Quantitative analysis of foot-and-mouth disease virus RNA duration in tissues of experimentally infected pigs

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Abstract

Quantitative analysis of the duration of foot-and-mouth disease virus (FMDV) RNA in tissues was carried out in pigs experimentally infected with FMDV O UKG 34/2001 and O SKR 1/2000. The results showed that the viral RNA was still detectable in cervical lymph nodes, mandibular lymph nodes and tonsils collected from both inoculated and contact pigs at 28 days post infection. There was no detectable viral RNA in the soft palate or pharynx, which are thought to be tissue sites for viral persistence in cattle. Further study is needed to clarify whether this difference has significance in terms of viral clearance in pigs.

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Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals caused by the foot-and-mouth disease virus (FMDV), genus Aphthovirus, family Picornaviridae (Martinez-Salas et al., 1993). Of considerable importance in the control of FMD is persistent infection that can occur following clinical or sub-clinical disease in both vaccinated and non-vaccinated ruminants (Alexandersen et al., 2003). Persistent infection in carrier cattle is defined as the presence of virus in oropharyngeal fluid samples more than 28 days after infection (Sutmoller and Gaggero, 1965). Many studies have tried to define the carrier state in pigs (Alexandersen et al., 2001, 2003) but only one publication supports the finding of FMDV carriers in this species (Mezencio et al., 1999). In the present study, viral RNA levels in various tissues were quantified over a 28 days period following initiation of infection.

The animals were housed in a category four bio-containment animal unit (Specified Animal Pathogens Order, DEFRA 1998). Animal experimentation was carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. Eighteen \((n = 18)\) Landrace × large white pigs, females, between 20 and 30 kg bodyweight were used. Briefly, two pigs were killed before inoculation and used as normal controls. Half of the remaining 16 pigs were inoculated by heel pad inoculation using 0.25 mL of FMDV O UKG 34/2001 inoculum containing 5.6 logs TCID\(_{50}\). The remaining pigs were kept in direct contact throughout the experiment. The pigs were monitored for clinical signs of disease daily throughout experiments. Clinical signs were noted and a score assigned to the individual signs as previously described (Quan et al., 2004).

One inoculated and one contact pig were selected at random and killed at 1–4, 7, 21 and 28 days post infection (dpi). Two animals were excluded from the study due to unexpected deterioration in health following infection. Tissue samples were collected and immediately put into RNA-later (Ambion), a tissue storage reagent, and stored at \(-20^\circ\text{C}\) until required. RNA was extracted from tissue samples and eluted in 50 μL of elution buffer as described previously (Zhang and Alexandersen, 2004). The level of viral RNA was then measured by a quantitative RT-PCR assay (Quan et al., 2004).
An increase in body temperature, viraemia and early lesions were observed in all inoculated pigs from around 1–2 dpi and in the contact pigs at around 2–3 dpi. The kinetics of FMDV RNA loads in tissues during acute infection are summarised in Fig. 1. The quantities of FMDV are expressed as natural logarithm (LN) genome copy per gram of tissue. Lymphoid tissue, tonsil, cervical lymph node (CLN) and mandibular lymph node (MLN) were tested for levels of viral RNA. The CLN sample was always collected from the same side as the inoculated foot. FMDV RNA was detected in tonsil, CLN and MLN samples of inoculated pigs from 1 dpi and contact pigs from 2 dpi. Peak viral RNA loads in these tissues occurred in inoculated pigs at 2 dpi (17.94, 18.48 and 14.49 LN genomes per gram, respectively). In contact pigs, peak viral RNA loads occurred in tonsil at 2 dpi (18.91 LN genomes per gram, in CLN at 3 dpi (16.83 LN genomes per gram and in MLN at 4 dpi (15.08 LN genomes per gram). For pharyngeal samples, viral RNA was detected in soft palate (SP) and pharynx samples collected from inoculated pigs from 1 dpi and contact pigs from 2 dpi. In inoculated pigs, viral RNA loads peaked at 2 dpi in the dorsal and ventral SP, oropharynx and nasopharynx. In contact pigs, viral RNA loads that peaked in the ventral SP, oropharynx and nasopharynx were measured at 2 dpi and in the dorsal SP at 3 dpi. Tongue and epithelium collected from interdigital area on foot, generally contained the highest levels of virus RNA of all of the tissue samples analysed. Peak viral loads were measured at 2 dpi for inoculated pigs and 3 dpi for contact pigs.

Tissues in the pharyngeal area have been shown to be important in FMDV infection during persistence in cattle (Zhang and Alexandersen, 2004). Therefore, the duration of FMDV infection was investigated in the pharyngeal region including dorsal SP, ventral VSP, nasopharynx and oropharynx, the lymphatic systems draining them (i.e., tonsil, MLN and RPLN), and from other non-pharyngeal tissues. The data are summarised in Fig. 1A (inoculated pigs) and 1B (contact pigs). At 21 dpi, CLN from inoculated and contact pigs (11.2 and 11.6 LN genomes per gram, respectively), MLN from inoculated and contact pigs (8.8 and 11.2 LN genomes per gram, respectively) and tonsil samples from one inoculated pig (8.9 LN genomes per gram) still contained a detectable level of viral RNA while tissues of the SP, pharynx, tongue and skin were negative. By 28 dpi, CLN from inoculated and contact pigs (10.9 and 12.1 LN genomes per gram, respectively), MLN from contact pigs (11.4 LN genomes per gram) and tonsil from inoculated and contact pigs (4.0 and 9.11 LN genomes per gram, respectively) contained a detectable level of viral RNA while tissues of the SP, pharynx, tongue and skin were negative.

Similar RNA levels and durations were observed in tissues from pigs infected with FMDV O SKR 1/2000. Four pigs were infected with FMDV O SKR1/2000 by heel pad inoculation as described above. One pig was selected at random and killed at 4, 5, 21 and 23 dpi. Viral RNA was detected in the CLN, MLN and tonsil at 4 days (18.1, 18.1 and 12.6 LN genomes per gram) at 5 dpi (18.6, 16 and 19.4 LN genomes per gram, respec-
tively); at 21 dpi (14.3, 14.9 and 14.1 LN genomes per gram, respectively) and in the MLN and tonsil at 23 dpi (13.1 and 12.8 LN genomes per gram, respectively). No viral RNA was detected in tissues of soft palate, nasopharynx, oropharynx, epithelia and tongue collected at 21 and 23 dpi.

Clearance of viral RNA from the tissues was largely independent of the peak level of viral RNA. The results are consistent with a previous study in cattle (Zhang and Alexandersen, 2004), where the epithelia of the skin and mouth contained the highest levels of viral RNA during the acute stage of infection, but the viral RNA in these tissues was cleared faster than in pharyngeal tissues. No clear explanations can be put forward for the differences in the sites of apparent FMDV RNA retention in cattle and pigs, which may be due to the cell type in the tissues or by the presence of local factors. For example, it has been reported that cytokines exert a profound effect on FMDV replication (Zhang et al., 2002). The present study suggests that FMDV RNA can persist in some pig tissues for up to 28 dpi. It will be important to examine in more detail the potential role of host factors in clearance of the virus during infection with FMDV.

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References