with the age. These serological results suggest that the EMC viral infection have been occurred among pigs in Japan.—**KEY WORDS:** encephalomyocarditis virus, pig, serological survey.

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Encephalomyocarditis (EMC) virus, a member of the family *Picornaviridae*, genus *Cardiovirus*, causes a fatal disease attributable to myocarditis and encephalitis in young pigs [1, 3, 9]. Recently EMC virus was proposed as a cause of swine reproductive disorders in Australia [8], Canada [2] and the United States [4, 5, 7]. However, it has been no evidence for the presence of EMC virus in Japan. The present study was undertaken to determine the evidence of EMC virus infection in pigs in Japan by neutralization (NT) test.

A total of 1502 sera were collected between 1990 and 1991 from healthy pigs one-month to 4-year-old which had been no herd history of clinical evidence on EMC virus infection at 65 farms in 22 prefectures which cover all areas of Japan. All sera were stored at -20°C until examined, and were heated at 56°C for 30 min before use. The cell lines HmLu-1 employed for the NT test were grown in Eagle's MEM containing 5% inactivated fetal

calf serum (FCS), 10% tryptose phosphate broth (TPB), and antibiotics. The cells were washed twice with the medium without serum before use. EMC-D variant virus used in this study was kindly supplied by Dr. Ji-Won Yoon, the University of Calgary, Calgary, Alberta, Canada [13]. The NT test was performed with microtiter method. In the wells, 0.025 ml volumes of serial 2-fold dilutions of the serum were made with the maintenance medium (MM) containing MEM, TPB, 0.5% FCS and antibiotics, and each serum dilution was mixed with an equal volume of MM containing 100 TCID₅₀ of virus. After incubation at 37°C for 60 min, the serum-virus mixtures were inoculated to HmLu-1 cell monolayers in 96-well plates. Serum dilutions were tested by duplicate and the NT antibody titer was expressed as the reciprocal of the highest serum dilution inhibiting CPE in at least one of the two wells. The titers of 1:4 or higher were taken as neutralizing titer positive.

Table 1. Neutralizing antibodies against EMC virus in pigs from different prefecture

Prefecture	No. of farms	No. of sera	Positive rate (%)	No. of sera with antibody titer										
				<4	4	8	16	32	64	128	256	512	1024	2048
Hokkaido	2	35	22.9	27	5	2	1							
Aomori	1	41	14.6	35	4	1				1				
Iwate	13	145	17.2	120	12	4	8	1						
Miyagi	5	49	16.3	41	8									
Akita	4	139	23.0	107	14	12	5		1					
Yamagata	5	125	24.0	95	18	4	6	1	1					
Fukushima	1	57	15.8	48	7	1			1					
Niigata	9	289	24.9	217	36	20	7	3	4	1	1			
Ibaraki	2	37	13.5	32	1	3	1							
Saitama	1	9	22.2	7	1	1								
Chiba	2	54	29.6	38	9	4	2	1						
Yamanashi	1	10	30.0	7	2		1							
Mie	1	28	32.1	19	6	2			1					
Tottori	4	99	49.5	50	34	7	6				1			1
Shimane	1	27	44.4	15	7	4		1						
Okayama	3	71	28.2	51	11	7		2						
Hiroshima	3	66	12.1	58	7			1						
Kagawa	1	18	27.8	13	3	2								
Ehime	2	49	53.1	23	16	5	2	2	1					
Kochi	2	43	20.9	34	6	1	1	1						
Nagasaki	1	31	38.7	19	6	4	1		1					
Kumamoto	1	80	26.3	59	13	3	2	2	1					
Total	65	1502	25.8	1115	226	87	43	15	11	2	2			1

Table 2. Neutralizing antibodies against EMC virus from different age

Age in months	No. of sera	Positive rate (%)	GM ^{a)}	No. of sera with antibody titer										
				<4	4	8	16	32	64	128	256	512	1024	2048
1-2	194	13.4	2.2	168	23	1	1	1						
3-4	248	19.4	2.4	200	36	7	3	1	1					
5-12	647	26.1	2.8	478	90	41	20	6	8	2	2			
>13	413	34.9	3.1	269	77	38	19	7	2					1
Total	1502	25.8	2.7	1115	226	87	43	15	11	2	2			1

a) Geometric mean titer. The titer of <4 was taken as 1:2.

The results of the serological survey on EMC virus infection are summarized in Table 1. Three hundred eighty seven (25.8%) of the 1502 pigs from 55 (84.6%) of the 65 farms had neutralizing antibody against EMC virus. The antibody level, however, ranged between 4 and 2048. The positive rates were rather high in prefectures; Ehime (53.1%), Tottori (49.5%), Shimane (44.4%) and Nagasaki (38.7%) located in western and southern areas of Japan. The incidence of antibody by age of pigs is given in Table 2. The positive rates of neutralizing antibody were 13.4%, 19.4%, 26.1% and 34.9% in the age groups of 1-2 months, 3-4 months, 5-12 months and more than 12 months, respectively. The positive rate and geometric mean titer (GM) of antibody tended to increase with the age. Although more serological studies are necessary to obtain the precise epidemiological knowledge of the EMC virus, the serological results presented herein are suggesting that EMC viruses are widely disseminated among pigs in Japan, and EMC virus infection is being occurred primarily among young pigs (less than 12-month-old).

Also, normal pig sera from Britain [11] and Canada [10] showed the positive rates of antibody against EMC virus approximately 28 and 10%, respectively. Similar results were observed in Japan, as mentioned above. Any clinical evidence of EMC virus disease was not found in the serologically positive pigs in Japan and these countries. Recently Kim *et al.* [5] reported that there were variations in the pathogenicity depending on the virus strain. Thus, it is speculated that the strain of virus in Japan is less virulent, and/or epidemiology of EMC virus infection is a little difference. The forms of virus transmission might take place among pigs or from some reservoirs to pigs with a insufficient amount of virus to develop clinical signs of the infection.

Many EMC virus isolates were reported to be antigenically coincided and serologically indistinguishable [6]. Also, EMC virus is shown to be antigenically distinct from

62 known human enteroviruses [12] and no cross-reaction between EMC virus and other porcine enteroviruses has been reported. Consequently, we believe that the NT test results are specific and that the presence of EMC neutralizing antibodies indicates previous infection with the virus.

In this investigation, we provided the first serological evidence for the presence of EMC virus in pigs in Japan. From the result of this serological survey, it is presumed that the EMC viral infection have been occurred among pigs in Japan. However, further precise investigations are required on the existence, etiological role and pathogenicity of the virus.

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