Detection of foot-and-mouth disease virus in infected pigs by RT-PCR four weeks after challenge

K. ORSEL, H. I. J. ROEST, E. M. ELZINGA-BRIL, F. VAN HEMERT-KLUITENBERG, A. DEKKER

Veterinary Record (2008) **162,** 753-754

K. Orsel, DVM, PhD, Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands
H. I. J. Roest, DVM,
E. M. Elzinga-Bril, DVM, PhD,
F. van Hemert-Kluitenberg, DVM, PhD,
A. Dekker, DVM, PhD, Central Veterinary Institute of Wageningen University and Research Centre, PO Box 65, 8200 AB Lelystad, The Netherlands

Correspondence to Dr Dekker

FOOT-and-mouth disease (FMD) is a contagious viral disease of cloven-hoofed animals including ruminants and pigs. The occurrence of disease in livestock has a great economic impact, especially for exporting countries. Export limitations are based partly on the existence of FMD carrier animals. Carriers have been defined as animals in which virus is present more than 28 days after infection (Sutmoller and Gaggero 1965). Approximately 50 per cent of cattle become carriers of FMD virus (FMDV) after clinical or subclinical infection; this has been observed in both vaccinated and unvaccinated animals. Other ruminants, such as sheep, goats and African buffaloes, have also been recognised as carriers of FMDV.

A number of studies have tried to define a carrier state in pigs. Mezencio and others (1999) described the finding of carrier pigs by identifying FMDV RNA in sera from pigs 226 days after infection. However, Alexandersen and others (2003) showed that pigs cleared the virus within three to four weeks. This short communication describes a real-time reverse transcriptase (RT)-PCR study to identify FMDV RNA in the tonsils of pigs 31 to 32 days after initial inoculation with FMDV. The study focused on the tonsil because in ruminants the oropharynx is considered to be an important site of viral persistence.

Tonsils were available from two transmission experiments performed with pigs 10 weeks of age, as described by Orsel and others (2007). Twenty-five pigs were vaccinated with a standard dose of double oil emulsion FMD O_1Manisa vaccine 15 days before infection. The pigs were infected with FMDV O/NET/2001 either by intradermal inoculation in the bulbus of the heel or by contact with infected pigs. The pigs were slaughtered 30 to 32 days after infection.

A biopsy of 1 x 1 cm was collected from the central part of the left tonsil. Tissue suspensions were prepared by disrupting the fresh-frozen biopsy in phosphate-buffered saline

TABLE 1: Results of a reverse transcriptase-PCR (RT-PCR) assay for foot-and-mouth disease (FMD) in 25 pigs vaccinated against FMD virus and then infected with a strain of FMD, and 31 unvaccinated and infected pigs

	Vaccination		
RT-PCR result	Yes	No	Total
Positive	2	11	13
Negative	23	20	43
Total	25	31	56

TABLE 2: Individual test results for both vaccinated and non-vaccinated pigs

Animal identification*	Infection†	Unvaccinated RT-PCR on tonsil >28 days	CP LightCycler	Virus-positive in OPF (dpi)	Animal identification*	Infection†	Vaccinated RT-PCR on tonsil >28 days	CP LightCycler	Virus-positive in OPF (dpi)
8676	Yes	+	29·7	2-7	8666	Yes	+	28·9	3-7
8663	Yes	+	28·9	2-5	8668	Yes	+	28·7	3-7
8659	Yes	+	28·9	2-4	8658	Yes	–	–	2-5
8669	Yes	+	28·8	2-7	8662	Yes	–	–	2-8
8667	Yes	+	28·8	2-6	9521	Yes	–	–	2-5
8672	Yes	+	28·8	2-6	9524	Yes	–	–	1-6
8674	Yes	+	28·8	2-6	9527	Yes	–	–	7
8671	Yes	+	28·6	3-9	9530	Yes	–	–	4-7
8677	Yes	+	28·6	1-5	9536	Yes	–	–	2-5
8670	Yes	+	28·6	3-9	9537	Yes	–	–	1-5
9513	Yes	+	27·1	2-7	9539	Yes	–	–	2-7
8675	Yes	–	–	1-8	9542	Yes	–	–	3-9
8679	Yes	–	–	2-9	9544	Yes	–	–	4-8
8678	Yes	–	–	2-7	9522	Sub	–	–	1-8
9043	Yes	–	–	1-5	9523	Sub	–	–	1-6
9044	Yes	–	–	1-3	9525	Sub	–	–	1-6
9045	Yes	–	–	1-5, 9-10	9526	Sub	–	–	4-8
9046	Yes	–	–	2-8	9529	Sub	–	–	3-8
9047	Yes	–	–	3-10	9538	Sub	–	–	2-5
9048	Yes	–	–	2-6	9543	Sub	–	–	4-7
9506	Yes	–	–	1-5	9545	Sub	–	–	6
9509	Yes	–	–	2-5	8664	No	–	–	–
9510	Yes	–	–	3-6	8660	No	–	–	–
9511	Yes	–	–	2-8	9540	No	–	–	–
9512	Yes	–	–	2-7	9541	No	–	–	–
9514	Yes	–	–	3-8					
9515	Yes	–	–	2-6					
8681	Sub	–	–	–					
8665	No	–	–	–					
8661	No	–	–	–					
8680	No	–	–	–					

