DETECTION OF ANTIBODIES AGAINST 3D PROTEIN OF FOOT AND MOUTH DISEASE VIRUS BY A LIQUID PHASE BLOCKING SANDWICH ELISA

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The detection of antibodies (Ab) against the virus infection associated antigen (VIA) whose main component is the viral RNA polymerase (3D) has been used to study the presence of animals that have been infected with foot-and-mouth disease virus (FMDV). The Agar Gel Immunodifussion test (AGID) which employs the VIAA, has been a useful tool in monitoring Control and Erradication Programs for FMD, as an indirect indicator of viral activity in population of susceptible hosts. To improve the sensitivity of the AGID and to use a biologically safe antigen (Ag), we have previously developed a liquid - phase blocking sandwich (LPBS) ELISA using a bioengineered 3D protein (ELISA-3D). In this report we have extended its evaluation on sera from vaccinated animals. The method was also applied to epidemiological surveys in an endemic FMD area.

MATERIALS AND METHODS

Experimental animals: sera from 51 calves after vaccination with commercial tetravalent inactivated oil adjuvanted vaccines collected during one year and a half were included. These calves belong to the Mesopotamiam region free of FMD outbreaks since 1992. In addition, 15 unvaccinated sheep were used as controls of viral activity. Serum samples from cattle aged 6 - 12 months (n: 542) collected before full vaccination campaign of 1993-94 and 95 were also included. ELISA-3D: The detection of Ab against the 3D protein was carried out using the liquid-phase ELISA described previously, which uses as antigen the 3D protein of FMDV expressed in E. coli as a fusion protein with GST. AGID: The detection of Ab against the VIA antigen was carried out according to the method describes by Alonso et al (1984). Oesophagol - pharingeal fluid (OPF) samples: Samples of OPF were recovered from positive animals for VIA Ab at 90 days post-revaccination (dprv) by the probang cup method (n: 12).

RESULTS

It is well known that inactivated vaccines against FMDV can induce Ab against the VIA antigen, although the levels of Ab and their persistence is lower and shorter than in infected animals. In our assay, no anti-VIA and anti-3D Ab were detected after the initial vaccination. Following the 2nd vaccination positive animals were detected by both methods. The immune response disappeared 60-120 days post-revaccination (dprv) according to the AGID method and 90 -180 dprv when the ELISA-3D was used. After the third vaccination a strong and lasting response were detected in the different groups by both methods.The sentiinel group (15 sheep) were found negative by both methods for the entire duration of the assay. The samples of OPF taken from anti-VIA positives animals at 90 dprv were negative for FMDV isolation at 120, 150 and 180 dprv. Based on our results previously obtained working with vaccinated animals, we choose the group of animals ranging between 6 months to 1 year of age for seroepidemiological studies for viral activity evaluation. They had received at least one dose of vaccine 4 - 6 months prior of the sample collection. The prevalence of 3D Ab in this group of animals showed a decrease from 1993 to 1995 surveys. Comparison of the results with those obtained with the routinely used AGID test revealed a good correlation between both techniques.

DISCUSSION

Serological assays which detect Ab to non-structural proteins have shown specificity in assessing the post-infection state and offers various advantages for use in epidemiological surveys and in serology for certification of FMD free animals. The Ab response induced by some vaccines was transient and increased as the number of vaccinations increased. In conclusion, results obtained here suggests that the ELISA-3D could be applied as a complementary method in epidemiological studies assuming that a good record keeping system of vaccination is available and for certification of FMD free condition for international trade. The method is rapid and simple to perform, and additionally the use of a bioengineered antigen has advantages for practical application such as safety and a consistent source of antigen.

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