FOOT-AND-MOUTH DISEASE IN SWINE

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PUBLISHED IN: Frontiers in Veterinary Science

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88945-280-4
DOI 10.3389/978-2-88945-280-4

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FOOT-AND-MOUTH DISEASE IN SWINE

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Foot-and-mouth disease (FMD) is one of the most devastating diseases of livestock. The research topic here features nine studies supplementing the state-of-the art of the knowledge on the pathogenesis and epidemiology of FMD in swine.

Citation: Perez, A. M., Willeberg, P., eds. (2017). Foot-and-Mouth Disease in Swine. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-280-4

Table of Contents

frontiers in Veterinary Science

EDITORIAL
published: 21 August 2017
doi: 10.3389/fvets.2017.00133

Editorial: Foot-and-Mouth Disease in Swine

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Keywords: foot-and-mouth disease, swine, epidemiology, pathogeny

Editorial on the Research Topic

Foot-and-Mouth Disease in Swine

INTRODUCTION

Foot-and-mouth disease (FMD) is one of the most devastating diseases of livestock (1). The disease is caused by infection with a picornavirus, generically referred as FMD virus (FMDV), which is considered one of the most infectious agents affecting animals (2). FMD status affects national and international movement and trade of animals and animal products, and food animal trade is expected to play an important role in poverty alleviation (Perez). Applied knowledge about FMD pathogenesis and epidemiology is important in the design and implementation of effective prevention and control programs, minimizing detrimental effects of FMD outbreaks. Decision tools have been developed by applying simulation models based on characteristics of FMD pathogenesis and epidemiology. These tools are meant to be used by risk managers and risk communicators to help prioritize control options during an FMD epidemic and making the evidence available for all stakeholders [Willeberg et al.; (3)].

Much of the literature on FMD has focused on the pathogenesis and epidemiology of the disease in cattle. However, FMD also affects other food animal species, most notably, swine. This research topic contributes to the gain and dissemination of important knowledge on the dynamics of one of the most devastating diseases of livestock when occurring in the pig, a susceptible species for which limited information is available in the peer-reviewed literature. The ultimate objective of these original articles and reviews was to contribute preventing and mitigating the impact of FMD in swine, thus, promoting health and economic development of non-affected as well as affected countries and regions.

This research topic features nine studies supplementing the state-of-the-art of the knowledge on the pathogenesis and epidemiology of FMD in swine. Three papers focus on the analysis of experimental studies, which have been designed with the objective of gaining basic knowledge on the pathogenesis of the disease. Three other papers summarize the results of field studies and review fundamental features of FMD transmission and the effectiveness of FMD vaccination in swine. The last three papers describe the design and implementation of applied epidemiology approaches to prevent or mitigate the impact of FMD epidemics in disease-free regions.

EXPERIMENTAL STUDIES

The potential for FMDV transmission during the preclinical incubation period of infection was assessed in seven groups of pigs, which were sequentially exposed to a group of infected donor pigs (Stenfeldt et al.). Results demonstrated significant differences between contact-exposed

OPEN ACCESS

Edited and Reviewed by:
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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *27 June 2017*
Accepted: *03 August 2017*
Published: *21 August 2017*

Citation:
Perez AM and Willeberg PW (2017) Editorial: Foot-and-Mouth Disease in Swine. Front. Vet. Sci. 4:133. doi: 10.3389/fvets.2017.00133

groups, in the time between virus exposure to first detection of FMDV shedding, viremia, and clinical lesions. These results are important because they suggest that FMDV shedding in oropharyngeal fluids does not correlate well with clinical signs of FMDV infection in pigs, which may affect FMDV transmission, and hence the effectiveness of control strategies in the face of an FMD epidemic.

The extent to which maternally derived antibodies interfere with the protection conferred by FMD vaccination was assessed in piglets (Dekker et al.). Results suggest that immune responses in piglets with maternally derived antibodies vaccinated at 7 or 9 weeks of age are similar to those of piglets without maternal immunity that were vaccinated at 3 weeks of age. These results are important because they demonstrate that maternally derived antibody levels in piglets strongly depend on the antibody titer in the sow, so the optimal time for vaccination in piglets will be affected by the vaccination scheme and the quality of vaccine used in the sows.

A review of results from recent experimental studies suggested that pigs were more susceptible to FMDV infection *via* exposure of the upper gastrointestinal tract (oropharynx) than through virus inhalation (Stenfeldt et al.). Due to massive amplification and shedding of virus, acutely infected pigs constitute an important reservoir for amplification of virus over the course of an epidemic. However, infection is ultimately cleared due to a strong humoral response and there is no evidence of subclinical persistence of FMDV infection in pigs. In general, FMDV infection in pigs spreads rapidly among in-contact pigs and efficiency of transmission depends on a number of factors, including the virus strain and the intensity of exposure to the virus. Under experimental conditions, physical separation of pigs may be sufficient to prevent virus transmission, which, in the field, may result in different infection patterns between and among sections or rooms within pig farms.

DESCRIPTIVE STUDIES AND REVIEWS

Foot-and-mouth disease is still to be eradicated from many regions of the world; for example, FMD epidemics are recurrent in Israel and in many Middle Eastern countries (Elnekave et al.). Although, for cultural reasons, swine production is not prevalent in the Middle East, there is a large population of wild boars in the region. On assessing 120 wild boar (*Sus scrofa lybicus*) samples, 15 (12.5%) were found to be FMD seropositive. Most of the FMD-positive samples obtained from wild boar [13/15 (86.7%)] were collected during 2007, and because clinical signs of FMD infection were not evident in these animals, it is possible that, under certain conditions, wild boars may contribute to maintenance and spread of FMD infection in the region.

Foot-and-mouth disease control programs in endemic settings are largely based on the use of vaccines. However, recent FMD epidemics in Asia demonstrated that developing an adequate artificial immune response is challenging in pigs. The performance of FMDV vaccines has been reviewed to identify knowledge gaps and provide ideas to improve efficiency and efficacy of vaccination programs (Lyons et al.). Factors found to affect vaccine performance include potency, antigenic payload, formulation of the vaccine, antigenic match between the vaccine and heterologous circulating field strains, and the vaccine administration regime, i.e., timing, frequency, and herd-level coverage.

In countries free from FMD infection, such as the US, response strategies are required in early control of hypothetical incursions, and disease simulation models play a role in the design of prevention and mitigation activities. Values associated with the duration of the stages of FMD infection (latent period, subclinical period, incubation period, and duration of infection), the probability of transmission (within-herd and between-herd *via* spatial spread), and the diagnosis of FMD within a herd were evaluated using a combination of a meta-analysis of the peer-reviewed literature and elicitation of expert opinion (Kinsley et al.). Although most US swine practitioners believed that they could detect an FMD incursion relatively soon, some estimated that up to half of the herd would need to show clinical signs before detection *via* passive surveillance would occur, which suggests the need for disease awareness programs in FMD-free countries.

APPLIED STUDIES

The ultimate objective of epidemiological studies is to create the foundations for the design and implementation of strategies and policy to prevent or mitigate disease impact, including modeling and risk analysis techniques [Perez; Willeberg et al.; (3)]. The risk of introducing FMDV into Australia through illegal importation of infected meat was quantified for large-scale pig producers, small-scale producers (<100 sows) selling at sales yards and abattoirs, and small-scale producers selling through informal means (Hernández-Jover et al.). Risk was quantified using scenario trees and Monte Carlo stochastic simulation. Although risk was predicted to be extremely low for the three sectors of the pig industry, exposure through direct swill feeding was 10–100 times more likely to occur than through contact with infected feral pigs. Furthermore, the FMDV would be more likely to spread from small-scale farms selling at sales yards and abattoirs compared to other sectors. Factors most influential on the probability of FMDV spread from the first-case farm included the effectiveness of the farmer in early disease detection, the probability of FMDV spread through contaminated fomites, and contact with ruminants on the farm. These results stress again the importance of programs to facilitate awareness and promote early detection of the disease in the face of an epidemic, and also, the importance of biosecurity in preventing disease introduction and spread into FMD-free areas.

One of the most challenging aspects of FMD response plans in FMD-free countries is the design of plans to secure continuity of business (COB) while implementing control measures to keep the food system functional and mitigate the impact of the epidemic. Animal health emergency response plans have been designed in the US to mitigate the unintended negative consequence of an FMD epidemic to stakeholders (Goldsmith et al.; Patterson et al.). The COB principles and goals adopted by the United States Department of Agriculture for responding to foreign animal diseases, such as FMD, are to (1) detect,

control, and contain the disease in animals as quickly as possible; (2) to eradicate the disease using strategies that stabilize animal agriculture, the food supply, and the economy that protect public health and the environment; and (3) to provide science- and risk-based approaches and systems to facilitate COB for non-infected animals and non-contaminated animal products. A protocol has been developed to use proactive risk assessments (i.e., before an outbreak happens) to authorize specific movements from low-risk premises located in control areas that are not known to be infected (Goldsmith et al.). However, this requires a system of prioritization of different types of movements. Highest priority was given by the industry to movement of weaned pigs originating from multiple sow farm sources to an off-site nursery or wean-to-finish facility, the movement of employees or commercial crews, the movement of vaccination crews, the movement of dedicated livestock hauling trucks, and the movement of commercial crews such as manure haulers and feed trucks onto, off, or between sites. These critical movements provide an initial guide for prioritization of risk management efforts and resources to be better prepared in the event of an FMD outbreak in the US and other FMD-free countries with the ultimate objective of regaining disease-free status while mitigating the impact on the industry.

FINAL REMARKS

In summary, the articles in this research topic explore and discuss important aspects of FMDV infection in swine, highlighting features that differ from traditional knowledge on the pathogenesis and epidemiology of the disease, as observed in cattle. The research topic advances our understanding of challenges in the design and implementation of vaccination campaigns to control the disease, the importance of biosecurity measures to prevent and limit its spread, and the role that modeling and risk assessments may play in mitigating the economic impact of FMD epidemics in swine.

AUTHOR CONTRIBUTIONS

AP and PW co-edited the research topic and wrote this editorial.

ACKNOWLEDGMENTS

The authors thank the scientists for submitting their works, the reviewers for critically evaluating the manuscripts, and the Frontiers Editorial Office for their help in producing this research topic.

REFERENCES

1. James AD, Rushton J. The economics of foot and mouth disease. *Rev Sci Tech* (2002) 21(3):637–44. doi:10.20506/rst.21.3.1356
2. OIE Technical Disease Cards. *Foot-and-Mouth Disease* (2017). Available from: http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/FOOT_AND_MOUTH_DISEASE.pdf
3. Halasa T, Willeberg P, Christiansen LE, Boklund A, AlKhamis M, Perez A, et al. Decisions on control of foot-and-mouth disease informed using model predictions. *Prev Vet Med* (2013) 112:194–202. doi:10.1016/j.prevetmed.2013.09.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

REVIEW
published: 23 May 2016
doi: 10.3389/fvets.2016.00041

The Pathogenesis of Foot-and-Mouth Disease in Pigs

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The greatest proportion of foot-and-mouth disease (FMD) clinical research has been dedicated to elucidating pathogenesis and enhancing vaccine protection in cattle with less efforts invested in studies specific to pigs. However, accumulated evidence from FMD outbreaks and experimental investigations suggest that critical components of FMD pathogenesis, immunology, and vaccinology cannot be extrapolated from investigations performed in cattle to explain or to predict outcomes of infection or vaccination in pigs. Furthermore, it has been shown that failure to account for these differences may have substantial consequences when FMD outbreaks occur in areas with dense pig populations. Recent experimental studies have confirmed some aspects of conventional wisdom by demonstrating that pigs are more susceptible to FMD virus (FMDV) infection *via* exposure of the upper gastrointestinal tract (oropharynx) than through inhalation of virus. The infection spreads rapidly within groups of pigs that are housed together, although efficiency of transmission may vary depending on virus strain and exposure intensity. Multiple investigations have demonstrated that physical separation of pigs is sufficient to prevent virus transmission under experimental conditions. Detailed pathogenesis studies have recently demonstrated that specialized epithelium within porcine oropharyngeal tonsils constitute the primary infection sites following simulated natural virus exposure. Furthermore, epithelium of the tonsil of the soft palate supports substantial virus replication during the clinical phase of infection, thus providing large amounts of virus that can be shed into the environment. Due to massive amplification and shedding of virus, acutely infected pigs constitute a considerable source of contagion. FMDV infection results in modulation of several components of the host immune response. The infection is ultimately cleared in association with a strong humoral response and, in contrast to ruminants, there is no subclinical persistence of FMDV in pigs. The aim of this review is to provide an overview of knowledge gained from experimental investigations of FMD pathogenesis, transmission, and host response in pigs. Details of the temporo-anatomic progression of infection are discussed in relation to specific pathogenesis events and the likelihood of transmission. Additionally, relevant aspects of the host immune response are discussed within contexts of conventional and novel intervention strategies of vaccination and immunomodulation.

