Application of Latex Beads Agglutination Test for the Detection of the Antibody against Virus-Infection-Associated (VIA) Antigen of Foot-and-Mouth Disease (FMD) Virus

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ABSTRACT. Latex beads agglutination (LA) for the detection of the antibody against virus infection-associated (VIA) antigen of foot-and-mouth disease (FMD) virus was estimated using experimentally infected animals. The VIA antibody titer by the LA test were compared with the neutralization titer and the titer by agarose gel diffusion (AGD) test, which has been used as a standard method for VIA antibody titration. The latex beads were coated with VIA antigen in carbonate buffer solution (0.5M, pH9.6) for the test. The sensitivity of the LA test was clearly higher than that of the AGD test in the results for cattle and swine infected experimentally. The antibody was detected in the bovine serum obtained at the 13th week after inoculation by the LA but not by the AGD test. The LA test appears to be simple, rapid and sensitive for the detection of the antibody of FMD virus in the surveillance of FMD and the FMD quarantine of imported animals.

KEY WORDS: foot-and-mouth disease, latex beads agglutination test, VIA antigen.

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Virus-infection-associated (VIA) antigen of foot-and-mouth disease (FMD) was first described by Cowan and Graves in 1966 [1]. It was regarded that VIA antigen was a nonstructural antigen corresponding to viral RNA-dependent RNA polymerase [5], and that the antibody to VIA antigen (VIA-antibody) was produced in an animal infected or multi-vaccinated [2, 4]. Moreover, the VIA-antibody reacts with the VIA antigen from any type of FMD virus [1]. The agarose gel diffusion (AGD) test has been widely used as a standard method for the detection of VIA-antibody. The purpose of the present paper was to find a more sensitive method to replace the AGD test, which is not very sensitive. The latex beads agglutination method (LA) was evaluated as a method for the detection of VIA antibody.

The VIA antigen used in the experiments was obtained from inactivated FMD vaccine, as described before [6]. The molecular weight of the purified VIA antigen was approximately 56,000. The AGD tests were carried out on slide glass (76 × 26mm). Five ml of one per cent agar in 0.02 M tris buffer solution at pH 7.6 with 0.15 M NaCl was dispensed onto each slide glass. The test pattern consisted of a center well surrounded at a distance of 1.5 mm by six peripheral wells, each 2 mm in diameter. The center well was filled with VIA antigen. Two opposing peripheral wells were filled with positive serum (convalescent bovine serum) and the other four wells were used for detection of VIA-antibody in sera.

For sensitization of the latex beads, 37.5 ml of 1% Latex beads (Polybead, 0.96 µm in diameter, Polyscience, Inc.) in carbonate-bicarbonate buffer solution (pH 9.6) and VIA antigen, the titer of which was 1:4 in AGD, were mixed thoroughly. The mixture was kept overnight at 4°C. The excess

of VIA antigen was washed out with PBS (pH 7.3) three times. After the last washing, the latex beads were resuspended at a 0.2% concentration in same PBS containing 0.5% horse serum. Fifty microliters of a two-fold serial dilution of test serum was put in each well of a 96 well microplate (Round bottom), and then, 50 µl of 0.2% latex beads suspension was added into each well with diluted serum. They were mixed carefully and kept at room temperature. The results were judged by naked eye on the following day. The known positive and negative control sera were kept for each test.

A 96-well microplate was used for the neutralization test. The serum was serially two-fold diluted using Eagle's MEM without bovine serum. Twenty-five microliters of diluted serum was added into two wells and mixed with the same volume of homologous virus (200 TCID₅₀/0.1 ml) used for the experimental infection. After one hour incubation period, 0.1 ml of BHK cell suspension (25,000 cells/well) in Eagle's MEM containing 20% bovine serum was added to each well, and each plate was incubated in CO₂ incubator at 37°C. The two wells were used for one dilution of serum. The reciprocal of the maximum serum dilution inhibited showing CPE completely in one or two wells was judged to be the neutralization titer of each serum.

