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## BRIEF COMMUNICATION

# APPLICATION OF ELISA TO SERO-EPIDEMIOLOGICAL SURVEY OF JAPANESE ENCEPHALITIS IN SWINE

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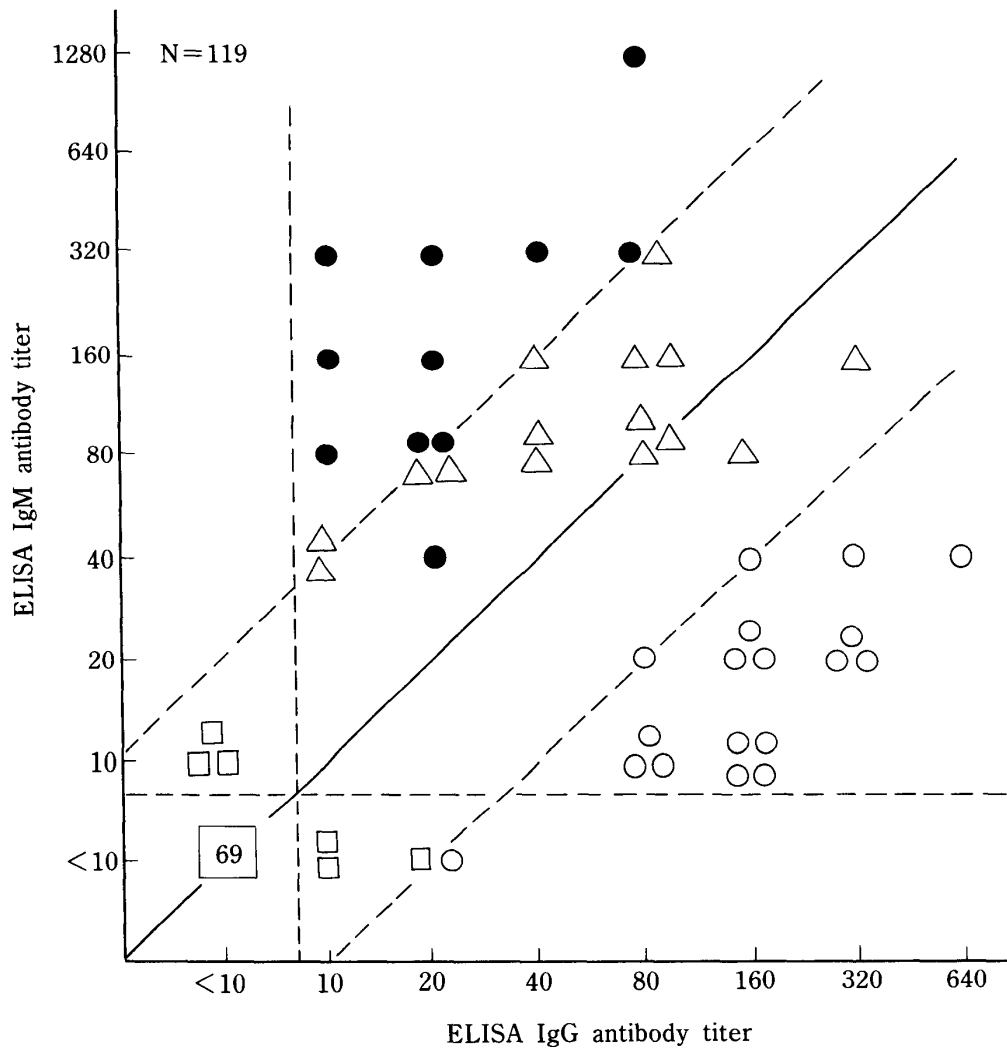
Key words: ELISA, Japanese encephalitis virus, swine

Swine are highly susceptible to Japanese encephalitis (JE) virus infection and play an important role as amplifiers to transmit JE virus to a number of vector mosquitoes, *Culex tritaeniorhynchus*.<sup>1,4,5,8)</sup> In Japan, quite a high proportion of swine has continued to acquire new JE infection every epidemic season, even though the number of human patients of JE have shown a tendency to decrease remarkably in recent years.<sup>2,3,7)</sup> Acquisition of JE antibody by a swine population has been observed at least three or four weeks earlier than in human instances of JE epidemic. Therefore, the sero-conversion to JE virus in swine is considered as a marker to predict JE epidemic in a human population.<sup>4,5)</sup> We reported that an enzyme-linked immunosorbent assay (ELISA) could detect and differentiate both IgM and IgG antibodies to JE virus in the sera collected from experimentally infected swine.<sup>6)</sup> This paper deals with the application of ELISA to a sero-epidemiological survey for the prediction of JE epidemic by detection of IgM and IgG antibodies of JE in swine sera obtained in an epidemic area.