Keywords: foot-and-mouth disease, foot-and-mouth disease virus, pigs, pathogenesis, host response, virus diseases, virology

OPEN ACCESS

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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *15 March 2016*
Accepted: *06 May 2016*
Published: *23 May 2016*

Citation:
Stenfeldt C, Diaz-San Segundo F, de los Santos T, Rodriguez LL and Arzt J (2016) The Pathogenesis of Foot-and-Mouth Disease in Pigs. Front. Vet. Sci. 3:41. doi: 10.3389/fvets.2016.00041

RELEVANCE

Foot-and-mouth disease (FMD) is recognized as one of the most contagious and economically important diseases of domestic livestock. The etiological agent, FMD virus (FMDV), an aphthovirus of the *Picornaviridae* family, is capable of infecting a multitude of cloven-hoofed animal species including both ruminants and suids (1, 2). Although domestic cattle are often prioritized with regards to FMD prevention and strategic countermeasures, it is important to recognize that pigs constitute a substantial proportion of agricultural production in large areas of the world. Even though cattle and pigs may be similarly susceptible to FMDV infection under most circumstances, there are critical differences in FMD pathogenesis and infection dynamics that emphasize the importance of species-specific experimental investigations and adaptation of countermeasure policies. Important distinctions between cattle and pigs in FMD pathogenesis events include variations in permissiveness to infection by different routes of virus exposure and thereby differences in the most likely mechanisms of virus transmission between animals. Furthermore, variations in the quantities of virus shed by aerogenous routes, as well as the capability of long-term persistence of infectious virus in tissues of ruminants, but not pigs, indicate important differences pertaining to risk assessments and practical management of infected or convalescent animals.

It is well known that the clinical severity of FMD may vary greatly depending both on the virus strain and the affected host species (1, 2). Acute clinical FMD has been reported to be more severe in pigs compared to ruminant species (1). Contrastingly, pigs are more efficient in complete clearance of the infection, and there is no subclinical "FMDV carrier state" in suids (3). It has also been widely accepted that while pigs are capable of generating large amounts of aerosolized virus, they are less susceptible to airborne infection compared to ruminants (4, 5). Demonstrated variability in host range of certain FMDV strains that are significantly attenuated in cattle, yet virulent in pigs provides additional evidence of the existence of host-specific differences in the molecular pathways of FMDV infection (6–9). Specifically, it was confirmed that a mutation within the FMDV 3A coding region was the determinant for the strictly porcinophilic phenotype of the serotype O FMDV that caused an outbreak in Taiwan in 1997 (8, 10).

A large proportion of experimental studies investigating FMDV pathogenesis and vaccinology have been performed in cattle. Furthermore, the guidelines for FMDV vaccine production published by the World Organization for Animal Health (OIE) only define procedures for efficacy testing in cattle (11). In many regions, it is common practice to vaccinate only cattle, but not pigs, based on the assumption that this practice may be sufficient to prevent dissemination of a potential outbreak. This premise may be misguided if extrapolated to regions with intensive pig production or substantial quantities of wild suids. Several experimental studies have demonstrated difficulty in achieving sufficient protection against clinical FMD in pigs by vaccination, especially when the virus challenge consisted of direct exposure to clinically infected pigs (12–15). Additionally, recent experiences from South Korea have shown that high quality FMDV vaccines with confirmed efficacy in cattle may fail to elicit sufficient levels of immunity (based on serum neutralization testing) when administered to pigs in commercial production settings (16). These distinct, porcinocentric scenarios may be explained by species-specific differences in susceptibility to the virus or by differences in the host response to vaccination. Regardless of the causality, the documented variations between cattle and pigs in outcomes of both vaccination and infection suggest that FMD control policies may, justifiably, be based on species-specific data and should be adapted to account for the composition of the animal population in any given region. Such differences are also highly relevant for disease modeling, wherein it is critical to account for species-specific aspects of FMDV infection dynamics and transmission in order to precisely model distinct scenarios.

FMD IN PIGS

Routes of Infection

Early experimental studies performed by Terpstra (17) concluded that pigs were highly susceptible to FMDV *via* artificial aerosol exposure, while a 1000-fold higher inoculation dose was required to achieve successful infection by virus instillation in the oral cavity. This was subsequently contradicted in works by Alexandersen and Donaldson which demonstrated that pigs were largely resistant to FMDV infection by inhalation of naturally produced aerosols (4, 5). Additionally, more recent investigations have confirmed that the porcine upper respiratory tract (nasopharynx) is less permissive to inoculation by direct deposition of virus when compared to the upper gastrointestinal tract (oropharynx) (18, 19). Infection *via* the oral route is likely mediated by virus entry through the mucosal surfaces of the oropharyngeal tonsils rather than trough the lower gastrointestinal tract. This is supported by demonstrated tropism of tonsillar epithelium to primary FMDV infection (20) as well as the instability of FMDV at low pH (21, 22), which likely leads to dissociation of virus particles that reach the stomach. The predilection for virus entry *via* the porcine upper gastrointestinal tract is in direct contrast to primary FMDV infection of cattle, which has been demonstrated to occur in the upper respiratory tract (23–26). However, despite this apparent discrepancy in anatomic location, there are striking similarities in microanatomic characteristics of the epithelium that supports primary FMDV replication in both cattle and pigs (19, 23, 25). Specifically, in both species, primary infection occurs at distinct regions of epithelium overlaying mucosa-associated lymphoid tissue (MALT). In these regions (so-called reticular- or follicle-associated epithelium), the epithelium is intimately associated with the subjacent lymphoid follicles, the basement membrane is discontinuous, and there are abundant intraepithelial (transmigrating and resident) leukocytes.

The relative resistance of pigs to aerogenous FMDV infection has been further corroborated by several experimental studies, which have shown that physical separation of pigs is sufficient to prevent transmission of virus under experimental conditions (27–29). Contrastingly, direct contact exposure leads to rapid transmission of infection within groups of pigs that are housed together. Furthermore, it has been demonstrated that this system

of virus exposure is often sufficient to overcome vaccine protection (15) even though vaccination may reduce shedding of virus and thereby lower the transmission rate (12, 14). The efficiency of transmission of FMDV under experimental conditions varies between different strains of FMDV (30, 31). Additionally, external factors, such as housing density, the intensity of interactions between animals, and the duration of exposure, will directly influence the outcome of experimental transmission studies (30, 32, 33). Even though these findings strongly suggest that direct physical contact between pigs facilitates FMDV transmission, the specific route of virus entry during contact exposure has not been completely identified. The susceptibility of the porcine oropharyngeal mucosa to FMDV infection would support virus transmission *via* the oral route, e.g., from salivation and subsequent ingestion of shed virus during communal feeding. However, direct entry of virus through skin abrasions and punctures derived from biting or oral entry mediated through direct contact to exposed vesicular lesions on donor animals may also constitute likely transmission routes.

There are many options for challenge systems for FMD experimentation in pigs, which reflect the differences described above. FMDV infection in pigs is often achieved by intraepithelial injection of the heel bulb (27, 34–39). This technique is convenient for vaccine studies, as the pedal epithelium is highly permissive to FMDV infection, leading to substantial amplification of the injected virus at the inoculation site and consistently rapid progression of generalized FMD in susceptible animals. Despite the convenience and consistency of injection-based inoculation techniques, these systems are less appropriate for studies of disease pathogenesis as they are based on an artificial route of virus entry that bypasses the natural barrier of the mucosal immune system. As mentioned above, direct contact exposure to infected animals is highly efficient in generating infection in susceptible animals. However, critical factors, such as the dose and timing of virus challenge, are difficult to control in contact-based systems, which may lead to inconsistencies across studies or misinterpretations of experimental outcomes. Recent studies have demonstrated that controlled exposure of the porcine upper gastrointestinal tract by deposition of virus inoculum in the oropharynx of sedated pigs is highly efficient in generating consistent and synchronous clinical FMD and may thus be considered a valid alternative to the more traditional injection-based challenge systems (18–20).

Temporo-Anatomic Progression of Infection

Primary Infection (Pre-Viremia)

Relatively few experimental studies have been dedicated to investigation of the progression of FMDV infection in porcine tissues following natural or simulated natural virus exposure (17, 20, 34, 36, 40). There is general agreement across these investigations that epithelial tissues of the oropharynx constitute the main sites of virus replication during early infection, whereas abundant amplification of virus occurs in vesicular lesions at secondary (peripheral) replication sites (**Figure 1**). However, there are slight variations among published works regarding the interpretation of the precise events that constitute the initial phase of FMDV infection in pigs.

A recent investigation demonstrated specific predilection of primary FMDV infection to porcine paraepiglottic tonsils (**Figure 2A**). This was concluded based on consistent detection of FMDV RNA and infectious virus by qRT-PCR and virus isolation (VI), respectively, prior to the development of viremia and generalization of infection. Additionally, FMDV structural and non-structural viral proteins were localized to crypt epithelium of this specific tonsil by immunomicroscopy at 6–24 h post intraoropharyngeal inoculation (20). Early detection of FMDV RNA and infectious virus was more variable in the tonsil of the soft palate, lingual tonsil, and the dorsal soft palate, suggesting that these sites may also be potential sites of primary infection. A similar investigation performed by Murphy et al. (34) reported detection of FMDV RNA in tonsils, submandibular lymph nodes, spleen, liver, tongue, skin, and pharynx, prior to the detection of viremia. However, the earliest time point for tissue collection in this study was 24 h post contact exposure, which may account for the somewhat wider distribution of viral genome. Additionally, in this study, localization of viral replication was not confirmed by VI or microscopy. Similarly, an earlier investigation by Alexandersen et al. (36) concluded that the highest quantities of FMDV RNA during pre-clinical infection of contact exposed pigs were found in the dorsal soft palate and tonsil (24–48 h post exposure). Noteworthy for these latter two investigations is that the term "tonsil" is not further defined anatomically but may be assumed to represent the tonsil of the soft palate. However, there are multiple distinct tonsils in the porcine oropharynx, including the tonsil of the soft palate, lingual tonsil, and paraepiglottic tonsils (41).

An earlier study by Brown et al. (40) described an investigation of tissue distribution of FMDV RNA by *in situ* hybridization (ISH) in pigs infected by intraepithelial injection, as well as morphological characterization of microscopic lesions associated with the detection of viral genome. This study described widespread dissemination of FMDV genome in the epidermis from 24 to 96 hours post infection (hpi), at sites with or without visible FMDV-associated lesions (40). The study does not include determination of the onset of viremia and systemic dissemination of virus in relation to the time points for tissue collection. However, it is mentioned that pigs euthanized at 24 hpi, corresponding to the earliest time point investigated, were clinically depressed with marked vesicles at the epithelial inoculation sites on the snout and lips. The somewhat different findings between these published studies highlights the differences in experimental outcomes pertaining to experimental design, e.g., inoculation/exposure routes and time points included in the investigation, as well as methods used for virus detection. It is clear that detection of virus genome by qRT-PCR or ISH may lead to different outputs compared to VI or detection of antigen by immunomicroscopy. Combining multiple techniques incurs additional cost and time investment, but ultimately provides a more detailed and substantiated experimental output.

