DIAGNOSIS OF PERSISTING INFECTIONS OF FOOT-AND-MOUTH DISEASE VIRUS IN CATTLE: IG A ELISA, VIRUS ISOLATION AND RT-PCR.

P. Moonen, L. Jacobs, H. Costa, A. Crienen, and R. S. Schrijver
Institute for Animal Science and Health, Department of mammalian virology, Lelystad, The Netherlands

Key words: foot-and-mouth disease, persisting infection, diagnosis, RT-PCR, IgA ELISA

Introduction
Foot-and-mouth disease (FMD) is one of the most important diseases from both a veterinary as well as an economic point of view. Outbreaks with devastating economic consequences still occur and remain a terrible threat to countries that have eradicated the disease and to those that never had the disease. The main concern nowadays is how to prevent introduction and, in case of an outbreak, how to prevent spread of the virus. In this respect a critical issue is the occurrence of carrier animals and their risk in transmitting the virus (3, 6). Carrier animals are currently defined as animals from which FMDV can be isolated from oropharyngeal fluid (OPF) more than 28 days after infection. However, virus isolation (VI) from carriers is intermittent. To improve detection of carrier animals, virus isolation, polymerase chain reaction (PCR), IgA ELISA and virus neutralisation test (VNT) were compared. For that purpose animals were infected with FMDV Aτur 14/98 and samples were taken at regular intervals during an eight months period after infection.

Materials and methods
Fifteen cattle were vaccinated with Aτur 14/98: five animals with a full dose (nrs 1315-1319), five animals with an 1/4 dose (nrs 1320-1324) and five animals with 1/16 dose (nrs 1325-1329), two animals were not vaccinated. After three weeks all animals were infected with Aτur 14/98. Two months after infection a sentinel animal was placed between the infected cattle. The animals were sampled weekly; serum samples were tested in an IgA ELISA and VNT; sputum samples, collected using a probang sampler and diluted 1:1 in EMEM +2% FBS +2% antibiotics, were tested in a PCR and after treatment with 1,1,2-trichlorotrifluoroethane (5) in a virus isolation and IgA ELISA.

VI was performed in eightfold in microtiter plates by adding 50 µl sputum to 150 µl porcine kidney cells. After three days incubation the plates were read microscopically and, after staining with amidoblack, macroscopically. Supernatants were additionally tested in an IDAS ELISA for antigen content. RT-PCR as performed using primers and hybridisation probes selected on the 3D nucleotide sequence of Aτur 14/98 and were analysed using Lightcycler® technology (Roche). The isotype specific IgA ELISA was done essentially as described by van Zaane and IJzerman (6). The VNT was done as described by van Maanen et al. (2).

Results
The results of the VI are shown in figure 1. The animals vaccinated with the highest dose (nrs 1315-1319) shed virus for a longer time period than animals vaccinated with lower doses. Using RT-PCR more samples were found positive than using VI.

Figure 1: Results of the virus isolation and PCR in probang samples. Positive isolations and PCRs are depicted +, periods of intermittent positive findings are shaded light grey for VI and dark grey for PCR.

Of the 465 samples tested in VI and PCR, 35 were positive in both assays, 127 were positive in PCR but negative in VI and 43 were positive in VI and negative in PCR, 260 samples were negative in both assays (Fig. 2). Within 10 days after infection neutralisation titres in all animals raised from 1 \(10^{3}\) log after vaccination, to comparable levels of ca. 3.5 \(10^{3}\) log. Representative profiles of neutralisation titres and IgA responses detected in serum and probang samples are shown in figure 3.

Figure 2: Comparison of reactions of probang samples in virus isolation and PCR.

<table>
<thead>
<tr>
<th></th>
<th>VI+</th>
<th>VI-</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR+</td>
<td>35</td>
<td>127</td>
<td>162</td>
</tr>
<tr>
<td>PCR-</td>
<td>43</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In serum, shortly after infection, IgA levels raised, and decreased within 10 to 20 weeks to baseline levels. In sputum IgA levels were hardly detected. Only in a few cases OD450 values higher than 1 were reached. The sentinel animal was negative for VI, PCR, VNT and IgA in probang during the entire period of sampling.

**Discussion**

VI is the standard method to detect carriers of FMDV, however, other techniques may be more suited, such as assessment of specific antibody responses as suggested by Archetti et al. (1) or detection of viral RNA by RT-PCR (4). We found that detection of IgA responses in serum were not suitable to detect carriers. Although IgA antibodies were detected up to 150 days post infection, correlation between the rate of decline of the antibodies titres and the presence of virus in probang samples was not clear. IgA detection in probang samples was, in our hands, difficult. No, or only low levels were detected, and no correlation was found between virus detection in VI or RT-PCR and reaction in the IgA ELISA.

RT-PCR seemed to be a more suitable technique to detect carriers of FMDV. The number of positive samples found in RT-PCR was approximately 17% higher than the number of samples found in VI. Only 8% of the samples was positive in both, VI and the RT-PCR. Approximately 9% of the samples was positive in VI and negative in RT-PCR. This can be due to the low virus content of the sample and the smaller volume of the sample (10x) tested in the RT-PCR compared to VI. Approximately 27% of the samples was positive in the RT-PCR and negative in the VI. If virus is neutralised, it will not be detected in the VI but will be detected in the RT-PCR. In conclusion: RT-PCR is more sensitive than VI for detection of FMDV in probang samples and moreover more easy to perform and less time consuming.

**References**