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Infection dynamics of foot-and-mouth disease virus in pigs using two novel simulated-natural inoculation methods

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ABSTRACT

In order to characterize foot-and-mouth disease virus (FMDV) infection dynamics in pigs, two simulated-natural inoculation systems were developed and evaluated. Intra-oropharyngeal (IOP) and intra-nasopharyngeal (INP) inoculation both enabled precise control of dose and timing of inoculation while simulating field exposure conditions.

There were substantial differences between outcomes of infections by the two routes. IOP inoculation resulted in consistent and synchronous infection, whereas INP inoculation at similar doses resulted in delayed, or completely absent infection. All pigs that developed clinical infection had detectable levels of FMDV RNA in their oropharynx directly following inoculation. Furthermore, FMDV antigens were localized to the oropharyngeal tonsils suggesting a role in early infection.

The utility of IOP inoculation was further demonstrated in a vaccine-challenge experiment. Thus, the novel system of IOP inoculation described herein, offers a valid alternative to traditionally used systems for FMDV inoculation of pigs, applicable for experimental studies of FMDV pathogenesis and vaccinology.

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1. Introduction

Foot-and-mouth disease (FMD) is an acute vesicular infection caused by FMD-virus (FMDV), an *Aphthovirus* belonging to the family of *Picornaviridae* (Grubman and Baxt, 2004). Characteristic features of acute infection involve blanching and vesiculation within stratified squamous epithelia at lesion–predilection sites including coronary bands, oral cavity, and teats. These common features of the clinical infection can be observed across a wide range of cloven-hoofed animal species susceptible to the infection. There are, however, certain mechanisms within the pathogenesis of FMD that have proven to be more host-specific (Alexandersen and Mowat, 2005; Alexandersen et al., 2003b; Arzt et al., 2011).

Experimental FMDV-challenge of swine, often consists of either intradermal heel bulb (IDHB) inoculation and/or contact exposure (Alexandersen et al., 2001, 2003a; Eble et al., 2006, 2004; Mohamed et al., 2011; Pacheco and Mason, 2010; Pacheco et al., 2012). Direct IDHB inoculation is highly repeatable, and as such, offers a desired degree of control and consistency of the experimental model. This route of virus entry can, however, be suboptimal as it completely bypasses the natural barrier of mucosal immunity, a feature of the innate host response that is likely of critical importance during natural exposure conditions.

It has been widely accepted that pigs, although capable of generating large amounts of aerosolized virus when infected, are less susceptible to airborne infection when compared to cattle (Alexandersen et al., 2002; Alexandersen and Donaldson, 2002; Donaldson and Alexandersen, 2001; Donaldson et al., 2001). In contrast to recently gained knowledge characterizing acute FMDV-infection in cattle (Arzt et al., 2010; Pacheco et al., 2010a), the precise mechanisms involved in the very early stages of infection in pigs, including determination of the initial site of virus entry, have not yet been fully elucidated (Arzt et al., 2011). Previous investigations have suggested that following initial virus entry through lymphoid tissues of the pharyngeal region, substantial amplification of virus takes places at secondary lesion sites, with specifically high viral loads found within the skin covering the coronary bands (Alexandersen et al., 2001; Murphy et al., 2010).

As pigs have proven to be relatively resistant to FMDV infection through inhalation (Alexandersen et al., 2002, 2003a; Alexandersen and Donaldson, 2002; Donaldson and Alexandersen, 2001; Pacheco and Mason, 2010), they are more likely to acquire the infection through direct or indirect contact with infected animals or contaminated fomites (Alexandersen et al., 2003b; Donaldson

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et al., 2001). Infection models based on contact exposure of test animals to inoculated seeder animals provide an infectious route that closely mimics natural infection. However, a disadvantage of using this approach for experimental studies is the inability to control the dose and exact timing of virus exposure, resulting in a potential lack of consistency. An additional level of control may be included in the model, by limiting the amount of time during which test-animals are exposed to infected animals (Pacheco and Mason, 2010; Pacheco et al., 2012), or by changing the intensity of exposure by adapting the ratio between seeders and recipients (Quan et al., 2009). It has, however, been postulated that challenge models based on continuous direct contact between inoculated and in-contact animals may lead to an excessive magnitude of viral exposure (de Leeuw et al., 1979; Pacheco et al., 2010b), and recent studies have also indicated significant variations in transmission parameters between different strains of FMDV (Pacheco and Mason, 2010; Pacheco et al., 2012).

It is known that different routes of virus exposure may lead to substantial variations in the dynamics of infection (Donaldson and Ferris, 1980; Quan et al., 2004; Terpstra, 1972), and that unforeseen variation of the actual challenge dose, may lead to confounding, or misinterpretation of experimental outcomes (Eble et al., 2004; Orsel et al., 2007). There is therefore a need to develop standardized models for FMDV inoculation of swine that can be used for experimental studies investigating the pathogenesis of natural infection, as well as for testing and validating novel FMDV vaccines and biotherapeutics. It is desirable that a model for such purposes should be consistent and repeatable, with a possibility of controlling both timing and dose of inoculation, whilst also providing a close simulation of natural infection.