Viremia and Clinical Disease

In all *in vivo* studies, the onset of viremia is a critical milestone in FMD pathogenesis, as it accompanies a surge in contagion and

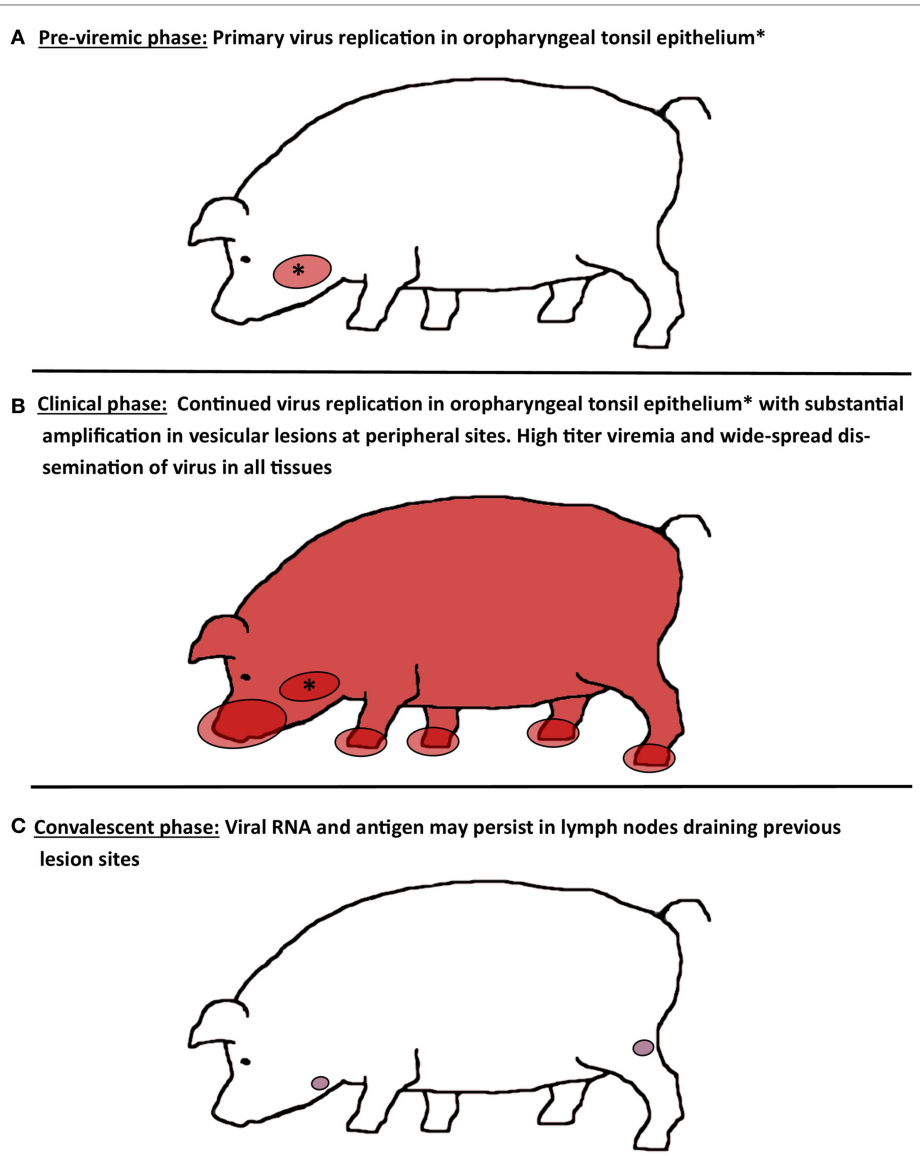

FIGURE 1 | Schematic illustration of virus distribution in tissues during distinct phases of FMD in pigs. **(A)** During the pre-viremic phase of infection, primary virus replication is localized to epithelium of oropharyngeal tonsils. **(B)** During the clinical phase of infection, FMDV can be recovered from essentially every tissue or organ sampled due to high titers of virus in blood. Virus replication in oropharyngeal tonsil epithelium continues, while substantial amplification of FMDV occurs in vesicular lesions on the feet, snout, and in the oral cavity. **(C)** After resolution of viremia and clinical disease, FMDV genome and antigen can be recovered from lymph nodes that drain lesion sites for up to 2 months. However, there is no persistence of infectious virus.

predicts the impending clinical syndrome. In pigs, viremia may be detected as early as 24 h after natural or artificial virus exposure, and it is associated with a substantial increase in shedding of infectious virus *via* the oropharyngeal route (3, 20, 30, 42). The onset of clinical FMD, which usually occurs approximately 24 h after detection of viremia, is characterized by fever, loss of appetite, and the appearance of vesicular lesions on feet, snout, and within the oral cavity (20, 30, 34). The initial phase of infection, consisting of the progression from primary, pre-viremic, infection to viremia and clinical disease may be prolonged following exposure to an FMDV strain of reduced virulence, or if exposure conditions are less stringent (e.g., suboptimal exposure route, low challenge dose, or time-limited exposure) (19, 27, 30, 33, 43).

During clinical FMD, the highest quantities of infectious virus are found in vesicular lesions in cornified epithelium of the feet (heel bulbs and coronary bands), on the snout and on the dorsal surface of the tongue (20, 34, 36, 40). It has recently been demonstrated that during clinical disease abundant virus replication occurs in epithelial crypts of the tonsil of the soft palate, and that microscopic vesicular lesions containing large quantities of viral protein can be detected at this site (**Figures 2B,C**) (20). During peak viremia, FMDV RNA and infectious virus can be recovered

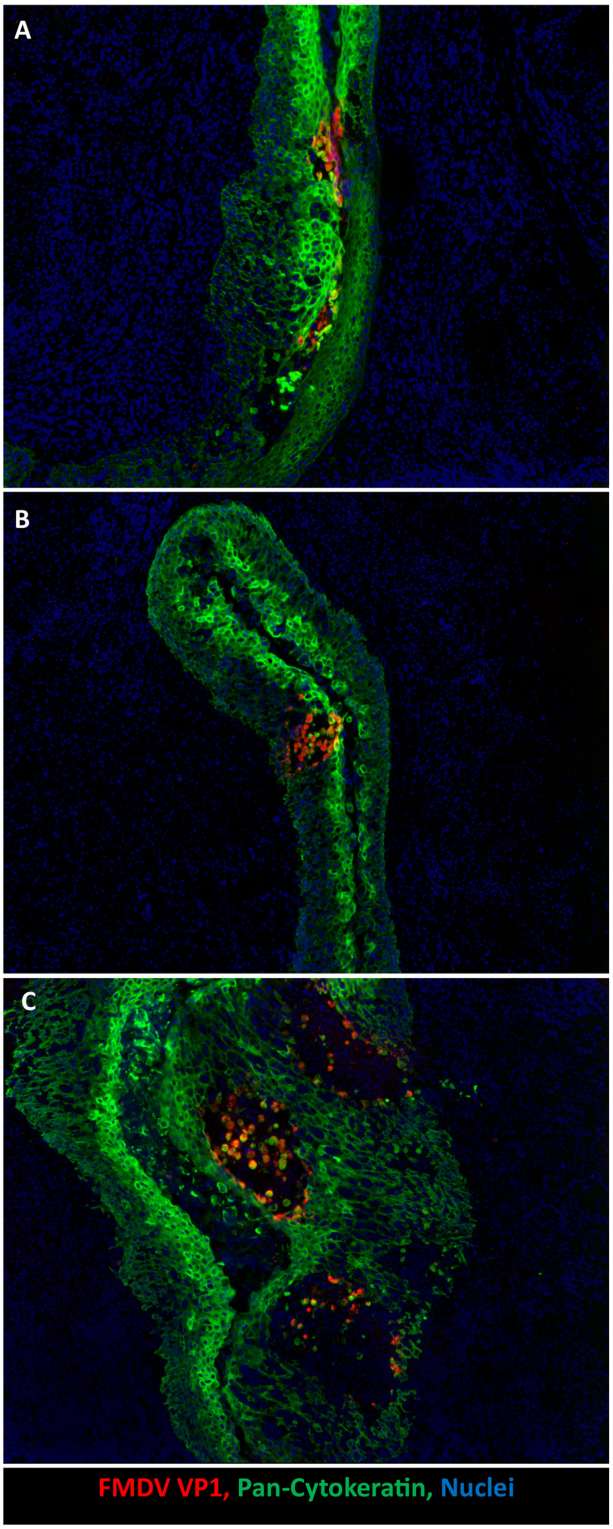

FIGURE 2 | Development of microvesicles within oropharyngeal tonsil epithelium during early infection. **(A)** Earliest detection of infection occurs within paraepiglottic tonsil at 24 h post intraoropharyngeal inoculation. FMDV antigen (red) in clusters of infected cytokeratin-positive (green) epithelial cells

(Continued)

FIGURE 2 | Continued

in superficial layers of crypt epithelium. **(B)** At 48 h post intraoropharyngeal inoculation, a single microvesicle is present within the tonsil of the soft palate. Focus of FMDV-infected (red) epithelial cells expanding through deeper layers of epithelium (green). **(C)** At 78 h post intraoropharyngeal inoculation, three distinct microvesicles are present within crypt epithelium of the tonsil of the soft palate. Sloughed FMDV VP1/cytokeratin double positive cells are present in vesicle lumen. 10× magnification.

from essentially any tissue sampled (**Figure 1**), but such detection represents intravascular, viremic FMDV rather than regional replication (20).

It is well established that clinically infected pigs release infectious FMDV in exhaled air at quantities substantially greater than cattle (43–46). However, the anatomic source of exhaled virus remains incompletely elucidated. Donaldson and Ferris (47) used an approach of direct air sampling from intact or intubated pigs to evaluate the sources of exhaled virus during different phases of FMD. This demonstrated that infectious FMDV was primarily recovered from the upper respiratory tract during early infection, but that both upper and lower segments of the respiratory tract contributed to exhaled virus during the clinical phase of FMD (47). Unfortunately, there was no collection or analysis of tissue samples in this study, and more detailed conclusions regarding the anatomic sites of released virus are therefore lacking. With the exception of Terpstra (17), tissue-based pathogenesis studies have failed to demonstrate substantial amplification of FMDV in porcine lungs (20, 34, 36).

The tonsil of the soft palate is the only tissue in the respiratory or gastrointestinal tract that has been shown to support substantial levels of FMDV replication (20, 36), and it is therefore the best candidate as the source of aerosolized FMDV derived from pigs. This tonsil is located at the dorsal boundary of the oropharynx and is therefore not within the direct route of exhaled air passing from the lungs through the nasopharynx and nasal cavity. However, the tonsil is anatomically continuous with the dorsal soft palate, and therefore FMDV originating from the tonsil may be aerosolized in the nasopharynx. Additionally, exhalation of air through the oropharynx and mouth, as would occur during vocalization, would pass directly across the surface of the tonsil of the soft palate and facilitate direct aerosolization. Another potential source of airborne virus is secondary resuspension of virus that has been shed into the environment in secretions and sloughed vesicles. However, this would not provide an explanation for the apparently higher quantities of aerogenous virus produced by pigs compared to cattle.

Despite relative resistance to FMDV infection *via* aerogenous exposure (4, 5), clinically infected pigs are a potential source of infection for exposed ruminants due to release of large amounts of aerosolized virus (44). Furthermore, the extent of virus dissemination in porcine tissues during viremia is noteworthy in that pigs and pork harvested during the viremic phase of disease contain massive loads of infectious FMDV. Thus, FMDV-infected pigs constitute a considerable source of contagion during the clinical phase of disease, and movement of live pigs or associated products can have substantial impact on disease spread. These aspects of FMD pathogenesis are of

critical importance for the establishment of efficient measures to control outbreaks. Stringent restrictions on movements of animals and animal products, as well as depopulation of infected premises are generally required in order to control dissemination of the disease, regardless of whether emergency vaccination is applied.

Clearance of Infection

The clinical phase of FMD subsides within approximately 7–14 days post infection (dpi). In the absence of complications due to secondary bacterial infections, adult pigs generally recover from FMDV infection, although severe foot lesions may cause enduring lameness and debilitation (**Figure 3**).

FMDV-neutralizing antibodies can be measured in serum of the infected pigs from approximately 4–7 dpi (39, 48). This is followed by a subsequent clearance of infectious virus from blood within approximately 7–14 dpi (3, 49). A single publication by Mezencio et al. (50) concluded that it was possible to detect FMDV RNA in porcine sera as late as 300 dpi. However, this finding has not been repeated or confirmed in any subsequent investigations. Consistent shedding of FMDV RNA can be detected in oral and nasal secretions for up to 14 dpi (3, 49), with some variation across different virus strains. It is likely that the infectiousness of shed virus and thereby contagion associated with pigs recovering from infection is substantially reduced concurrent with increasing titers of neutralizing antibodies in secretions. Nonetheless, despite a large number of published FMDV contact transmission studies, there is no detailed experimental investigation that has precisely documented the duration of infectiousness of FMDV-infected pigs.