Sera were collected in 1980 from swine at a slaughterhouse in Shizuoka prefecture. ELISA procedure to detect anti-JE IgG and IgM antibodies was described previously.<sup>6)</sup> Hemagglutination inhibition (HI) titers of the sera were also tested before and after 2-mercaptoethanol (2-ME) treatment.

The sera were classified into three groups according to the sensitivities of HI antibodies to 2-ME. ELISA IgG and IgM titers were compared in three groups of sera to see the specificity and sensitivity of ELISA (Fig. 1). In a total 119 sera, 43 out of 46 ELISA IgM antibody positive sera (93.5%,  $\geq 1:10$ ) associated with ELISA

FIGURE 1 Correlation of ELISA IgG and IgM antibody titers against JE virus in swine sera from Shizuoka prefecture



Each symbol shows an individual serum of which HI titer is 2-ME resistant (○), 2-ME not classified (△), 2-ME sensitive (●), and  $\leq 1:10$  (□).

IgG antibody ( $\geq 1:10$ ). Similarly, 43 sera out of 47 ELISA IgG positives (91.5%) had ELISA IgM antibody. These results indicate that the sero-conversions followed by the JE virus infection could be detected practically in either ELISA IgM or IgG test. Similar antibody rates were obtained in ELISA and HI test. Of 119 sera, 50 sera (42.0%) were positive ( $\geq 1:10$ ) either in ELISA IgG and IgM antibodies and 44 sera (37.0%) were positive in HI test. Predominant immunoglobulin (Ig) class of JE antibody was determined according to ELISA IgG and IgM titers and was compared

with 2-ME sensitivity of HI antibody. Most of the 2-ME sensitive sera (10 of 11) were ELISA IgM type sera of which ELISA IgM titer was equal to or higher than 4 fold of ELISA IgG titer. All of the 18 2-ME resistant sera were ELISA IgG type sera of which ELISA IgG titer was equal to or higher than 4 fold of the ELISA IgM titer. Of the 15 sera not classified by 2-ME, nine (60.0%) had almost equal titers in both IgG and IgM antibodies, and six (40.0%) were ELISA IgM type sera of which ELISA IgM titer was 4 fold of ELISA IgG titer. The results show that ELISA is as sensitive as HI test and that classification of Ig class by ELISA corresponded well with 2-ME sensitivity of the HI antibody.

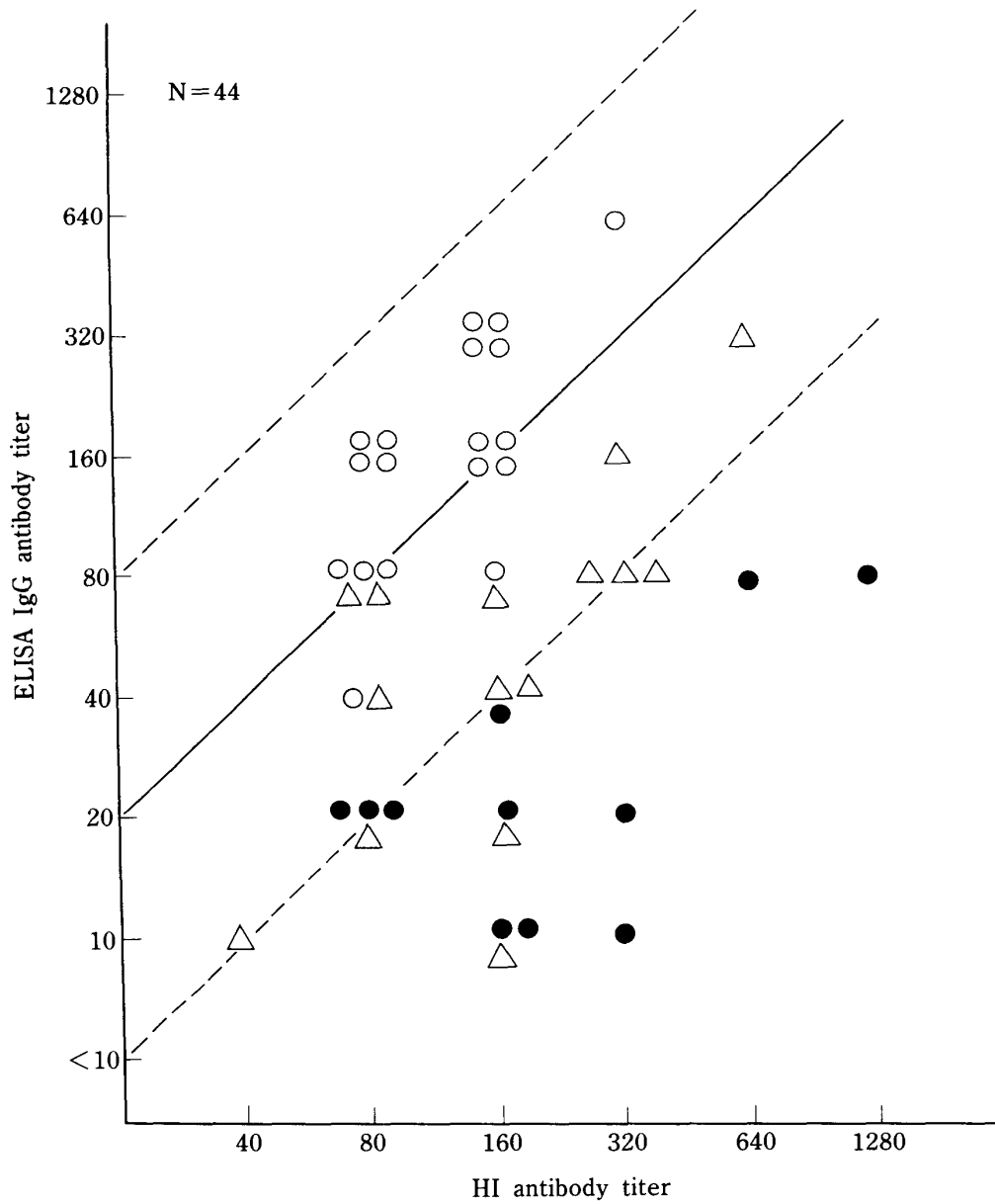
Next, ELISA IgM titers were compared with HI titers to see the correlation of both titers (Fig. 2). The close correlation of both titers was observed only in the group of sera of which HI titers were 2-ME sensitive. ELISA IgG titers were also compared with HI titers to see the correlation of both titers (Fig. 3). In the group of 2-ME resistant sera, ELISA IgG titers were almost equal to the HI titers, and a close correlation was observed between both titers. These results combined with the data of Fig. 1 show that ELISA could successfully differentiate IgG and IgM antibody to JE in swine sera.

