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EXPERIMENTALLY INDUCED DISEASE

Infection Dynamics of Foot-and-Mouth Disease Virus in Cattle Following Intranasopharyngeal Inoculation or Contact Exposure

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Summary

For the purpose of developing an improved experimental model for studies of foot-and-mouth disease virus (FMDV) infection in cattle, three different experimental systems based on natural or simulated natural virus exposure were compared under standardized experimental conditions. Ante-mortem infection dynamics were characterized in cattle exposed to FMDV through a novel, simulated natural intranasopharyngeal (INP) inoculation system or through standardized and controlled systems of within- or between-species direct contact exposure (cattle-to-cattle or pig-to-cattle). All three systems were efficient in causing synchronous, generalized foot-and-mouth disease in cattle exposed to one of three different strains of FMDV representing serotypes O, A and Asia1. There was more within-group variation in the timing of clinical infection following natural and simulated natural virus exposure systems when compared with the conventionally used system of needle inoculation (intraepithelial lingual inoculation). However, the three optimized exposure systems described herein have the advantage of closely simulating field conditions by utilizing natural routes of primary infection, thereby facilitating engagement of mucosal host defence mechanisms. Overall, it is concluded that INP inoculation and standardized systems of direct contact exposure provide effective alternatives to conventional (needle) inoculation systems for studies in which it is desirable to simulate the natural biology of FMDV infection.

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Keywords: animal models; cattle; foot-and-mouth disease virus; pathogenesis

Introduction

Foot-and-mouth disease virus (FMDV), a single-stranded positive-sense RNA virus belonging to the *Aphthovirus* genus of the Picornaviridae family, is the causative agent of foot-and-mouth disease (FMD), a highly infectious vesicular disease of cloven hoofed animals. FMD is a disease of substantial socio-economic impact because the FMD status of any geographical region defines access to international markets for trade in agricultural products. The economic implications of this disease, combined with its capacity for rapid transmission within and between a wide range of susceptible host species, demonstrate the importance of FMD as a constant threat to multinational livestock industries as well as small-scale farmers (Arzt *et al.*, 2010b; Knight-Jones and Rushton, 2013).

Multiple experimental models for FMD studies in cattle have been developed with the goals of closely simulating naturally occurring disease mechanisms. Additional requirements for optimal experimental systems include providing consistent and reproducible results with minimal variation in temporal or quantitative measures of infection dynamics across experimental subjects. Conventional experimental models most often involve virus challenge through

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intraepithelial injection in the tongue (intraepithelial lingual [IEL], often described incorrectly as intradermal lingual [IDL]) (Henderson, 1949; Charleston *et al.*, 2011; Arzt *et al.*, 2014). Although this approach provides a highly consistent and reproducible model for the disease, there are disadvantages with using this system for studies of FMD pathogenesis and vaccinology as it by-passes the host's natural mucosal barrier. Even though it can be argued that viral entry through pre-existing abrasions in the oral cavity may occur during natural conditions, it has been demonstrated that the more common route of infection in cattle is viral entry through the upper respiratory tract (Donaldson *et al.*, 2001; Donaldson and Alexandersen, 2002) with primary infection occurring within the nasopharyngeal mucosa (Burrows *et al.*, 1981; Arzt *et al.*, 2010a; Pacheco *et al.*, 2010; Stenfeldt *et al.*, 2015a).

Experimental systems based on contact transmission provide the closest simulation of truly natural routes of virus exposure. However, contact systems have two major drawbacks: (1) dose and timing of virus exposure cannot be precisely controlled, and (2) additional experimental animals are required as virus donors, which is undesirable in consideration of cost and ethical concerns. Therefore, experimental studies based on contact exposure need to be carefully executed in order to minimize the effects of any extrinsic factors that might influence the interaction between animals and thereby alter the resulting 'dose' of virus exposure.

Contact transmission has been achieved by co-housing experimental animals with animals of the same or different species that have previously been infected by direct inoculation. Published studies have included cattle-to-cattle (Graves *et al.*, 1971; Orsel *et al.*, 2005; Howey *et al.*, 2012) and/or pig-to-pig transmission (Alexandersen *et al.*, 2001; Eble *et al.*, 2004; Pacheco and Mason, 2010; Pacheco *et al.*, 2012), with fewer studies describing interspecies transmission (i.e. pig-to-cattle or cattle-to-pig; Blackwell *et al.*, 1982; Gomes and Auge de Mello, 1994; Pacheco *et al.*, 2016). Within any contact system, efficiency of FMD transmission will depend on the biological characteristics of the specific FMDV strain involved (Pacheco and Mason, 2010) and the conditions of contact and interaction between animals (Quan *et al.*, 2009; Pacheco *et al.*, 2012). Additionally, relevant intrinsic viral properties that may affect transmission include possible variations in host range (Brooksby, 1950; Dunn and Donaldson, 1997), virulence and environmental stability of the specific virus strain. Additional variations in exposure conditions may be adjusted to simulate distinct transmission scenarios; for instance, continuous direct contact (Zhang *et al.*, 2006; Stenfeldt *et al.*, 2011) would be expected to deliver a higher effective challenge dose compared with time-limited exposure of hours (Burrows *et al.*, 1981; Quan *et al.*, 2009) or days duration (Cox *et al.*, 2005; Juleff *et al.*, 2013). Animals may be separated by gates or unoccupied space to simulate indirect exposure (Donaldson and Ferris, 1980; Bouma *et al.*, 2004). Some investigators have utilized three-fold (tertiary) contact systems to ensure that the experimental subjects are exposed to donors that were infected by natural contact exposure (Charleston *et al.*, 2011).

Similar to contact-based challenge systems, simulated natural exposure systems strive to closely simulate the natural biology of primary FMDV infection. Such systems allow precise control of timing and dosage of the virus challenge, while preserving the natural engagement of the mucosal immune system and reducing the numbers of study animals required. For cattle, FMDV inoculation by a natural (i.e. aerogenous) route has been demonstrated by delivery of naturally generated aerosol using an improvised apparatus (Donaldson *et al.*, 1987), artificially generated aerosol using improvised apparatus (McVicar and Eisner, 1983; Donaldson *et al.*, 1987; Brown *et al.*, 1992, 1996), artificially generated aerosol using standardized apparatus (Pacheco *et al.*, 2010; Arzt *et al.*, 2010a), or by deposition of virus moistened gauze on the nasal planum (Korn, 1957). Other investigators have inoculated FMDV using an intranasal delivery system developed for use in man (Pacheco *et al.*, 2010) or by deposition of virus suspension using tubing with a length of 5 cm (Sutmoller *et al.*, 1968) or 10 cm (McVicar *et al.*, 1970; Graves *et al.*, 1971). Other authors have described using intranasal inoculation without giving specific details of depth of instillation (McVicar and Sutmoller, 1974; Salt *et al.*, 1996; Sanz-Parra *et al.*, 1999; Bouma *et al.*, 2004; Orsel *et al.*, 2005, 2007).

In the current study, recently acquired knowledge gained from studies of FMDV dynamics in cattle (Pacheco *et al.*, 2010; Arzt *et al.*, 2010a, 2014) and contact transmission in pigs and cattle (Pacheco and Mason, 2010; Pacheco *et al.*, 2012, 2016) were elaborated up on in order to develop standardized FMDV challenge systems for cattle based on time-limited direct contact with infected pigs or continuous contact with infected cattle. Furthermore, a novel system based on direct deposition of virus within the bovine nasopharynx is described as an alternative simulated natural system that provides precise control of the challenge dose, while also engaging natural mucosal defences at the primary site of infection.

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