In contrast to ruminant species, pigs that survive FMDV infection efficiently clear infectious virus from all tissues after resolution of the clinical disease (3). A study by Rodriguez-Calvo et al. (49) demonstrated that infectious serotype C FMDV could be recovered from porcine tonsils as late as 17 dpi, postulating existence of a putative FMDV carrier state in pigs. A subsequent investigation, using five different strains of FMDV, demonstrated that it was not possible to recover infectious FMDV from any porcine tissues harvested beyond 28 dpi, corresponding to the commonly acknowledged threshold for FMDV persistence (3). However, the same investigation showed that (non-infectious) FMDV RNA could be detected within porcine lymphoid tissue for up to 60 dpi, with highest detection prevalence and most abundant RNA quantities found in the popliteal lymph node that drains the hind feet (**Figure 1**). Concurrent detection of FMDV structural protein and absence of non-structural protein in popliteal lymph nodes by immunomicroscopy supported the conclusion that viral degradation products may persist in lymphoid organs beyond clearance of infectious virus (3). Detection of FMDV RNA in lymph nodes harvested from both domestic and feral pigs after resolution of clinical disease has been demonstrated in several studies (51–53). However, there is no convincing report documenting isolation of infectious FMDV from porcine tissues beyond 17 dpi.

FMDV Myocarditis in Pigs

Although FMD-related mortality among adult pigs is generally low, mortality rates may be higher in juvenile animals (1), and there are often reports of sporadic deaths occurring during experimental studies (6, 19, 31, 54, 55). FMD-related deaths are often attributed to acute viral myocarditis, even in cases when the precise cause of death has not been definitively determined. However, it is well established that acute FMDV infection may cause infection of the myocardium, leading to heart failure and

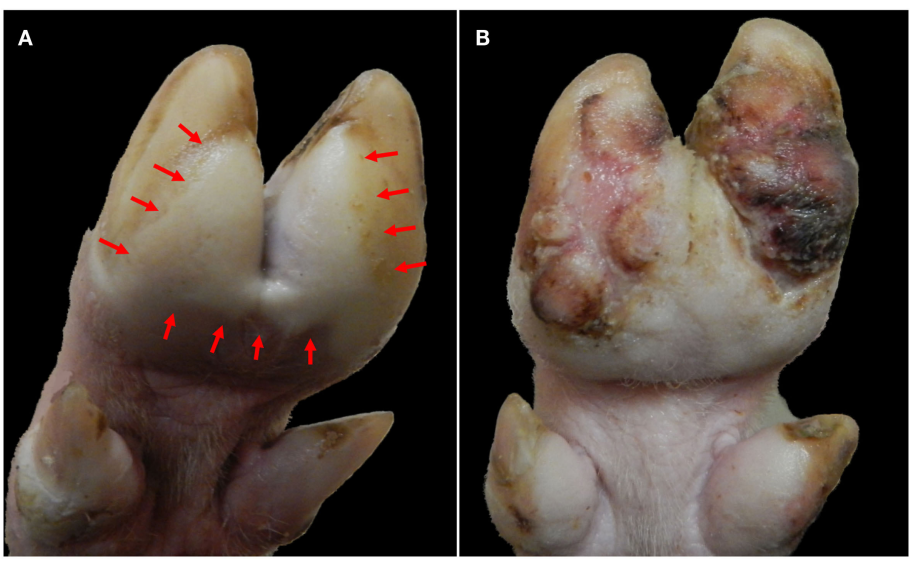

FIGURE 3 | Progression of foot lesions in pig infected with FMDV A_{24} Cruzeiro at 2 (A) and 24 (B) days after intraoropharyngeal inoculation. **(A)** Vesicular lesion on solar aspect of hind foot at 2 dpi. Blanching (white) epithelium (delineated within arrows) extends across both heel bulbs and interdigital skin with clear demarcation from normal skin. **(B)** The same animal at 24 dpi. Proliferative dyskeratotic scar tissue has replaced sloughed epithelium.

sudden death (56–58). The gross pathological findings associated with FMDV-induced myocarditis may range from complete absence of visible lesions to distinct areas of pallor on the cardiac surface that extend into subjacent myocardium (**Figure 4A**). Effusion in thoracic and/or abdominal cavities may occur in subacute or chronic cases indicating congestive heart failure, but are often absent in acute, rapidly progressing cases. The terms "tiger stripes" or "tiger heart" are commonly used to describe gross pathological changes associated with FMDV myocarditis. It is our opinion that these are inappropriate and often confusing descriptions as the myocardial pallor induced by FMDV myocarditis rarely assumes a striped pattern. In contrast, stripe-like pale coloration of the myocardial surface is often found as part of normal anatomy associated with superficial vessels and epicardial fat.

Histological findings suggestive of FMDV-induced myocarditis may be predominantly acute and necrotizing or subacute–chronic with various hallmarks of inflammation (**Figures 4B,C**). Generally, necrosis and inflammation coexist in every lesion with continuum of severity. Inflammation typically includes lymphohistiocytic infiltration and edema, whereas cardiomyocyte degeneration and necrosis may occur as individual cells, small clusters, or may be regionally extensive (**Figure 4C**) (58). Regions of architectural disruption may have evidence of viral replication including presence of viral antigens and nucleic acids detectable by immunomicroscopy and *in situ* hybridization, respectively (**Figure 4D**).

Viral loads in the myocardium are massive, often approaching levels otherwise only found in vesicular lesions (58). The prevalence of FMDV myocarditis varies between different strains of FMDV as well as the age of the infected host (2). Interestingly, despite extensive investigation, we have not found evidence of FMDV replication in myocardium of any infected pigs that did not display clinical signs of heart failure.

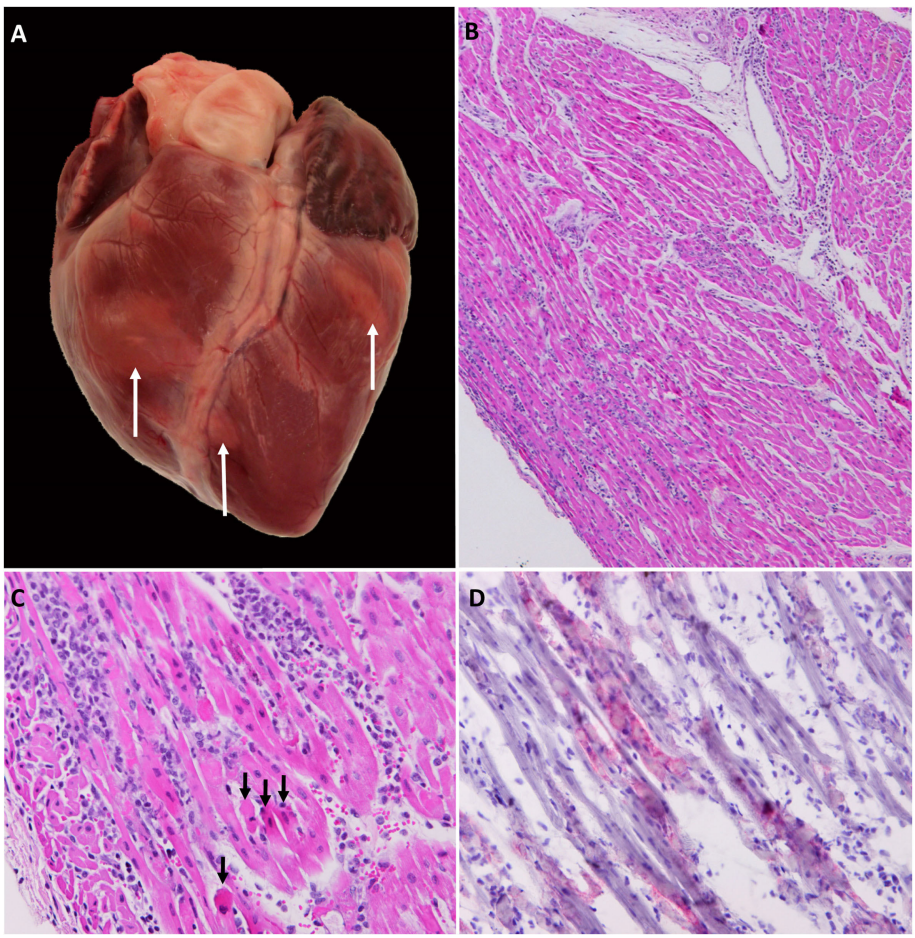

FIGURE 4 | Gross, histological, and immunomicroscopic characterization of FMDV myocarditis. **(A)** Gross image of porcine heart with confirmed FMDV-associated myocarditis. Multifocal pallor on the surface of both right and left ventricles (arrows). **(B,C)** Right ventricle of pig found dead at 5 days post infection with FMDV A_{24} Cruzeiro. Interstitial edema and mixed mononuclear infiltrates consisting of lymphocytes, large macrophage-like cells, and scarce neutrophils. Myocyte necrosis and fragmentation (arrows). Hematoxylin and eosin **(B)** 4× magnification, **(C)** 20× magnification. **(D)** Immunohistochemical staining demonstrating localization of FMDV within cardiomyocytes. Anti-FMDV capsid monoclonal antibody (red). Micropolymer alkaline phosphatase. Gill's hematoxylin counterstain. 20× Magnification. Gross image **(A)** was edited to reduce artifactual glare from flash; lesion areas were not modified.

Since FMDV RNA and infectious virus can be recovered from essentially any tissue harvested during the viremic phase of infection, isolation of FMDV from the heart of a pig with FMD is not sufficient for a diagnosis of FMDV myocarditis. A tentative diagnosis of myocarditis may be based on clinical history of unexpected death in an animal confirmed to be infected with FMD combined with gross findings of (multi)focal mycocardial pallor (**Figure 4A**). Viral loads in myocardium, which are higher than those detected in serum, provide further support. But, confirmation of FMDV myocarditis requires histopathological identification of lesions combined with immunohistochemical confirmation of presence of FMDV. Additionally, it is noteworthy that identical clinical and histopathological findings have been documented following infection of pigs with encephalomyocarditis virus (EMCV) (59, 60), a related Picornavirus with worldwide distribution (61).

HOST RESPONSE TO FMDV IN PIGS

FMD virus has been demonstrated to modulate the host immune response *via* several mechanisms. Thus, understanding host/pathogen interactions and elucidating the contributions of innate versus adaptive immune responses have become a central topic in FMDV research. Although the host response to FMDV infection is incompletely elucidated, in recent years several studies in swine and cattle have been published, some of them with controversial results. These differences directly reflect the existence of species-specific variations in both systemic and cellular responses to infection (62) that ultimately justifies continued investigation in both suids and ruminants.

Cellular and Humoral Immune Response

During the early phase of viral infections, interactions between pathogens and cellular components of the innate immune response, such as natural killer (NK) cells, dendritic cells (DCs), and macrophages, define the cellular and humoral adaptive immune response that is believed to ultimately clear the infection. During FMDV replication in pigs, the virus may come in contact with antigen-presenting cells (APCs), either as a result of lytic infection of epithelial cells and subsequent phagocytosis of damaged tissue (63) or by direct infection of immune cells through an antibody-dependent internalization process [macrophages (64, 65) or DCs (66, 67)]. Although the interactions between FMDV and APCs have been shown to be abortive and no virions are produced (63, 67), there are functional consequences that affect the host response. During acute infection, the virus stimulates DCs to produce interleukin (IL)-10, thus directing the adaptive immune response toward a stronger humoral rather than a T-cell-mediated response (67). FMDV also blocks the ability of porcine DCs to differentiate into mature conventional DCs (67) and impairs the response to stimulation by TLR ligands (68).

