Differences in detection of foot-and-mouth disease virus RNA in oral swabs and probangsamples during experimental infection of cattle and pigs

Stenfeldt, Anna Carolina; Lohse, Louise; Belsham, Graham

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

DTU Vet
National Veterinary Institute

Differences in detection of foot-and-mouth disease virus RNA in oral swabs and probang samples during experimental infection of cattle and pigs

Carolina Stenfeldt, Louise Lohse, Graham J Belsham

National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark,

Introduction

Reliable systems for rapid and efficient laboratory evaluation of suspected cases of FMD is of crucial importance for veterinary preparedness in FMD-free nations. In countries where FMD is endemic, it is important that the FMDV strains that are currently circulating, can be identified using sampling regimes that are suitable for epidemiological field surveys. In such settings, it is also relevant to consider the possibility of differentiating between animals that are subclinically infected (albeit potentially in an early phase of infection) from persistently infected carriers (beyond 28 days of infection).

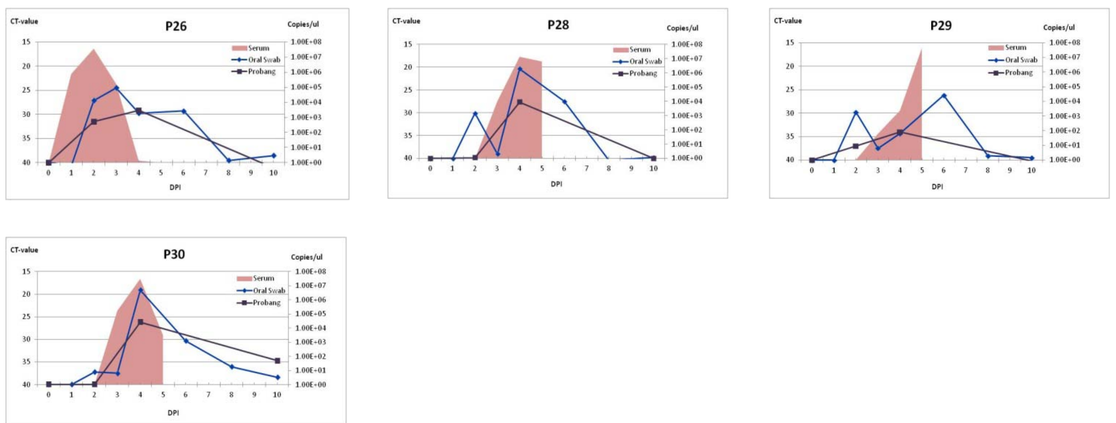
For laboratory detection of FMDV, analysis of oropharyngeal fluid / probang samples is a well proven approach that is also recommended by OIE technical guidelines. Although useful for virus detection, the collection of probang samples requires a certain degree of technical skill, as well as the necessity of keeping the animals restrained during the procedure.

During recent years, it has become more common to replace probang samples with the more easily collected oral swabs for both experimental studies, as well as in epidemiological field surveys. This sampling approach is simple and less time consuming, it does not require any specific instruments or specific training of staff, and can therefore be an attractive alternative in order to detect FMDV excretion in cattle and pigs.

Study Design

In this study, the comparative utility, for detection of FMDV RNA, of oral swabs and probang samples derived from cattle and pigs experimentally infected with serotype O FMDV, was evaluated. Using an accredited qRT-PCR assay to analyze both sample types, the aim of the study was to evaluate how well the detection of FMDV RNA in oral swabs corresponds to the levels of viral RNA measured in probang samples collected simultaneously. In addition to analyzing samples from the acute phase (up to 10 dpi) of FMDV infection in both cattle and pigs, samples were collected during the persistent phase of the infection in cattle in order to evaluate the applicability of using qRT-PCR assays for FMDV RNA within mouth swab samples for the detection of FMDV-carriers. The results obtained were compared to the duration of FMDV RNA detection in serum samples from both cattle and pigs.