Test sera used for the detection of VIA-antibody were obtained from four cattle and four pigs infected experimentally. Two (No. 628 and 629) of the cattle were observed for 18 weeks after infection with FMD virus O_{Bangkok60}. The other two (Nos. 601 and 602) were vaccinated with inactivated vaccine Type O_{Bangkok60} and inoculated with the homologous live virus at the 3rd week after the vaccination, and observed for 13 weeks. Pigs (Nos. 6228 and 6209) were inoculated with FMD

Fig. 1. Development of antibodies in cattle inoculated with FMD virus type O_{Bangkok60}-.

virus, A_{Nokornpathom82} and observed for 8 weeks. The other two pigs (Nos. 6103 and 6154) were inoculated with same type of FMD virus after two vaccinations with inactivated FMD virus, A_{Nakompatom82} on the 3rd and 4th weeks (one week interval) before the inoculation.

The results obtained from the four cattle infected experimentally are shown in Fig. 1. Cattle Nos. 628 and 629 were observed until the 18th week after the inoculation. Their serum neutralization (SN) titer rose in the first week after the inoculation, and reached a maximum (1:512) in the 6th week. Two cattle keep the SN antibody at a titer of more than 1:32 on the 18th week after the inoculation (the end of this experiment). The VIA-antibody was first detected in the 2nd week after the inoculation both in the AGD and LA tests, and reached a maximum one or two weeks later in both tests. The titer of antibody in the LA test was always higher (from 2 to 64 times) than in the AGD test. Cattle Nos. 602 and 601 were vaccinated once before the virus inoculation. The SN antibodies were detected at the time of challenge inoculation (their titer; 1:4 each). The SN antibody titer rose much rapidly in the first week (1:512 each) after the inoculation and reached a maximum (1:1,024) in the 2nd week. Although the titer gradually decreased, the SN antibody was still detected on the 13th

week after the challenge. The VIA-antibody was first observed in the 2nd week after inoculation, and was already the maximum. The titers in the AGD test were 1:1 and 1:4 in Nos. 602 and 601, respectively. The titer in the AGD test was maintained until the 5th week in No. 602 and the 11th week in No. 601 cattle. The VIA-antibody titers in both cattle were also detected as a higher titer in the LA test than in the AGD test. The VIA-antibody was detected in the second week after inoculation in both tests, but the LA titers were 1:16 in both cattle, which was 16 or 4 times as high as in the AGD test. The titer in LA test was not only higher but continued for a longer period, until 12th week or later in No. 602.

Four pigs were used for the next experiment. Two of them (Nos. 6228 and 6209) were inoculated with FMD virus and observed for 7 weeks. The other two pigs (No. 6103 and No. 6154) were previously vaccinated and inoculated with challenge FMD virus. Their antibody development was examined by AGD, LA and SN titers for seven weeks. The results are shown in Fig. 2. Pig Nos. 6228 and 6209 showed the SN antibody (1:64) in the first week. The antibody titer increased but never reached more than 1:256 until the 7th week. In the LA test, the development pattern of the antibodies was similar to that in the AGD test, but the titer in LA was 4–32 times as high

Fig. 2. Development of antibodies in swine inoculated with FMD virus type A_{Nakoknatom82}.

as in the AGD test. The VIA-antibody was detectable in the LA test in the 6th or 7th week. Pigs Nos. 6103 and 6154, vaccinated previously, showed the SN antibodies (1:8) at the time of the challenge inoculation. The titer reached a maximum in the 2nd week in the AGD and LA tests, and was detectable even in the 7th week (the end of this experiment). The titer in the LA test was 2–8 times as high as in the AGD test.

The VIA-antibodies were detected in animals infected or multi-vaccinated, regardless of which FMD serotype was involved [4]. The VIA-antibody detection has proven to be very useful as an economical way to examine large numbers of serum samples in order to detect animals having the FMD virus. The SN antibody was more sensitive and continued for more long period, but, SN antibody is specific to a subtype of virus particle, which is different from nonstructural antigen (VIA antigen). Therefore, SN antibody and VIA-antibody are very different from each other and are difficult to compare. The AGD and LA tests detect the antibody to the same antigen, and in the present experiments both tests were compared. Good results for the VIA-antibody detection by the LA test were obtained in this experiment. The LA test was much more sensitive than the AGD test for both cattle and swine serum in this study. Although, the VIA-antibody detection by the LA test requires overnight incubation before judgement, a new

application of this method would overcome this demerit. If agglutination of LA beads on the slide glass becomes possible, the LA test will become more practical and useful. For this reason, kinds and sizes of latex beads and the quantity of VIA antigen for coating them should be investigated in further studies.

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