A distinct subset of DCs; plasmocytoid DCs (pDCs), are susceptible to FMDV infection *in vitro* (66). pDCs internalize immunoglobulin-bound FMDV immune complexes *via* FcγRII surface receptors, and uptake of these complexes results in abortive virus replication (66). Similarly, studies in pigs have shown that these cells are directly affected by FMDV, as the infection leads to depletion of pDCs in peripheral blood, and the remaining pDCs are less capable of producing interferon (IFN)-α in response to *ex vivo* stimulation by TLR ligands or FMDV (69). Porcine Langerhan cells (LCs), identified as a langerin-expressing subset of DCs found in the epidermis (70), are also affected by FMDV infection (68, 71). Although these cells constitutively express type I IFN, *in vitro* studies have demonstrated that FMDV is able to attach to, and become internalized by LCs; however, there is no evidence of internalization leading to replication of viral RNA or production of viral proteins (71). Furthermore, LCs from FMDV-infected pigs produced less IFN-α after *ex vivo* stimulation, although the cells' ability to present antigen was retained (68).

Natural killer cells also play a critical role during the initial host response to pathogens. Although *in vitro* stimulation of porcine NK cells using pro-inflammatory cytokines induces lysis of FMDV-infected cells and increased expression of IFN-γ (72), *in vivo* studies have demonstrated that NK cells from swine infected with FMDV have a reduced capacity to lyse target cells and secrete IFN-γ (72). NK cell dysfunction during the viremic phase of acute infection suggests that FMDV can effectively block NK function, thereby evading the host's immune system and promoting virus replication and dissemination within the host.

Another mechanism whereby FMDV may evade the porcine cellular immune response is the induction of severe lymphopenia and lymphoid depletion during peak viremia. The lymphopenia is accompanied by a long-lasting suppression of T-cell function, as T-cells have been shown to respond poorly to mitogen stimulus even after the lymphopenia is resolved (73, 74). However, the mechanisms by which the virus induces this immunosuppression are not completely understood. Lymphocyte depletion and T-cell dysfunction may be caused by viral replication in lymphocytes as has been described during FMDV serotype C infection in swine (73), as well as in *in vitro* experiments investigating FMDV infection of bovine peripheral blood mononuclear cells (PBMCs) (75). However, subsequent studies concluded that active infection of lymphocytes by FMDV could not be demonstrated when pigs were infected with other FMDV serotypes (69, 74). Furthermore, these investigations concluded that FMDV infection was not associated with cell death, suggesting that lymphopenia during FMDV infection might not be related to virus-mediated killing. Additionally, it cannot be ruled out that FMDV-associated lymphopenia may represent a shift of circulating lymphocytes from circulating pool to marginating and tissue-residing pools. An additional mechanism that may contribute to the diminished T cell response during FMDV infection could be related to the elevated amounts of IL-10 produced by conventional DCs that, as mentioned above, has been reported to have immunosuppressive functions *in vivo* for FMDV (67) and for other viruses (76).

Despite the apparent inhibitory actions of FMDV on the cellular host response during early infection, pigs are capable of mounting a substantial humoral response within few days of infection, and there is no documentation of any long-term negative effects on the immune system in pigs that survive FMDV infection. In fact, the high levels of IL-10 during acute infection may skew the adaptive immune response toward a stronger humoral rather than a T-cell-mediated cellular response. The serological response of naive pigs to FMDV infection consists of

a rapid surge of anti-FMDV IgM that peaks at 7 dpi and subsequently declines to baseline levels by approximately 4 weeks after infection (39). The IgM response is followed by a sustained anti-FMDV IgG response, which remains at high titers beyond 28 days (39). High titers of neutralizing antibodies can be detected as early as 4–7 days after infection, and unpublished results from our laboratory have confirmed that neutralizing antibody titers remained at high levels as late as 100 days after infection with FMDV A_{24} Cruzeiro. To the best of our knowledge, there is no published documentation of the duration of immunity following FMDV infection in pigs.

Systemic Antiviral Host Response

Type I, II, and III IFNs, including IFN-α, -β, -γ, and -λ, are critical components of the innate host response to viral infection. Induction of IFN pathways involve initial recognition of pathogen-associated molecular patterns (PAMPs) by cellular pattern-recognizing receptors (PRRs), such as the family of toll-like receptors (TLRs) and cytosolic sensors, eventually leading to the activation of interferon-stimulated genes (ISGs) and production of a variety host proteins with antiviral functions (77–82).

FMD virus has been shown to partially counteract the innate immune response *in vitro* by blocking the expression of IFN (83, 84). Similarly, *in vivo* in pigs, it has been shown that during acute infection FMDV suppresses IFN-α production by skin, myeloid, and plasmacytoid DCs (68, 69, 74). However, it has also been reported that FMDV infection induces a systemic IFN response, which coincides with the onset of viremia (69).

Although the extent of endogenous IFN response in FMDV-infected pigs is incompletely understood, it has been thoroughly documented that FMDV replication in pigs is highly sensitive to the exogenous administration of type I, II, and III IFNs delivered using recombinant vector constructs (83, 85–88). Specifically, pigs pretreated with human adenovirus serotype 5 (Ad5) vectors expressing either porcine IFN-α (Ad5–poIFN-α), porcine IFN-β (Ad5–poIFN-β), porcine IFN-γ (Ad5–poIFN-γ), or porcine IFN-λ (Ad5–poIFN-λ) were efficiently protected against challenge with different FMDV serotypes at 1 day after IFN delivery (37, 88–91) and IFN-induced protection has been demonstrated to last approximately 3–5 days (90). Interestingly, combination of type I and type II IFN results in synergistic anti-FMDV activity *in vivo*; swine inoculated with a combination of Ad5–poIFN-α and Ad5–poIFN-γ, at doses that alone do not protect against FMDV, are completely protected against clinical disease and do not develop viremia (88). More recently, a similar approach using an Ad5 that expressed porcine IFN-α and IFN-γ bicistronically also showed an enhancement of the antiviral activity as compared to Ad5 constructs that only expressed either IFN alone (92). Studies aimed at elucidating the mechanisms by which IFN protects swine against FMD have demonstrated that protection of swine inoculated with Ad5–poIFN-α correlated with recruitment of skin DCs (38), which showed a partial maturation phenotype with increased expression of CD80/86 and decreased phagocytic activity (93).

Administration of Ad5–IFN, type I, II, or III to cattle or pigs leads to induction of numerous genes in association with protection against FMDV (38, 88, 93). However, by directly administering IFN constructs, the natural pathways of interaction of unique viral molecules (or PAMPs) with specific PRRs present in host cells are bypassed. Therefore, to induce a broader, enhanced, and prolonged antiviral response, treatment of animals with various PAMPs could potentially result in a positive feedback induction of additional IFN production (94, 95). Recently, two different strategies have successfully exploited this concept in pigs: (i) the use of double stranded RNA, poly IC, in combination with IFN treatment (96) and (ii) expression of a constitutively active transcription factor, IRF7/3 (5D) fusion protein delivered with the Ad5 vector platform (97).

The value of enhanced understanding of IFN and ISG effects upon FMDV replication in pigs derives from the potential to develop combined-delivery products containing FMDV vaccines and select immunomodulatory constructs. Such products could provide rapid onset and broad protection that could prevent primary virus infection prior to the development of vaccine-induced antibodies. Additionally, enhancement of specific pathways of the innate host response may also serve to strengthen the adaptive immune response, ultimately leading to an overall improved vaccine response.

CONCLUDING REMARKS

To summarize the consensus interpretation of typical FMD pathogenesis in pigs, the majority of experimental investigations suggest that primary FMDV replication during pre-viremic infection occurs in the oropharynx. More detailed investigations have identified epithelial crypts of oropharyngeal tonsils as preferred site of primary infection. Oropharyngeal shedding of virus increases substantially concurrent with the development of viremia, which occurs approximately 24 h prior to appearance of clinical FMD lesions. Abundant quantities of infectious virus can be recovered from essentially all tissues harvested during peak virema, although virus replication in the oropharynx and in vesicular lesions on the feet, snout, and in the mouth constitute the most significant sources of contagion during the clinical phase of disease. FMDV modulates the host immune response and causes severe lymphopenia during acute infection. However, there is a strong humoral immune response, and virus is cleared from circulation within 2 weeks of infection. FMDV RNA and structural antigen may be recovered from lymphoid tissues for several weeks after resolution of the clinical disease, but there is no evidence of the existence of an FMDV carrier state in pigs. These distinct aspects of FMD in pigs should be considered in the development and deployment of response policies and in the modeling of FMD in pigs.

AUTHOR CONTRIBUTIONS

CS planned the work and drafted the manuscript. FD drafted the section on host response. TS contributed scientific contents. LR contributed scientific contents. JA planned and coordinated the work and contributed in writing the manuscript. All authors have critically reviewed and revised the manuscript and approved the final product.

ACKNOWLEDGMENTS

Professor Poul Hyttel is acknowledged for contributing the pig illustration for **Figure 1**.

FUNDING

This work was funded in part by ARS-CRIS Project 1940-32000-057-00D. Additional funding came from an interagency agreement with the Science and Technology Directorate of the U.S. Department of Homeland Security (award numbers HSHQPM-13-X-00131 and HSHQPM-13-X-00113) and Specific Cooperative Agreement # 58-1940-4-003 between USDA and University of Connecticut. CS is a recipient of a Plum Island Animal Disease Center Research Participation Program fellowship, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement with the U.S. Department of Energy.