* Identification numbers as described by Orsel and others (2007)
† Infection sites: No Uninfected, Yes Clinically infected, Sub Subclinically infected
CP Crossing point (second derivative), OPF Oropharyngeal fluid, dpi Days postinoculation

containing 2 per cent fetal calf serum and 10 per cent mixed antibiotics using the MagNA Lyser (Roche). All the samples were handled in a class II laminar flow cabinet to prevent contamination. The tissue suspensions were tested by virus isolation on a monolayer of secondary lamb kidney cells. In total, 200 µl of tonsil tissue suspension was added to one well. After one hour's incubation, the wells were washed with fresh medium and 2·5 ml of fresh medium was added. The cells were macroscopically observed for cytopathic effects (cpe) for two days. If no cpe was observed, the cells and supernatant were frozen and thawed, and 200 µl of this suspension was tested in the same way. All incubations were performed at 37°C in a humidified atmosphere containing 5 per cent carbon dioxide.

The tonsil suspensions were also tested by automated real-time RT-PCR. RNA isolation was performed as described in detail by Moonen and others (2003). The RT-PCR was performed using the LightCycler system (Roche) with hybridisation probes performed in a closed glass capillary, thereby minimising the risk of cross-contamination. The RT-PCR for FMDV is ISO 17025 accredited and validated within the laboratory facilities of CIDC-Lelystad.

No virus was isolated from the tonsil samples from the pigs. In contrast, two of the 25 vaccinated animals and 11 of 31 non-vaccinated control animals tested positive by RT-PCR (Tables 1, 2). The difference between the number of positive samples from the vaccinated and non-vaccinated pigs was significant (P=0·024) Fisher's exact test. All RT-PCR-positive samples originated from pigs that showed clinical signs and from which virus had been isolated from oropharyngeal fluid (OPF) samples collected by swabbing during the acute stage of FMD infection, but from which no virus had been detected in OPF samples for at least the last 22 days of the study period. It was not clear what the biological relevance was of the low amounts of viral RNA, since no viable virus was detected.

In cattle with persistent FMDV infection, the viral titre is usually low, detectable only intermittently and declines over time (Alexandersen and others 2002, Moonen and others 2004, Zhang and others 2004). In the present study, FMD viral RNA was tested for in the tonsils of pigs only at one time after infection; it would be useful to assess virus excretion and RT-PCR test results in a group of infected, vaccinated and non-vaccinated pigs to monitor the dynamics of viral RNA clearance and the exact location of viral persistence in the oropharynx.

References

ALEXANDERSEN, S., ZHANG, Z. & DONALDSON, A. (2002) Aspects of the persistence of foot-and-mouth disease virus in animals – the carrier problem. *Microbes and Infection* **4,** 1099

ALEXANDERSEN, S., ZHANG, Z., DONALDSON, A. I. & GARLAND, A. J. (2003) The pathogenesis and diagnosis of foot-and-mouth disease. *Journal of Comparative Pathology* **129,** 1-36

MEZENCIO, J. M., BABCOCK, G. D., KRAMER, E. & BROWN, F. (1999) Evidence for the persistence of foot-and-mouth disease virus in pigs. *Veterinary Journal* **157,** 213-217

MOONEN, P., BOONSTRA, J., VAN DER HONING, R. H., LEENDERTSE, C. B., JACOBS, L. & DEKKER, A. (2003) Validation of a LightCycler-based reverse transcription polymerase chain reaction for the detection of foot-and-mouth disease virus. *Journal of Virological Methods* **113,** 35-41

MOONEN, P., JACOBS, L., CRIENEN, A. & DEKKER, A. (2004) Detection of carriers of foot-and-mouth disease virus among vaccinated cattle. *Veterinary Microbiology* **103,** 151-160

ORSEL, K., DE JONG, M. C., BOUMA, A., STEGEMAN, J. A. & DEKKER, A. (2007) Foot-and-mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* **25,** 6381-6391

SUTMOLLER, P. & GAGGERO, A. (1965) Foot-and-mouth disease carriers. *Veterinary Record* **77,** 968-969

ZHANG, Z., MURPHY, C., QUAN, M., KNIGHT, J. & ALEXANDERSEN, S. (2004) Extent of reduction of foot-and-mouth disease virus RNA load in oesophageal-pharyngeal fluid after peak levels may be a critical determinant of virus persistence in infected cattle. *Journal of General Virology* **85,** 415-421

Detection of foot-and-mouth disease virus in infected pigs by rt-pcr four weeks after challenge

K. Orsel, H. I. J. Roest, E. M. Elzinga-Bril, et al.

Veterinary Record 2008 162: 753-754
doi: 10.1136/vr.162.23.753