DEVELOPMENT AND EVALUATION OF IGM ELISA FOR THE DETECTION OF FMDV SPECIFIC IGM ANTIBODIES IN BOVINE AND OVINE SERA

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Introduction
FMDV infected cattle and pigs usually develop obvious signs of the disease but in sheep and goats diagnosis is more difficult because the manifestation of the disease is often mild. Therefore, an easy laboratory diagnostic assay is necessary to detect disease in early phase of outbreak. It is well known that the first serum neutralisation antibodies are IgM that appears within 7 days following infection or vaccination. Therefore the main aim of this study was to develop and evaluate IgM assay for detection of early FMDV infection and to find out whether this assay can be used as a DIVA test.

Materials and methods
An indirect ELISA was developed for the detection of FMDV specific IgM antibodies in bovine and ovine sera from FMDV vaccinated and subsequently infected experimental animals (cattle=50 and sheep=36) as well as from field outbreaks animals (n=521) with and without previous vaccination status.

Results
234 naïve cattle and 36 naïve sheep serum samples were used to find out the normal frequency distribution and 100% specificity of the assay is obtained at a cut off of 0.3 OD. High level of IgM antibody was evident on 7th day of post-infection in unvaccinated animals. The vaccinated animals revealed high titre of IgM antibody starting from 7 days of post-vaccination up to end of second week and in some case up to end of 3rd week of post-vaccination. Vaccinated and subsequently contact challenged transiently infected animals were seen positive in IgM assay from 7 to 21 days of post-challenge and if the animal acquired a carrier status the IgM antibody level increased further. Sera obtained from early phase of outbreaks were found positive whereas sera from late phase of outbreak were found mostly negative in IgM assay.

Conclusions
The IgM test has potential to identify early infection in field outbreaks, particularly in unvaccinated populations of sheep where lesions are very difficult to find out. Though conventional FMD vaccine induces IgM antibody in early stage of vaccination and the antibody does not persist long and only reappears if the animal receives FMDV infection, the test has potential to identify infection in vaccinated population as a DIVA screening or confirmatory test.

1. INTRODUCTION:

During and following the 2001 FMD outbreak in the UK and the Netherlands, there has been a growing demand for vaccination as an alternative to large-scale slaughter for the control of FMD. To demonstrate the absence of infection in vaccinated population, serological surveillance needs to be based on the detection of antibodies to the non-structural proteins of FMDV. ELISAs that measure antibodies to FMDV NSPs can be used for differentiating infection in vaccinated animals (DIVA) as purified FMDV vaccine elicits antibody against only structural proteins whereas natural infection elicits antibodies against both structural and non-structural proteins. Available NSP antibody tests were validated in Brescia, Italy under the scope of an EU funded International research group, a consortium of European reference laboratories (Brocchi et al., 2006). The
specificity of the tests ranged between 97 to 98% whereas the sensitivity of the tests to detect viral carriers in vaccinated and subsequently infected cattle ranged from 68 to 94% depending on the test used. The workshop concluded that two tests should be carried out simultaneously to increase the specificity up to 99.99%. Therefore, there is a need to develop further serological test which can work as a confirmatory test to existing NSP antibody test. Further, FMDV infected cattle and pigs usually develop obvious signs of the disease but in sheep and goats diagnosis is more difficult because the manifestation of the disease is often mild. Therefore, an easy laboratory diagnostic assay is necessary to detect disease in early phase of outbreak. It is well known that the first serum neutralisation antibodies are IgM that appears within first week following infection or vaccination (Collen, T. 1994). Therefore the main aim of this study was to develop and evaluate IgM ELISA for detection of early FMDV infection and to find out whether this assay can be used as a DIVA test.

2. MATERIALS AND METHODS

IGM ELISA

An indirect ELISA for the detection of IgM antibodies to structural proteins was developed. Semi-purified, concentrated, inactivated FMDV vaccine antigen in blocking buffer was added to alternate columns of 96 well ELISA plates (Nunc™, Denmark) that had been pre-coated with a polyclonal rabbit anti-FMDV antibody, as in the IgA ELISA [Parida et al., 2006]. After washing, 50 µl of diluted test serum was added in blocking buffer in the plates and then incubated for one hour at 37°C. After a further wash step, specific bovine IgM was detected using a polyclonal rabbit anti-bovine IgM HRPO conjugate. After a final wash the test was developed by the addition of substrate. The reaction was stopped after suitable colour development by the addition of 1M sulphuric acid and the plates read on a multi-channel spectrophotometer at 490nm (A_{490}). ELISA results were expressed as optical density values after subtracting the OD of without antigen well from OD of antigen positive well.

Test serum samples

Serum samples were collected from vaccinated challenged cattle (Cox et al., 2005 & 2006; Parida et al., 2006) and sheep (Parida et al., 2008) experiments conducted at Pirbright high containment isolation facility. Serum samples from 2001 UK outbreak were also used in the assay.

3. RESULTS AND DISCUSSION

Serum from 234 naïve cattle and 36 sheep were used to find out the normal frequency distribution (Fig. 1) and at a cut off of 0.3 OD value 100% specificity was obtained.

![Frequency Distribution](image.png)

**Fig 1:** Normal frequency distribution of negative sera originated from cattle and sheep.
High level of IgM antibody was observed during first week of post-infection which stayed up to 3rd week post-infection in unvaccinated sheep and cattle (Fig 2).

Fig 2: IgM antibody titre remains high in unvaccinated infected sheep (A) and cattle (B) post-challenge.

The vaccinated animals revealed high titre of IgM antibody starting from 7 days of post-vaccination up to end of second week and after challenge if the cattle acquired a carrier status the IgM antibody level increased further. All the 9 carriers detected by virus isolation and real-time RT-PCR were detected by IgM assay where as IgA and Cedi NSP test could detect only 7 carriers (Parida et al., 2005 and 2006). However, one carrier, UV 2 which was detected only twice by RT-PCR after 28 days post-challenge was undetected by Cedi test, but detected by IgM test at some time points. Therefore IgM test may have potential as a confirmatory test to the NSP test.

Fig. 3: High IgM antibody titre observed in vaccinated cattle during 2nd week of vaccination and after challenge if the animals become FMDV carrier.

The serum samples were also analysed from cattle vaccinated 3 times with 21dys intervals. IgM antibody was observed in all the 6 multiply vaccinated animals after 4 days of first vaccination and the level of antibody continued to stay up to end of second week of vaccination. There was no elevated IgM response observed after the second vaccination though 2 out of six cattle showed high IgM antibodies after 3rd vaccination. However 5 out of 6 vaccinated cattle scored positive in IgM assay after challenge though all the 6 animals were free from clinical lesions and persistent infection.
521 serum samples of ovine origin submitted in the early stage of 2001 outbreak were analysed in the IgM assay. These samples were originated from in and around the farms where clinical lesions were seen. 16 samples were scored positive in IgM test. Another 147 serum samples from sheep from 2001 outbreak those were submitted after the outbreak during serosurveillance period were found negative in the IgM assay. This results correlates well with the results obtained from experimental samples that IgM detect infection in early days of infection.

4. CONCLUSION:

Levels of FMDV specific IgM antibodies become elevated after vaccination, during acute phase of infection and continue to be higher in FMDV carrier animals. Therefore IgM assay may be a useful tool to detect disease in early phase of outbreak, especially in sheep.

5. RECOMMENDATION:

- The IgM detection test may have potential to work as DIVA test for the detection of infection in vaccinated population.

6. ACKNOWLEDGEMENT

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7. REFERENCES