REFERENCES

1. Alexandersen S, Zhang Z, Donaldson AI, Garland AJM. The pathogenesis and diagnosis of foot-and-mouth disease. *J Comp Pathol* (2003) **129**(1):1–36. doi:10.1016/S0021-9975(03)00041-0
2. Arzt J, Baxt B, Grubman MJ, Jackson T, Juleff N, Rhyan J, et al. The pathogenesis of foot-and-mouth disease II: viral pathways in swine, small ruminants, and wild-life; myotropism, chronic syndromes, and molecular virus-host interactions. *Transbound Emerg Dis* (2011) **58**(4):305–26. doi:10.1111/j.1865-1682.2011.01236.x
3. Stenfeldt C, Pacheco JM, Smoliga G, Bishop E, Pauszek S, Hartwig E, et al. Detection of foot-and-mouth disease virus RNA and capsid protein in lymphoid tissues of convalescent pigs does not indicate existence of a carrier state. *Transbound Emerg Dis* (2014) **63**(2):152–64. doi:10.1111/tbed.12235
4. Alexandersen S, Donaldson AI. Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. *Epidemiol Infect* (2002) **128**(2):313–23. doi:10.1017/S0950268801006501
5. Donaldson AI, Alexandersen S. Relative resistance of pigs to infection by natural aerosols of FMD virus. *Vet Rec* (2001) **148**(19):600–2. doi:10.1136/vr.148.19.600
6. Pacheco JM, Gladue DP, Holinka LG, Arzt J, Bishop E, Smoliga G, et al. A partial deletion in non-structural protein 3A can attenuate foot-and-mouth disease virus in cattle. *Virology* (2013) **446**(1–2):260–7. doi:10.1016/j.virol.2013.08.003
7. Yang PC, Chu RM, Chung WB, Sung HT. Epidemiological characteristics and financial costs of the 1997 foot-and-mouth disease epidemic in Taiwan. *Vet Rec* (1999) **145**(25):731–4.
8. Dunn CS, Donaldson AI. Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. *Vet Rec* (1997) **141**(7):174–5. doi:10.1136/vr.141.7.174
9. Uddowla S, Pacheco JM, Larson C, Bishop E, Rodriguez LL, Rai DK, et al. Characterization of a chimeric foot-and-mouth disease virus bearing a bovine rhinitis B virus leader proteinase. *Virology* (2013) **447**(1–2):172–80. doi:10.1016/j.virol.2013.08.035
10. Donaldson AI. Foot-and-mouth disease in Taiwan. *Vet Rec* (1997) **140**(15):407.
11. OIE. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015: 215 Foot-and-Mouth Disease*. Paris: World Organization for Animal Health (2015). p. 145–73.
12. Parida S, Fleming L, Oh Y, Mahapatra M, Hamblin P, Gloster J, et al. Reduction of foot-and-mouth disease (FMD) virus load in nasal excretions, saliva and exhaled air of vaccinated pigs following direct contact challenge. *Vaccine* (2007) **25**(45):7806–17. doi:10.1016/j.vaccine.2007.08.058
13. Barnett PV, Cox SJ, Aggarwal N, Gerber H, McCullough KC. Further studies on the early protective responses of pigs following immunisation with high potency foot and mouth disease vaccine. *Vaccine* (2002) **20**(25–26):3197–208. doi:10.1016/S0264-410X(02)00242-6
14. Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* (2007) **25**(34):6381–91. doi:10.1016/j.vaccine.2007.06.010
15. de Leeuw PW, Tiessink JW, van Bekkum JG. The challenge of vaccinated pigs with foot-and-mouth disease virus. *Zentralbl Veterinarmed B* (1979) **26**(2):98–109. doi:10.1111/j.1439-0450.1979.tb00798.x
16. Lyoo YS. Experience on outbreaks and controlling FMD in Korea raised a requirement of major modification to establish effective measures to FMD. *Global Foot-and-Mouth Disease Research Alliance Scientific Meeting*. Hanoi (2015).
17. Terpstra C. Pathogenesis of foot-and-mouth disease in experimentally infected pigs. *Bull Off Int Epizoot* (1972) **77**(5):859–74.
18. Fukai K, Yamada M, Morioka K, Ohashi S, Yoshida K, Kitano R, et al. Dose-dependent responses of pigs infected with foot-and-mouth disease virus O/JPN/2010 by the intranasal and intraoral routes. *Arch Virol* (2015) **160**(1):129–39. doi:10.1007/s00705-014-2239-4
19. Stenfeldt C, Pacheco JM, Rodriguez LL, Arzt J. Infection dynamics of foot-and-mouth disease virus in pigs using two novel simulated-natural inoculation methods. *Res Vet Sci* (2014) **96**(2):396–405. doi:10.1016/j.rvsc.2014.01.009
20. Stenfeldt C, Pacheco JM, Rodriguez LL, Arzt J. Early events in the pathogenesis of foot-and-mouth disease in pigs; identification of oropharyngeal tonsils as sites of primary and sustained viral replication. *PLoS One* (2014) **9**(9):e106859. doi:10.1371/journal.pone.0106859
21. Newman JF, Rowlands DJ, Brown F. A physico-chemical sub-grouping of the mammalian picornaviruses. *J Gen Virol* (1973) **18**(2):171–80. doi:10.1099/0022-1317-18-2-171
22. Bachrach HL, Breese SS Jr, Callis JJ, Hess WR, Patty RE. Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde. *Proc Soc Exp Biol Med* (1957) **95**(1):147–52. doi:10.3181/00379727-95-23148
23. Arzt J, Pacheco JM, Rodriguez LL. The early pathogenesis of foot-and-mouth disease in cattle after aerosol inoculation: identification of the nasopharynx as the primary site of infection. *Vet Pathol* (2010) **47**(6):1048–63. doi:10.1177/0300985810372509
24. Pacheco JM, Arzt J, Rodriguez LL. Early events in the pathogenesis of foot-and-mouth disease in cattle after controlled aerosol exposure. *Vet J* (2010) **183**(1):46–53. doi:10.1016/j.tvjl.2008.08.023
25. Stenfeldt C, Eschbaumer M, Pacheco JM, Rekant SI, Rodriguez LL, Arzt J. Pathogenesis of primary foot-and-mouth disease virus infection in the nasopharynx of vaccinated and non-vaccinated cattle. *PLoS One* (2015) **10**(11):e0143666. doi:10.1371/journal.pone.0143666
26. Burrows R, Mann JA, Garland AJ, Greig A, Goodridge D. The pathogenesis of natural and simulated natural foot-and-mouth disease infection in cattle. *J Comp Pathol* (1981) **91**(4):599–609. doi:10.1016/0021-9975(81)90089-X
27. Pacheco JM, Mason PW. Evaluation of infectivity and transmission of different Asian foot-and-mouth disease viruses in swine. *J Vet Sci* (2010) **11**(2):133–42. doi:10.4142/jvs.2010.11.2.133
28. van Roermund HJ, Eble PL, de Jong MC, Dekker A. No between-pen transmission of foot-and-mouth disease virus in vaccinated pigs. *Vaccine* (2010) **28**(28):4452–61. doi:10.1016/j.vaccine.2010.04.019
29. Eble P, de Koeijer A, Bouma A, Stegeman A, Dekker A. Quantification of within- and between-pen transmission of foot-and-mouth disease virus in pigs. *Vet Res* (2006) **37**(5):647–54. doi:10.1051/vetres:2006026
30. Pacheco JM, Tucker M, Hartwig E, Bishop E, Arzt J, Rodriguez LL. Direct contact transmission of three different foot-and-mouth disease virus strains in swine demonstrates important strain-specific differences. *Vet J* (2012) **193**(2):456–63. doi:10.1016/j.tvjl.2012.01.012
31. Pacheco JM, Lee KN, Eschbaumer M, Bishop EA, Hartwig EJ, Pauszek SJ, et al. Evaluation of infectivity, virulence and transmission of FDMV field strains of serotypes O and A isolated in 2010 from outbreaks in the Republic of Korea. *PLoS One* (2016) **11**(1):e0146445. doi:10.1371/journal.pone.0146445
32. Quan M, Murphy CM, Zhang Z, Durand S, Esteves I, Doel C, et al. Influence of exposure intensity on the efficiency and speed of transmission of

foot-and-mouth disease. *J Comp Pathol* (2009) **140**(4):225–37. doi:10.1016/j.jcpa.2008.12.002
33. Quan M, Murphy CM, Zhang Z, Alexandersen S. Determinants of early foot-and-mouth disease virus dynamics in pigs. *J Comp Pathol* (2004) **131**(4):294–307. doi:10.1016/j.jcpa.2004.05.002
34. Murphy C, Bashiruddin JB, Quan M, Zhang Z, Alexandersen S. Foot-and-mouth disease viral loads in pigs in the early, acute stage of disease. *Vet Rec* (2010) **166**(1):10–4. doi:10.1136/vr.b5583
35. Eble PL, Bouma A, de Bruin MG, van Hemert-Kluitenberg F, van Oirschot JT, Dekker A. Vaccination of pigs two weeks before infection significantly reduces transmission of foot-and-mouth disease virus. *Vaccine* (2004) **22**(11–12):1372–8. doi:10.1016/j.vaccine.2003.11.003
36. Alexandersen S, Oleksiewicz MB, Donaldson AI. The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT-PCR. *J Gen Virol* (2001) **82**(Pt 4):747–55. doi:10.1099/0022-1317-82-4-747
37. Dias CC, Moraes MP, Segundo FD, de los Santos T, Grubman MJ. Porcine type I interferon rapidly protects swine against challenge with multiple serotypes of foot-and-mouth disease virus. *J Interferon Cytokine Res* (2011) **31**(2):227–36. doi:10.1089/jir.2010.0055
38. Diaz-San Segundo F, Moraes MP, de Los Santos T, Dias CC, Grubman MJ. Interferon-induced protection against foot-and-mouth disease virus infection correlates with enhanced tissue-specific innate immune cell infiltration and interferon-stimulated gene expression. *J Virol* (2010) **84**(4):2063–77. doi:10.1128/JVI.01874-09
39. Pacheco JM, Butler JE, Jew J, Ferman GS, Zhu J, Golde WT. IgA antibody response of swine to foot-and-mouth disease virus infection and vaccination. *Clin Vaccine Immunol* (2010) **17**(4):550–8. doi:10.1128/CVI.00429-09
40. Brown CC, Olander HJ, Meyer RF. Pathogenesis of foot-and-mouth disease in swine, studied by in-situ hybridization. *J Comp Pathol* (1995) **113**(1):51–8. doi:10.1016/S0021-9975(05)80068-4
41. Horter DC, Yoon KJ, Zimmerman JJ. A review of porcine tonsils in immunity and disease. *Anim Health Res Rev* (2003) **4**(2):143–55. doi:10.1079/AHRR200358
42. Grau FR, Schroeder ME, Mulhern EL, McIntosh MT, Bounpheng MA. Detection of African swine fever, classical swine fever, and foot-and-mouth disease viruses in swine oral fluids by multiplex reverse transcription real-time polymerase chain reaction. *J Vet Diagn Invest* (2015) **27**(2):140–9. doi:10.1177/1040638715574768
43. Alexandersen S, Quan M, Murphy C, Knight J, Zhang Z. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J Comp Pathol* (2003) **129**(4):268–82. doi:10.1016/S0021-9975(03)00045-8
44. Donaldson AI, Alexandersen S, Sorensen JH, Mikkelsen T. Relative risks of the uncontrollable (airborne) spread of FMD by different species. *Vet Rec* (2001) **148**(19):602–4. doi:10.1136/vr.148.19.602
45. Donaldson AI, Herniman KA, Parker J, Sellers RF. Further investigations on the airborne excretion of foot-and-mouth disease virus. *J Hyg (Lond)* (1970) **68**(4):557–64. doi:10.1017/S0022172400042480
46. Sellers RF. Quantitative aspects of the spread of foot and mouth disease. *Vet Bull* (1971) **41**(6):431–9.
47. Donaldson AI, Ferris NP. Sites of release of airborne foot-and-mouth disease virus from infected pigs. *Res Vet Sci* (1980) **29**(3):315–9.
48. Eble PL, de Bruin MG, Bouma A, van Hemert-Kluitenberg F, Dekker A. Comparison of immune responses after intra-typic heterologous and homologous vaccination against foot-and-mouth disease virus infection in pigs. *Vaccine* (2006) **24**(9):1274–81. doi:10.1016/j.vaccine.2005.09.040
49. Rodriguez-Calvo T, Diaz-San Segundo F, Sanz-Ramos M, Sevilla N. A replication analysis of foot-and-mouth disease virus in swine lymphoid tissue might indicate a putative carrier stage in pigs. *Vet Res* (2011) **42**(1):22. doi:10.1186/1297-9716-42-22
50. Mezencio JM, Babcock GD, Kramer E, Brown F. Evidence for the persistence of foot-and-mouth disease virus in pigs. *Vet J* (1999) **157**(3):213–7. doi:10.1053/tvjl.1999.0357
51. Mohamed F, Swafford S, Petrowski H, Bracht A, Schmit B, Fabian A, et al. Foot-and-mouth disease in feral swine: susceptibility and transmission. *Transbound Emerg Dis* (2011) **58**(4):358–71. doi:10.1111/j.1865-1682.2011.01213.x
52. Orsel K, Roest HI, Elzinga-Bril EM, van Hemert-Kluitenberg F, Dekker A. Detection of foot-and-mouth disease virus in infected pigs by RT-PCR four weeks after challenge. *Vet Rec* (2008) **162**(23):753–4. doi:10.1136/vr.162.23.753
53. Zhang Z, Bashiruddin JB. Quantitative analysis of foot-and-mouth disease virus RNA duration in tissues of experimentally infected pigs. *Vet J* (2009) **180**(1):130–2. doi:10.1016/j.tvjl.2007.11.010
54. Lohse L, Jackson T, Botner A, Belsham GJ. Capsid coding sequences of foot-and-mouth disease viruses are determinants of pathogenicity in pigs. *Vet Res* (2012) **43**:46. doi:10.1186/1297-9716-43-46
55. Wilna V, Hong NT, Geoffrey FT, Jacqueline MM, Jianning W, Van Phuc K, et al. Efficacy of a high potency O1 Manisa monovalent vaccine against heterologous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination. *Vaccine* (2015) **33**(24):2778–85. doi:10.1016/j.vaccine.2015.04.045
56. Gulbahar MY, Davis WC, Guvenc T, Yarim M, Parlak U, Kabak YB. Myocarditis associated with foot-and-mouth disease virus type O in lambs. *Vet Pathol* (2007) **44**(5):589–99. doi:10.1354/vp.44-5-589
57. Karapinar T, Dabak DO, Kuloglu T, Bulut H. High cardiac troponin I plasma concentration in a calf with myocarditis. *Can Vet J* (2010) **51**(4):397–9.
58. Stenfeldt C, Pacheco JM, Borca MV, Rodriguez LL, Arzt J. Morphologic and phenotypic characteristics of myocarditis in two pigs infected by foot-and mouth disease virus strains of serotypes O or A. *Acta Vet Scand* (2014) **56**:42. doi:10.1186/s13028-014-0042-6
59. Gelmetti D, Meroni A, Brocchi E, Koenen F, Cammarata G. Pathogenesis of encephalomyocarditis experimental infection in young piglets: a potential animal model to study viral myocarditis. *Vet Res* (2006) **37**(1):15–23. doi:10.1051/vetres:2005041
60. Gwathmey JK, Nakao S, Come PC, Goad ME, Serur JR, Als AV, et al. An experimental model of acute and subacute viral myocarditis in the pig. *J Am Coll Cardiol* (1992) **19**(4):864–9. doi:10.1016/0735-1097(92)90533-S
61. Carocci M, Bakkali-Kassimi L. The encephalomyocarditis virus. *Virulence* (2012) **3**(4):351–67. doi:10.4161/viru.20573
62. Toka FN, Golde WT. Cell mediated innate responses of cattle and swine are diverse during foot-and-mouth disease virus (FMDV) infection: a unique landscape of innate immunity. *Immunol Lett* (2013) **152**(2):135–43. doi:10.1016/j.imlet.2013.05.007
63. Rigden RC, Carrasco CP, Summerfield A, KC MC. Macrophage phagocytosis of foot-and-mouth disease virus may create infectious carriers. *Immunology* (2002) **106**(4):537–48. doi:10.1046/j.1365-2567.2002.01460.x
64. McCullough KC, Parkinson D, Crowther JR. Opsonization-enhanced phagocytosis of foot-and-mouth disease virus. *Immunology* (1988) **65**(2):187–91.
65. Mason PW, Grubman MJ, Baxt B. Molecular basis of pathogenesis of FMDV. *Virus Res* (2003) **91**(1):9–32. doi:10.1016/S0168-1702(02)00257-5
66. Guzylack-Piriou L, Bergamin F, Gerber M, McCullough KC, Summerfield A. Plasmacytoid dendritic cell activation by foot-and-mouth disease virus requires immune complexes. *Eur J Immunol* (2006) **36**(7):1674–83. doi:10.1002/eji.200635866
67. Diaz-San Segundo F, Rodriguez-Calvo T, de Avila A, Sevilla N. Immunosuppression during acute infection with foot-and-mouth disease virus in swine is mediated by IL-10. *PLoS One* (2009) **4**(5):e5659. doi:10.1371/journal.pone.0005659
68. Nfon CK, Ferman GS, Toka FN, Gregg DA, Golde WT. Interferon-alpha production by swine dendritic cells is inhibited during acute infection with foot-and-mouth disease virus. *Viral Immunol* (2008) **21**(1):68–77. doi:10.1089/vim.2007.0097
69. Nfon CK, Toka FN, Kenney M, Pacheco JM, Golde WT. Loss of plasmacytoid dendritic cell function coincides with lymphopenia and viremia during foot-and-mouth disease virus infection. *Viral Immunol* (2010) **23**(1):29–41. doi:10.1089/vim.2009.0078
70. Valladeau J, Ravel O, Dezutter-Dambuyant C, Moore K, Kleijmeer M, Liu Y, et al. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* (2000) **12**(1):71–81. doi:10.1016/S1074-7613(00)80160-0
71. Bautista EM, Ferman GS, Gregg D, Brum MC, Grubman MJ, Golde WT. Constitutive expression of alpha interferon by skin dendritic cells confers resistance to infection by foot-and-mouth disease virus. *J Virol* (2005) **79**(8):4838–47. doi:10.1128/JVI.79.8.4838-4847.2005
72. Toka FN, Nfon C, Dawson H, Golde WT. Natural killer cell dysfunction during acute infection with foot-and-mouth disease virus. *Clin Vaccine Immunol* (2009) **16**(12):1738–49. doi:10.1128/CVI.00280-09