3A



3B

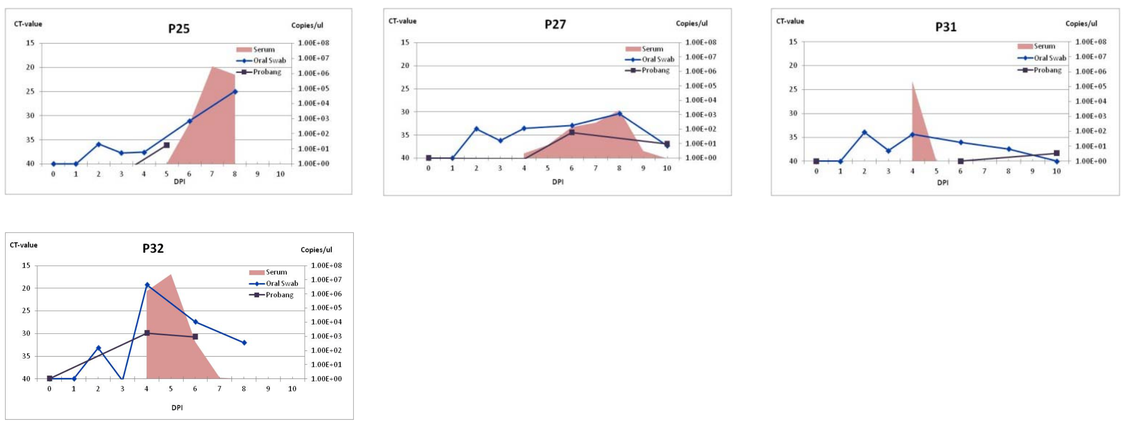
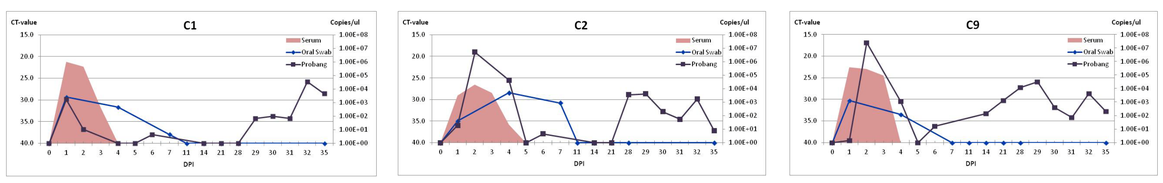


Figure 3 A-B; Detection of FMDV RNA in samples from experimentally infected pigs.

Viral RNA in serum (copies of FMDV genome/µl of serum), oral swab samples and probang samples (Ct values), measured by qRT-PCR. Pigs numbered 26, 28, 29 and 30 were infected through direct heel bulb inoculation (part A), whereas pigs numbered 25, 27, 31 and 32 were infected through contact exposure (part B).

Blood samples were collected on 0, 1, 2, 3, 4, 5, 7 and 10 dpi from inoculated pigs and on 0, 4, 5, 6, 7, 8, 9 and 10 dpi from contact pigs. Oral swabs were collected on a daily basis, and probang samples were collected on 0, 2, 4 and 10 dpi from the directly inoculated animals, and on 0, 4, 6 and 10 dpi from contact infected animals. Pigs numbered 25 and 32 were euthanized at 8 dpi for welfare reasons.

1A



1B

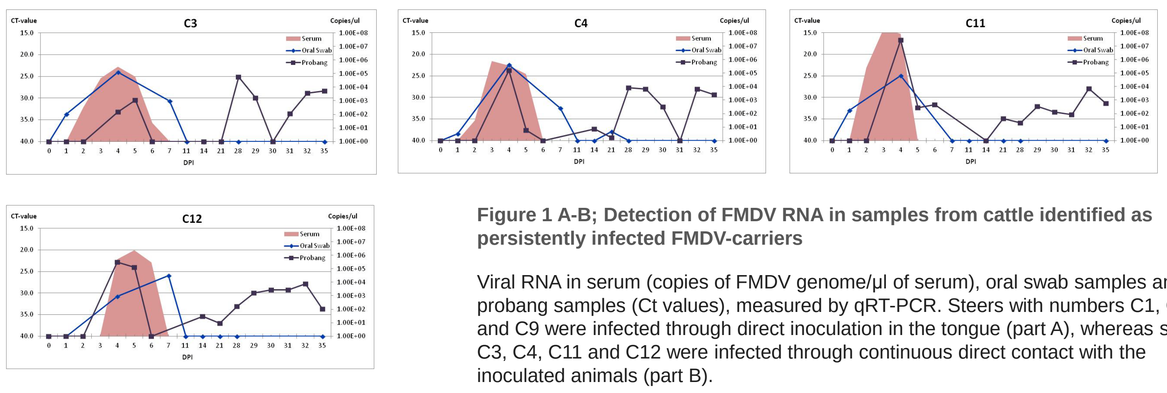
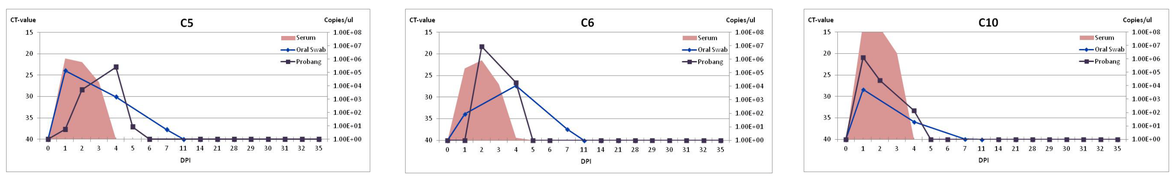