The purpose of the current work was to characterize clinical progression and viral dynamics of FMDV-infection in pigs that were inoculated by two simulated natural routes capable of contrasting infection via the upper respiratory and upper gastrointestinal tracts (nasopharynx vs. oropharynx). In order to accomplish this goal, two novel challenge methods, consisting of direct intra-oropharyngeal (IOP) and intra-nasopharyngeal (INP) inoculation were developed and optimized. The dynamics of infection were compared across the novel systems using FMDV O1 Manisa and FMDV A24 Cruzeiro. Data from these studies was subsequently compared to previously performed studies from our laboratory (Pacheco et al., 2012), in which IDHB inoculation and contact exposure were used to infect pigs with the same FMDV isolates. Following optimization of these two novel inoculation systems in naïve animals, IOP inoculation was further tested through application in a vaccine-challenge experiment.

2. Materials and methods

2.1. Virus strains

The FMDV strains O1 Manisa and A24 Cruzeiro, used in the studies described herein, were cattle derived viruses that were passaged once in pigs for adaptation to this host species. Detailed information regarding the generation of virus stocks has been published previously (Pacheco et al., 2012). Inoculation doses were calculated using pre-determined 50% pig-heel infectious doses per ml ($PHID_{50}$), a system based on *in vivo* titrations in porcine heel bulb epithelium (Pacheco and Mason, 2010).

2.2. Animal experiments

All animal studies were performed within high containment (BSL-3) animal facilities at Plum Island Animal Disease Center. Experimental protocols were subjected to prior approval by the Plum Island Animal Disease Center Institutional Animal Care and Use Committee (IACUC), which functions to ensure ethical and humane treatment of experimental animals. In order to minimize variation between experiments, all experimental animals (castrated male Yorkshire pigs, weighing approximately 25–30 kgs upon delivery) were obtained from the same vendor (Animal Biotech Industries Inc., Danboro, PA). Following an initial clinical examination, pigs were allowed an acclimation period of approximately 1–2 weeks prior to initiation of experiments.

2.2.1. Inoculation of naïve pigs

Initial experiments included detailed investigations of FMD infection dynamics in naïve pigs infected through either intra-nasopharyngeal (INP) or intra-oropharyngeal (IOP) inoculation, using two different strains of FMDV. FMDV O1 Manisa was inoculated at doses of 10, 100, and 1000 $PHID_{50}$, defined as low, medium and high doses (corresponding to 3.45, 4.45 and 5.45 Log_{10} PFU in BHK-21 cells (Pacheco et al., 2012)). Similar investigations were performed using FMDV A24 Cruzeiro, at low and high doses (10 and 100 $PHID_{50}$, respectively, corresponding to 5.95 and 6.95 Log_{10} PFU in BHK-21 cells (Pacheco et al., 2012)). Details regarding total animal numbers included in each dose/route-group, for both virus strains, are given in Table 1. Experimental groups of 4 pigs, all subjected to the same inoculation procedure, were housed in separate isolation rooms. Each room housed two different dose-groups, which were separated by double fencing at a distance of approximately 2 m. Animal handling was controlled in manners to avoid potential carry-over of virus between different dose groups. A limited number of additional animals were included in some experiments for the purpose of investigating tissue distribution of virus during early infection. These animals were euthanized at pre-determined time points, 24–48 h post infection (hpi), and were subjected to a standardized necropsy protocol including extensive collection of tissue samples (limited description herein).

2.2.2. Intra-nasopharyngeal inoculation

For the INP inoculation, pigs were deeply sedated using a mixture of Telazole, Ketamine and Xylazine, (3, 8 and 4 mg/kg, respectively) and placed in sternal recumbency. Two ml of inoculum was deposited into the nasopharynx, by use of a flexible, 14 gauge, plastic catheter inserted through one nostril. Prior to inoculation, the length of the catheter was measured to match the approximate distance from the external nares to the medial canthus of the eye to ensure deposition of inoculum in the nasopharynx, rather than within the anterior nasal cavity or trachea. Pigs were kept with their heads in a stable position for 1 min following inoculation in order to prevent premature loss of inoculum through the nares.

2.2.3. Intra-oropharyngeal inoculation

For IOP inoculation, sedated pigs (see above) were placed in dorsal recumbency. Two ml of inoculum was deposited directly onto the tonsil of the soft palate (TSP) (Horter et al., 2003), using a stainless steel cannula. Correct deposition of inoculum was ensured through visualization of the tonsil as the inoculum was deposited. Pigs were kept in dorsal recumbency, with the head in

Table 1
Number of animals included in each virus/dose group for INP and IOP inoculation of naïve pigs.

Virus	Inoculation route and dose ($PHID_{50}$)					
	IOP			INP		
	10	100	1000	10	100	1000
FMDV O1 Manisa	2	4 (+2*)	2	2	4	2
FMDV A24 Cruzeiro	2	2 (+4*)		2	2	

* Euthanized at 24–48 hpi for pathogenesis studies.

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