Detection of Antibody to Japanese Encephalitis Virus (JEV) by Enzyme-Linked Immunosorbent Assay (ELISA)

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To measure levels of antibody to Japanese encephalitis virus (JEV) in equine serum, an enzyme-linked immunosorbent assay (ELISA) was applied. Purified JEV particles of BMIII strain, derived from Beijing-1 strain, were physically adsorbed onto an immunoplate and the ability to react with anti-JEV antibody was tested. Whole virus particles reacted with the antibody, but the NP-40 lysed antigen did not. The ELISA titer correlated with the virus neutralization (VN) titer and the hemagglutination inhibition (HI) titer, although correlation coefficients were not very high. When the time course of the sera of immunized horses was measured by these three assays, the ELISA response was detected later than the VN and HI responses in 3 of 4 horses. This system can be useful for screening antibody to JEV.

Key words: Japanese encephalitis, equine, ELISA

Japanese encephalitis (JE) is caused by JEV and transmitted by mosquitoes. Many species of vertebrates including horses are susceptible to this disease. Horses infected with JEV usually do not show clinical signs, although once clinical signs appear the mortality rate is high. Epidemics of JE sometimes occurred until 1948, when an inactivated vaccine was developed; thereafter, the occurrence of epidemics gradually decreased. To prevent epidemics, it is important to keep high levels of antibody. For the determination of antibody levels, the enzyme-linked immunosorbent assay (ELISA) is a suitable system to measure many samples in a relatively short period of time. There are many reports on the detection of antibody to JEV by ELISA, but not in horses [1–3, 6–7]. In the present study we have developed an ELISA system mainly for racing horses as part of their medical inspection.

BMIII strain of JEV was grown on MPKIIIs, a line cell derived from porcine kidney cells. The culture supernatant was concentrated 20 times by ultrafiltration; the concentrate was further concentrated by ultracentrifugation at 28,000 rpm in a TST28, 38 rotor (Contron). The pellet was resuspended in PBS and was centrifuged at 36,000 rpm for 2 hr in an SW40 rotor (Beckmann) with a discontinuous sucrose gradient of 35%, 50% and 58%. Interface fractions between 35% and 50%, and 50% and 58% were collected and then dialyzed against PBS. Both fractions were pooled and used as antigen for ELISA.

The ELISA procedure was as follows. Virus particle antigen, whose HA titer was 32,000, was diluted 80 times with 0.05 M carbonate buffer (pH 9.6) and added to flat plates (Nunc) at 50 µl/well. The plates were freeze-dried. The antigen-coated plate was treated with 1% gelatin solution at 37°C for 1 hr to minimize nonspecific binding. The test serum was diluted 800 times with 1% bovine serum albumin (BSA) in PBS and then added to the plate at 50 µl/well and incubated at 37°C for 40 min. After rinsing, 50 µl of horseradish peroxidase-labeled anti-horse IgG (H+L) was added to each well and the plate was incubated at 37°C for 20 min. After rinsing, the colorimetric reaction was initiated by adding 50 µl of TM102 (Intergen, Milford) to each well and then incubating the plate at room temperature for 10 min. The reaction was stopped by adding 1 M sulfuric acid, and the optical density (OD) of the yellow color was measured at 450 nm. These OD values were taken as...
ELISA titer in this paper.

The virus was first lysed with 1% NP-40 and its ability to react with anti-JEV antibody was tested. Neither positive serum nor negative serum reacted with the lysed antigen. The reason why lysed antigen was unreactive is unclear.

Secondly, whole virus antigen was coated onto the immunoplate and its reactivity was tested. Because the optical density was highest at 40–80 times dilution of the antigen, 80 times diluted whole virus antigen was used for the following experiment.

To test the reliability of the ELISA system, sera from 40 field horses and 47 experimentally infected horses were assayed by the virus neutralization (VN) test, the hemagglutination inhibition (HI) test and ELISA. The correlation coefficients \( r \) between ELISA and VN, and ELISA and HI were 0.517 and 0.631, respectively [Figs. 1 and 2]. These values were relatively low compared with the correlation coefficient between VN and HI (0.835). We propose three reasons why the \( r \) values were not very high. First, there were 15 samples whose ELISA titers were more than 2.0. For the calculation, these samples were taken as 2.0, which may have lowered the \( r \) value. Second, in this experiment, the ELISA titer was measured for only one serum dilution. The ELISA titers were linear but the VN and HI titers fit a logarithmic function, and so we removed the 15 samples and plotted the ELISA titers logarithmically. The \( r \) values between ELISA and VN, and ELISA and HI were 0.644 and 0.721, respectively. It seems that the ELISA titer can be correlated to the VN and HI titers. Third, nonspecific reactions were high. Miyata et al. prepared ELISA antigen from JEV-infected mouse brain and observed similar nonspecific reactions, and they suggested that these nonspecific reactions may be due to a mouse brain component in the viral antigen [1]. We used a virus grown on a cell culture, but we could not eliminate nonspecific reactions.

