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The comparative utility of oral swabs and probang samples for detection of foot-and-mouth disease virus infection in cattle and pigs

Carolina Stenfeldt [1], Louise Lohse, Graham J. Belsham *

National Veterinary Institute, Technical University of Denmark (DTU-Vet), Division of Virology, Lindholm, DK-4771 Kalvehave, Denmark

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ABSTRACT

Foot-and-mouth disease virus (FMDV) RNA was measured using quantitative reverse transcription-PCR (qRT-PCR) assays in oral swab and probang samples collected from cattle and pigs during experimental infections with serotype O FMDV. During acute infection, FMDV RNA was measurable in oral swabs as well as in probang samples from both species. FMDV RNA 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 days post infection (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 28 dpi.

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.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral infection capable of rapid spread amongst cloven-hoofed animal species. The causative agent; FMD-virus (FMDV), is a positive stranded RNA virus (an *Aphthovirus*) within the family *Picornaviridae*. There are seven immunologically distinct serotypes of FMDV: O, A, C, Asia 1, and Southern African Territories (SAT)-1, -2 and -3, with multiple strains within each serotype (Knowles and Samuel, 2003).

FMD is most commonly reported in domestic species, such as cattle, pigs and sheep, although the infection is also known to occur in many other species of animals (Alexandersen et al., 2003b). Outbreaks of FMD are of huge socio-economic impact for affected countries and the infection is often described as being the single most important constraint on international trade in animal products. Introduction of FMD into an area previously free of the infection will lead to implementation of drastic intervention strategies, with culling of susceptible animals, as well as prolonged restrictions on animal movements and trade, often with devastating consequences for the agricultural industries of the country. Keeping an adequate level of preparedness, with rapid and efficient systems for

* Corresponding author. Tel.: +45 35 88 79 85; fax: +45 35 88 79 01.
E-mail addresses: Carolina.Stenfeldt@ars.usda.gov (C. Stenfeldt), loloh@vet.dtu.dk (L. Lohse), grbe@vet.dtu.dk (G.J. Belsham).
[1] Current address: USDA-ARS, Plum Island Animal Disease Center, P.O. Box 848, Greenport, NY 11944, USA.

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laboratory evaluation of suspected cases of FMD is therefore of high importance for countries in which international trade in animal products constitutes a significant part of the agricultural industry.

In cattle, FMDV infection is clinically manifested through a transient increase in body temperature with inappetence and development of characteristic vesicular lesions in areas of cornified epithelium, primarily within the oral cavity, as well as on the coronary bands and udders (Alexandersen et al., 2003b). A short period of viremia (approximately 2–3 days) is counteracted by a rapid rise in neutralizing anti-FMDV antibodies that efficiently clears the virus from the circulation. At the peripheral sites of replication (e.g. the feet and coronary bands), virus may be found until approximately 14 days post infection (dpi), at which time the clinical signs of infection are usually completely cleared (Alexandersen et al., 2003b). Pigs are generally more severely affected by the clinical infection than cattle. Even though the fever response may be less marked in pigs when compared to cattle, pigs usually display a higher degree of lameness, which is often associated with "thimbling" of claws as a consequence of severe coronary band lesions.

In ruminant species, in particular cattle and buffalo, FMDV is capable of causing a persistent subclinical infection during which the animals may continue to shed infectious virus (albeit at a rather low level) beyond 28 dpi and then for prolonged periods of time (up to 3 years in cattle and 5 years in buffalo) (Condy et al., 1985; Moonen and Schrijver, 2000). It has been generally accepted, that pigs do not become persistently infected by FMDV (reviewed by Alexandersen et al., 2003b) although, it has been indicated, by a number of recent reports, that this is an area that may need further research (Mohamed et al., 2011; Orsel et al., 2008; Rodriguez-Calvo et al., 2011; Zhang and Bashiruddin, 2009).

The persistently infected, so called "carriers" of FMDV, have high titers of anti-FMDV antibodies in their circulation and it is, therefore, not possible to discriminate animals that are carriers of FMDV from those that have been efficient in clearing the infection through serological screening. Virus excretion in FMDV-carriers has been reported to be intermittent, and at low levels (Alexandersen et al., 2002), and detection of FMDV-carriers within a larger group of animals by screening for the presence of infectious virus in pharyngeal excretions, has therefore been deemed unreliable. Recent studies have, however, demonstrated that under experimental conditions, using sensitive and specific real time quantitative RT-PCR (qRT-PCR) analysis of oesophagal–pharyngeal fluid (OPF) samples, it is possible to detect the maintenance of FMDV RNA in persistently infected cattle until 100 dpi (Stenfeldt and Belsham, 2012). In sharp contrast to this, in the animals that did not develop into FMDV-carriers, virus RNA became undetectable in OPF by approximately 14 dpi.

According to technical instructions from the World Organization for Animal Health (OIE), laboratory diagnosis of FMDV should be performed using either epithelium samples harvested from acute lesions, or samples of OPF, generally referred to as probang-samples (Sutmoller and Gaggero, 1965). Probang samples are harvested by means of a probang cup, a small metal cup with an attached shaft, which is introduced into the pharyngeal region of the animal in order to collect fluid excretions. Although a well-proven approach used for sampling, 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. It is also known that farmers have been reluctant to allow collection of probang samples from their animals, as they believed that the sampling procedure could injure the animals.

During recent years, it has become more common to replace probang samples with the more easily collected oral swabs for both experimental studies (Alexandersen et al., 2003a; Orsel et al., 2009; Pacheco and Mason, 2010), as well as in epidemiological field surveys (Jamal et al., 2011; Klein et al., 2008). 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.

During this study, we have collected both oral swabs and probang samples from cattle and pigs that have been experimentally infected with serotype O FMDV (strain O-UKG 34/2001). Using an accredited qRT-PCR assay to analyze both sample types, the aim of the study has been 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 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.

2. Materials and methods

2.1. Animal experiments and samples

This study is based on the results from two experimental infection studies, performed in cattle and pigs. The experiments were performed in biosecure research facilities at DTU-Vet, Lindholm Island, Denmark, in accordance with the requirements of the Danish Animal Experiments Inspectorate (License 2008/561-1541).

2.2. Cattle experiment

The general experimental protocol used for the cattle experiment has been described in detail elsewhere (Stenfeldt et al., 2011). In brief, the experiment included 12, 5–6 months old steers, that were infected with FMDV O UKG 34/2001 (original inoculum obtained from IAH-Pirbright, UK, and then passaged once in cattle), either through subepithelial injection, in the tongue, of approximately $10^{6.9}$ $TCID_{50}$ in a volume of 0.5 ml, or through continuous direct contact with inoculated animals.

All animals were monitored daily, with measurements of rectal temperature and observation of clinical signs.