73. Diaz-San Segundo F, Salguero FJ, de Avila A, de Marco MM, Sanchez-Martin MA, Sevilla N. Selective lymphocyte depletion during the early stage of the immune response to foot-and-mouth disease virus infection in swine. *J Virol* (2006) **80**(5):2369–79. doi:10.1128/JVI.80.5.2369-2379.2006
74. Bautista EM, Ferman GS, Golde WT. Induction of lymphopenia and inhibition of T cell function during acute infection of swine with foot and mouth disease virus (FMDV). *Vet Immunol Immunopathol* (2003) **92**(1–2):61–73. doi:10.1016/S0165-2427(03)00004-7
75. Joshi G, Sharma R, Kakker NK. Phenotypic and functional characterization of T-cells and in vitro replication of FMDV serotypes in bovine lymphocytes. *Vaccine* (2009) **27**(48):6656–61. doi:10.1016/j.vaccine.2009.08.107
76. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med* (2006) **12**(11):1301–9. doi:10.1038/nm1492
77. Der SD, Zhou A, Williams BR, Silverman RH. Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. *Proc Natl Acad Sci U S A* (1998) **95**(26):15623–8. doi:10.1073/pnas.95.26.15623
78. Iversen MB, Ank N, Melchjorsen J, Paludan SR. Expression of type III interferon (IFN) in the vaginal mucosa is mediated primarily by dendritic cells and displays stronger dependence on NF-kappaB than type I IFNs. *J Virol* (2010) **84**(9):4579–86. doi:10.1128/JVI.02591-09
79. Takaoka A, Yanai H. Interferon signalling network in innate defence. *Cell Microbiol* (2006) **8**(6):907–22. doi:10.1111/j.1462-5822.2006.00716.x
80. Wu J, Chen ZJ. Innate immune sensing and signaling of cytosolic nucleic acids. *Annu Rev Immunol* (2014) **32**:461–88. doi:10.1146/annurev-immunol-032713-120156
81. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat Immunol* (2010) **11**(5):373–84. doi:10.1038/ni.1863
82. Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* (2014) **32**:513–45. doi:10.1146/annurev-immunol-032713-120231
83. Chinsangaram J, Piccone ME, Grubman MJ. Ability of foot-and-mouth disease virus to form plaques in cell culture is associated with suppression of alpha/beta interferon. *J Virol* (1999) **73**(12):9891–8.
84. de Los Santos T, de Avila Botton S, Weiblen R, Grubman MJ. The leader proteinase of foot-and-mouth disease virus inhibits the induction of beta interferon mRNA and blocks the host innate immune response. *J Virol* (2006) **80**(4):1906–14. doi:10.1128/JVI.80.4.1906-1914.2006
85. Chinsangaram J, Koster M, Grubman MJ. Inhibition of L-deleted foot-and-mouth disease virus replication by alpha/beta interferon involves double-stranded RNA-dependent protein kinase. *J Virol* (2001) **75**(12):5498–503. doi:10.1128/JVI.75.12.5498-5503.2001
86. Diaz-San Segundo F, Weiss M, Perez-Martin E, Koster MJ, Zhu J, Grubman MJ, et al. Antiviral activity of bovine type III interferon against foot-and-mouth disease virus. *Virology* (2011) **413**(2):283–92. doi:10.1016/j.virol.2011.02.023
87. Kim SM, Park JH, Lee KN, Kim SK, You SH, Kim T, et al. Robust protection against highly virulent foot-and-mouth disease virus in swine by combination treatment with recombinant adenoviruses expressing porcine alpha and gamma interferons and multiple small interfering RNAs. *J Virol* (2015) **89**(16):8267–79. doi:10.1128/JVI.00766-15
88. Moraes MP, de Los Santos T, Koster M, Turecek T, Wang H, Andreyev VG, et al. Enhanced antiviral activity against foot-and-mouth disease virus by a combination of type I and II porcine interferons. *J Virol* (2007) **81**(13):7124–35. doi:10.1128/JVI.02775-06
89. Chinsangaram J, Moraes MP, Koster M, Grubman MJ. Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease. *J Virol* (2003) **77**(2):1621–5. doi:10.1128/JVI.77.2.1621-1625.2003
90. Moraes MP, Chinsangaram J, Brum MC, Grubman MJ. Immediate protection of swine from foot-and-mouth disease: a combination of adenoviruses expressing interferon alpha and a foot-and-mouth disease virus subunit vaccine. *Vaccine* (2003) **22**(2):268–79. doi:10.1016/S0264-410X(03)00560-7
91. Perez-Martin E, Diaz-San Segundo F, Weiss M, Sturza DF, Dias CC, Ramirez-Medina E, et al. Type III interferon protects swine against foot-and-mouth disease. *J Interferon Cytokine Res* (2014) **34**(10):810–21. doi:10.1089/jir.2013.0112
92. Kim SM, Kim SK, Park JH, Lee KN, Ko YJ, Lee HS, et al. A recombinant adenovirus bicistronically expressing porcine interferon-alpha and interferon-gamma enhances antiviral effects against foot-and-mouth disease virus. *Antiviral Res* (2014) **104**:52–8. doi:10.1016/j.antiviral.2014.01.014
93. Diaz-San Segundo F, Dias CC, Moraes MP, Weiss M, Perez-Martin E, Owens G, et al. Venezuelan equine encephalitis replicon particles can induce rapid protection against foot-and-mouth disease virus. *J Virol* (2013) **87**(10):5447–60. doi:10.1128/JVI.03462-12
94. Honda K, Taniguchi T. IRFs: master regulators of signalling by toll-like receptors and cytosolic pattern-recognition receptors. *Nat Rev Immunol* (2006) **6**(9):644–58. doi:10.1038/nri1900
95. Marie I, Durbin JE, Levy DE. Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor-7. *EMBO J* (1998) **17**(22):6660–9. doi:10.1093/emboj/17.22.6660
96. Dias CC, Moraes MP, Weiss M, Diaz-San Segundo F, Perez-Martin E, Salazar AM, et al. Novel antiviral therapeutics to control foot-and-mouth disease. *J Interferon Cytokine Res* (2012) **32**(10):462–73. doi:10.1089/jir.2012.0012
97. Ramirez-Carvajal L, Diaz-San Segundo F, Hickman D, Long CR, Zhu J, Rodriguez LL, et al. Expression of porcine fusion protein IRF7/3(5D) efficiently controls foot-and-mouth disease virus replication. *J Virol* (2014) **88**(19):11140–53. doi:10.1128/JVI.00372-14

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 30 June 2016
doi: 10.3389/fvets.2016.00052

Proper Timing of Foot-and-Mouth Disease Vaccination of Piglets with Maternally Derived Antibodies Will Maximize Expected Protection Levels

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We investigated to what extent maternally derived antibodies interfere with foot-and-mouth disease (FMD) vaccination in order to determine the factors that influence the correct vaccination for piglets. Groups of piglets with maternally derived antibodies were vaccinated at different time points following birth, and the antibody titers to FMD virus (FMDV) were measured using virus neutralization tests (VNT). We used 50 piglets from 5 sows that had been vaccinated 3 times intramuscularly in the neck during pregnancy with FMD vaccine containing strains of FMDV serotypes O, A, and Asia-1. Four groups of 10 piglets were vaccinated intramuscularly in the neck at 3, 5, 7, or 9 weeks of age using a monovalent Cedivac-FMD vaccine (serotype A TUR/14/98). One group of 10 piglets with maternally derived antibodies was not vaccinated, and another group of 10 piglets without maternally derived antibodies was vaccinated at 3 weeks of age and served as a control group. Sera samples were collected, and antibody titers were determined using VNT. In our study, the antibody responses of piglets with maternally derived antibodies vaccinated at 7 or 9 weeks of age were similar to the responses of piglets without maternally derived antibodies vaccinated at 3 weeks of age. The maternally derived antibody levels in piglets depended very strongly on the antibody titer in the sow, so the optimal time for vaccination of piglets will depend on the vaccination scheme and quality of vaccine used in the sows and should, therefore, be monitored and reviewed on regular basis in countries that use FMD prophylactic vaccination.