To estimate the time of onset of JE epidemic in swine population, antibody rates and type of antibody class to JE virus were determined by ELISA in the sera collected sequentially at a slaughterhouse located in Shizuoka prefecture in 1980 (Table 1). The sera from July 29th and August 5th were negative to JE virus in ELISA. Of the 30 sera collected from September 2nd, 21(70.0%) were positive in ELISA, 11(52.4%) were IgM type, 7(33.3%) were mixed type, and 3(14.3%) were IgG type. And of the 29 sera collected from September 30th, 23(79.3%) were positive in ELISA, IgM type sera decreased to 5(21.7%) and IgG type sera increased to 7(30.4%).

The results presented here show that ELISA was applicable to a sero-epidemiological survey in which the assay could separately detect JE IgG and IgM antibodies in sera from swine naturally infected in an epidemic area. The type of antibody class and titer of antibody corresponded well both in the ELISA and HI test. Differentiation of antibodies to IgG and IgM type is useful to estimate the time of onset of JE epidemic in a swine population. We demonstrated that IgM antibody appeared at 5 to 8 days after JE virus inoculation in the sera from experimentally infected swine.<sup>6)</sup> And IgG antibody appeared at 3 to 4 weeks after JE virus inoculation of the swine. These results, combined with the data in Table 1, suggest that the onset of JE epidemic started at the middle of August in the swine population studied in 1980. ELISA is a rapid and simple assay which requires no acetone treatment of the sera, which is essential in the HI test. Thus, ELISA has a great advantage over the latter especially when used in a mass survey of sera under limited time and space conditions.



FIGURE 3 Correlation of HI antibody titers with ELISA IgG antibody titers against JE virus in swine sera from Shizuoka prefecture



Each symbol shows an individual serum of which HI titer is 2-ME resistant (○), 2-ME not classified (△), and 2-ME sensitive (●).

TABLE 1 *Appearance of ELISA JE antibody in swine sera from Shizuoka prefecture and classification of antibody class in the sera.*

PREDOMINANT TYPE OF ANTIBODY CLASS IN ELISA	NO. OF POSITIVE SERUM/TOTAL NO. OF SERUM COLLECTED ON (%)			
	July 29	August 5	September 2	September 30
IgM	0/30 (0)	0/30 (0)	11/30 (52.4 <sup>1)</sup> )	5/29 (21.7)
Both	0/30 (0)	0/30 (0)	7/30 (33.3)	11/29 (47.8)
IgG	0/30 (0)	0/30 (0)	3/30 (14.3)	7/29 (30.4)
Antibody positive rate in ELISA	0/30 (0)	0/30 (0)	21/30 (70.0)	23/29 (79.3)

1) Positive serum number per total number of positive serum.

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