Figure 1 A-B; Detection of FMDV RNA in samples from cattle identified as persistently infected FMDV-carriers

Viral RNA in serum (copies of FMDV genome/µl of serum), oral swab samples and probang samples (Ct values), measured by qRT-PCR. Steers with numbers C1, C2 and C9 were infected through direct inoculation in the tongue (part A), whereas steers C3, C4, C11 and C12 were infected through continuous direct contact with the inoculated animals (part B).

2A



2B

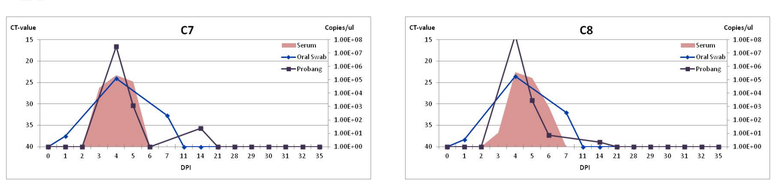


Figure 2 A-B; Detection of FMDV RNA in samples from cattle that did not become persistently infected carriers of FMDV

Viral RNA in serum (copies of FMDV genome/µl of serum), oral swab samples and probang samples (Ct values), was measured by qRT-PCR. Animals were identified as persistently infected carriers upon detection of FMDV RNA in a minimum of 4 probang samples that had been collected on or beyond 28 dpi. Steers with numbers C5, C6 and C10 were infected through direct inoculation in the tongue (part A), whereas steers C7 and C8 were infected by contact exposure (part B).

Results

During acute infection, FMDV RNA was measurable in oral swabs as well as in probang samples from both cattle and pigs. FMDV genomes could be detected in oral swabs and probang samples from a time point corresponding to the onset of viremia in directly inoculated animals, whereas animals which were infected through contact exposure had low levels of FMDV RNA in oral swabs before viral RNA could be measured in serum.

Analysis of samples collected from cattle persistently infected with FMDV showed that it was not possible to detect FMDV RNA in oral swabs harvested beyond 10 dpi, despite the presence of FMDV RNA in probang samples that had been collected as late as 35 dpi. An interesting feature of the persistent infection in the cattle was the apparent decline in the level of FMDV RNA in probang samples after the acute phase of infection, which was followed by a marked rise again (in all the carrier animals) by 28dpi.

Conclusion

Results from this study indicate that qRT-PCR analysis of oral swabs is a useful approach in order to achieve a time efficient and reliable initial diagnosis of acute FMD in cattle and pigs, whereas probang sampling is essential for the detection of cattle that are persistently infected “carriers” of FMDV.

The lack of detectable FMDV genome in oral swabs derived from cattle, that were identified as persistently infected carriers based on analysis of simultaneously collected probang samples, could potentially be related to the reported low rate of transmission of infection from carrier cattle to naïve animals. There could also be a potential application for the herein reported relationship in timing between FMDV RNA detection in oral swabs and probang samples in cattle, for approximation of the phase of infection (i.e. acute/subclinical versus persistent) of cattle that are sampled during epidemiological field surveys.