To observe the time course of the antibody response, 4 horses were immunized and serum samples were collected weekly. In horse #1, VN and HI antibody titers rose 2 weeks after the first immunization, but the ELISA antibody titer rose only after the second immunization [Fig. 3-1]. In horse #2, all three antibody titers rose simultaneously [Fig. 3-2]. In horse #3, although the three antibody response curves were similar, the ELISA antibody response was delayed 3 days compared with the VN and HI antibody responses [Fig. 3-3].
Fig. 3-1. Time course of ELISA, HI and VN titers of JEV immunized horse #1. Horse #1 was injected intramuscularly (IM) and intravenously (IV) with $10^{9.0}$ TCID$_{50}$ of live at strain on day 0, IM with $10^{9.0}$ mouse lethal dose 50 (MLD$_{50}$) of live Beijing-1 strain on day 28, and IM with $10^{8.7}$ MLD$_{50}$ of killed Beijing-1 strain at day 70. The ELISA titers were expressed as OD$_{450}$ of 800 fold dilution of the samples. The ELISA titers more than 2.0 were taken as 2.0. The HI test was done by the macro-method described by Clark and Casals [4] with JaGAr#01 strain as the HA antigen. HI titers of less than 10 were taken as 1. The VN test was done by the 50% plaque reduction method described by T. Kumanomido et al. [5] with slight modification, with Beijing-1 strain as the control virus. Titors are expressed as the log$_{10}$ of the serum dilution which neutralizes 50% of the plaques. Abbreviations L: live virus, K: killed virus.

Fig. 3-2. Time course of ELISA, HI and VN titers of JEV immunized horse #2. Horse #2 was injected IM and IV with $10^{7.0}$ TCID$_{50}$ of live at strain on day 0, IM with $10^{8.0}$ MLD$_{50}$ of live Beijing-1 strain on day 28, and IM with $10^{8.7}$ MLD$_{50}$ of killed Beijing-1 strain on day 70. The ELISA titers were expressed as OD$_{450}$ of 800 fold dilution of the samples. The ELISA titers more than 2.0 were taken as 2.0. The HI test was done by the macro-method described by Clark and Casals [4] with JaGAr#01 strain as the HA antigen. HI titers of less than 10 were taken as 1. The VN test was done by the 50% plaque reduction method described by T. Kumanomido et al. [5] with slight modification, with Beijing-1 strain as the control virus. Titors are expressed as the log$_{10}$ of the serum dilution which neutralizes 50% of the plaques. Abbreviations L: live virus, K: killed virus.

Fig. 3-3. Time course of ELISA, HI and VN titers of JEV immunized horse #3. Horse #3 was injected IM and IV with $10^{9.0}$ MLD$_{50}$ of live Beijing-1 strain on day 0 and IM with $10^{9.0}$ MLD$_{50}$ of live Beijing-1 strain on day 28. The ELISA titers were expressed as OD$_{450}$ of 800 fold dilution of the samples. The ELISA titers more than 2.0 were taken as 2.0. The HI test was done by the macro-method described by Clark and Casals [4] with JaGAr#01 strain as the HA antigen. HI titers of less than 10 were taken as 1. The VN test was done by the 50% plaque reduction method described by T. Kumanomido et al. [5] with slight modification, with Beijing-1 strain as the control virus. Titors are expressed as the log$_{10}$ of the serum dilution which neutralizes 50% of the plaques. Abbreviations L: live virus, K: killed virus.

Fig. 3-4. Time course of ELISA, HI and VN titers of JEV immunized horse #4. Horse #4 was injected IM and IV with $10^{9.0}$ MLD$_{50}$ of live Beijing-1 strain on day 0 and IM with $10^{9.0}$ MLD$_{50}$ of live Beijing-1 strain on day 28. The ELISA titers were expressed as OD$_{450}$ of 800 fold dilution of the samples. The ELISA titers more than 2.0 were taken as 2.0. The HI test was done by the macro-method described by Clark and Casals [4] with JaGAr#01 strain as the HA antigen. HI titers of less than 10 were taken as 1. The VN test was done by the 50% plaque reduction method described by T. Kumanomido et al. [5] with slight modification, with Beijing-1 strain as the control virus. Titors are expressed as the log$_{10}$ of the serum dilution which neutralizes 50% of the plaques. Abbreviations L: live virus, K: killed virus.
may be because in our ELISA system IgM antibody can not be detected. In horse #4, the ELISA antibody titer was highest at day 28, which was two weeks later than the first peaks of VN and HI antibody titer (Fig. 3-4).

In conclusion, we have developed a quick ELISA system whose total reaction time is 70 min to detect the anti-JEV antibody mainly in racing horses using whole virus particle antigen.

ELISA titers correlated with VN and HI titers, but there were some problems.

1. The correlation coefficients between ELISA and VN, and ELISA and HI were not very high.
2. There was one sample which showed high (0.416) nonspecific ELISA titers.
3. In some individuals ELISA titers were detectable later than VN and HI titers.

For the above reasons this ELISA system may not be suitable for measuring antibodies as individuals. But it can be useful as a first screening or for measuring the antibody level as a group.

References