Keywords: FMD, vaccine, maternal antibodies, porcine, timing of vaccination

OPEN ACCESS

Edited by:
Andres M. Perez, University of Minnesota, USA

Reviewed by:
Cristobal Cristian Verdugo, Austral University of Chile, Chile
Nick Lyons, The Pirbright Institute, UK

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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *01 March 2016*
Accepted: *15 June 2016*
Published: *30 June 2016*

Citation:
Dekker A, Chénard G, Stockhofe N and Eblé PL (2016) Proper Timing of Foot-and-Mouth Disease Vaccination of Piglets with Maternally Derived Antibodies Will Maximize Expected Protection Levels. Front. Vet. Sci. 3:52. doi: 10.3389/fvets.2016.00052

INTRODUCTION

Foot-and-mouth disease (FMD) is a contagious disease of ruminants and pigs caused by FMD virus (FMDV). The disease is considered a major threat to commercially kept ruminants and pigs. As transmission of FMD occurs even when animal movement is prohibited, the major transmission routes most likely include people moving between farms. "Stamping out" in a small radius around infected farms has recently been applied in several outbreaks, but this involves many people moving between potentially infected farms. Therefore, a control measure that requires fewer people, such as vaccination, is preferred. Furthermore, from an ethical point of

view, vaccination is preferred to stamping out farms at risk (1). However, maternally derived antibodies can interfere with the development of vaccine-induced immunity (2, 3). There has been discussion whether FMDV oil vaccines in pigs can induce immunity irrespective of maternally derived antibodies but Francis and Black (4) showed that maternally derived antibodies hinder the development of protective immunity. In cattle, it has been shown that a heterologous strain within the same serotype can induce an immune response in calves with maternally derived antibodies (5), so the immune response is not necessarily blocked by maternally derived antibodies. In addition, in pigs with maternally derived antibodies, a response to influenza vaccination can also be measured in the presence of maternally derived antibodies. However, the response is lower and will probably not protect (6). One of the options to boost immunity levels is repeated vaccination, i.e., first vaccination in the presence of maternally derived antibodies, to prime the immune system, and a second vaccination 1 or 2 months later. However, the costs of two vaccine administrations are high, not only due to the cost of vaccine but also the logistics and labor costs, which are often higher. Therefore, it may be preferable to optimize the timing of a single vaccination.

The objective of this study was to determine the factors that influence the optimal age for FMDV vaccination of piglets. We measured the neutralizing antibody response in piglets born to vaccinated sows at 3, 5, 7, and 9 weeks of age. The neutralizing antibody titer was compared with non-vaccinated piglets from the same sows, as well as with vaccinated piglets born from non-immune sows.

MATERIALS AND METHODS

Vaccine

The antigens used in the vaccines in this study were produced on an industrial scale using baby hamster kidney (BHK) cells. The antigens were inactivated with binary ethyleneimine (BEI) and concentrated approximately 100 times by two cycles of polyethylene glycol (PEG) precipitation. The antigen concentration was determined by sucrose gradient analysis (7). The oil vaccines were formulated using a mineral oil as adjuvant in a double oil emulsion, as previously described (8). The vaccines were formulated to contain at least six PD_{50} per dose (i.e., six times the dose that protects 50% of the cattle against virulent challenge in the tongue). One trivalent vaccine batch was used for the sows and one monovalent vaccine batch was used for the piglets. A single dose was 2 ml.

Vaccination of Sows

The sows (SPF pigs TN20 and TN70 from the genetics company Topigs Norsvin) used in this study were available from a vaccine safety test. The sows had not been vaccinated against FMD before beginning the study and were free of antibodies against FMDV. The sows were vaccinated intramuscularly with trivalent FMDV vaccine containing O Manisa, Asia-1 Shamir, and A TUR/14/98 antigen. The sows were vaccinated at day 36, 57, and 85 of gestation. Piglets were born after 112–114 days of gestation.

Vaccination Piglets

A total of five vaccinated sows were selected that had nine or more piglets. From each sow, two piglets with maternally derived antibodies were selected randomly and assigned to one of the five groups of piglets (except in Group 5 where one sow supplied three piglets and one sow only one piglet). Two non-vaccinated sows supplied each five piglets for Group 6 (vaccinated piglets without maternally derived antibodies).

Piglets with maternally derived antibodies in Groups 1, 2, 3, and 4 were vaccinated intramuscularly with a single dose of monovalent FMD vaccine containing A TUR/14/98 at 3, 5, 7, and 9 weeks of age, respectively. The piglets that were used as vaccination control (Group 6) were vaccinated at 3 weeks of age. Serum samples were collected weekly up to 6 weeks after vaccination.

Virus Neutralization Test

Sera were tested for virus neutralizing antibodies against FMDV A TUR/14/98, O Manisa, and Asia-1 Shamir, using primary porcine kidney cells (9). Twofold dilutions of the serum samples were tested starting with undiluted serum. Titers were expressed as $\log_{10}$ of the reciprocal of the dilution that inhibited virus growth in 50% of the wells. For calculation of the mean titers and for the use in statistical tests, we used 0 for the observations with a $\log_{10}$ titer of <0.30.

Statistical Analysis

Because the same animals were sampled at different time points, we analyzed the antibody response in the sows by forward selection in a linear mixed-effects model (10, 11) using the virus neutralization tests (VNT) titer as continuous response variable, the time after vaccination and strains as possible explanatory nominal variables. The animal ID was included as random variable (model M1). The relation between neutralizing antibody titer in the sows 3 weeks after the last vaccination (approximately 30 weeks of gestation), and the antibody titer in the piglets just after colostrum uptake was analyzed by linear regression. Using forward selection, the effect of serotype was analyzed (M2).

The median time for which neutralizing antibody titers in the piglets were detected (titer ≥ 0.3) was analyzed in a logistic mixed-effects model (10, 11). Whether or not a neutralizing antibody titer was observed was the binary result variable. Possible nominal explanatory variables used were group, serotype, and mother, whereas the possible continuous explanatory variables were age and neutralizing antibody titer at birth. The piglet was entered as random variable (M3).

The half-life of the neutralizing antibody titers was analyzed in a linear mixed-effects model (10, 11). The neutralizing antibody titer measured was the result variable. Possible nominal explanatory variables used were group, serotype, and mother, whereas the possible continuous explanatory variables were age, neutralizing antibody titer of the dam, and neutralizing antibody titer at birth. The piglet was entered as random variable (M4). In the abovementioned analyses, the best fitting model was selected in a forward selection procedure using the Aikaike information criterion (12, 13). Analysis was performed using R version 3.2.3 and lme4 library version 1.1.10 using default settings.

RESULTS

The neutralization titers observed in the sows are given in **Figure 1**. The linear mixed-effects model (M1, **Table 1**) showed that the neutralizing antibody response was significantly higher 2–10 weeks after vaccination compared with the titer at the time of vaccination, but differed significantly between strains. The titer induced by serotype Asia-1 was approximately 0.5 $\log_{10}$ lower than the titers against A TUR/14/98 and O Manisa (**Table 1**).

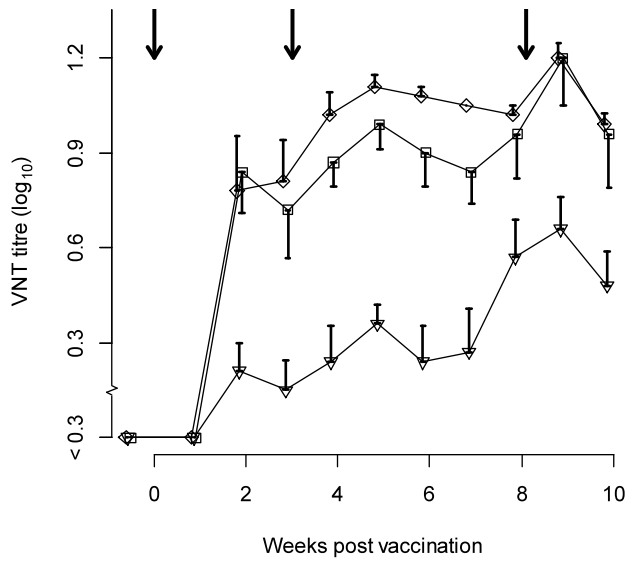

FIGURE 1 | Titers observed in sows ($n = 5$) vaccinated with trivalent FMD vaccine (at least six PD_{50} per dose) three times during gestation. Antigens included in the vaccine were type A TUR/14/98 (diamonds), O Manisa (squares), and Asia-1 Shamir (triangles). The arrows indicate the time of vaccination. The error bars (only one direction to avoid overlap) indicate the SEM.

TABLE 1 | Final selected linear mixed-effects model (M1) using the VNT titer in the sows ($n = 5$) as continuous response variable, the time after vaccination, and strains as possible explanatory nominal variables.

Random effects	Variance	SD	
Sow	0.007	0.08	
Residual	0.05	0.23	
Fixed effects	**Estimate**	**SE**	**t-Value**
Intercept	−0.3	0.07	−4.5
0.9 weeks post vaccination	0.0	0.08	0.0
1.9 weeks postvaccination	0.6	0.08	7.4
2.9 weeks postvaccination	0.6	0.08	6.8
3.9 weeks postvaccination	0.7	0.08	8.6
4.9 weeks postvaccination	0.8	0.08	9.9
5.9 weeks postvaccination	0.7	0.08	9.0
6.9 weeks postvaccination	0.7	0.08	8.7
7.9 weeks postvaccination	0.9	0.08	10.3
8.9 weeks postvaccination	1.0	0.08	12.4
9.9 weeks postvaccination	0.8	0.08	9.8
Strain A TUR/14/98	0.5	0.04	12.4
Strain O Manisa	0.5	0.04	10.8

The animal was included as random variable.

Table 2 shows the neutralizing antibody titers against A TUR/14/98 in the piglets in the various groups. In the piglets with maternally derived antibodies (Groups 1–5), the mean neutralizing antibody titer ($\log_{10}$) against A TUR/14/98 was 1.7 (SEM 0.05) at birth. The mean neutralizing antibody titer ($\log_{10}$) against O Manisa was slightly higher (mean, 2.0 SEM 0.06) and lower for serotype Asia-1 Shamir (mean, 1.3 SEM 0.06). The neutralizing antibody titer of the piglets was strongly correlated with the neutralizing antibody titer of the sows. On average, a 1 $\log_{10}$ higher antibody titer in the sows resulted in a 1 $\log_{10}$ higher antibody titer in the piglets (univariate linear regression, data not shown). However, the relation was different for each serotype, and an interaction effect was found between serotype and the neutralization titer of the dam (M2, **Table 3**). The interaction effect was caused by the fact that the titers in the dam for type A TUR/14/98 were 0.9 or 1.05, and no relation between antibody titer in the dam and the piglet for this serotype could be determined (**Table 3**).

The duration for which maternally derived antibodies could be detected differed for the different serotypes (**Figure 2**). The logistic mixed-effects model (M3, **Table 4**) showed that the presence of VNT titres was statistically dependent on the titer at birth, age, the serotype with an interaction effect between age, and serotype. The median time that antibodies are present increases with approximately 29–53 days when the titer at birth is 1 $\log_{10}$ higher, depending on the serotype. For piglets with a neutralizing antibody titer of 2 $\log_{10}$ at birth (the mean titer for O Manisa in the dataset), the model produced a median time that neutralizing antibodies could be detected of 9, 14, and 9 weeks, respectively, for serotype A TUR/14/98, O Manisa, and Asia-1 Shamir. Interestingly, when the analysis was performed with only the non-vaccinated piglets (Group 5), a significant interaction effect between age and serotype was found, indicating that the median time for which antibodies are present are different for different serotypes even if the piglets were born from the same mother and started with the same neutralizing antibody titer at birth (results not shown).

The total number of sera tested in Groups 1–5 was 421; the number of left censored data (sera with a titer <0.3) was different for each serotype 57 for O Manisa, 71 for A TUR/14/98, and 254 for Asia-1. However, none of the piglets had a titer <0.3 at the beginning of the study. In the linear mixed-effects model, to study of the decline of neutralizing antibody titers in the piglets (M4, **Table 5**), only sera with a titer of 0.3 or higher were included. The analysis showed that the antibody titer depended on the age of the piglets, the titer of birth, the titer of the dam, and the serotype. There were interaction effects found between serotype and age, titer at birth and age, and titer of the sow and serotype. The interaction effect between both serotype and titer at birth with age shows that half-life of neutralizing antibodies detected in our study depends on the serotype and the titer at birth. The estimated half-life of neutralizing antibodies for a piglet that started with a neutralizing antibody titer of 2 $\log_{10}$ (based on the observed mean titer in week 1 for O Manisa) was 11, 16, and 12 days for, respectively, serotype A TUR/14/98, O Manisa, and Asia-1.

TABLE 2 | Age and neutralizing antibody titer against the strain used for vaccination (A TUR/14/98).

Group	Number	Age of vaccination (days)			VNT titer (A TUR/14/98) at birth			VNT titer (A TUR/14/98) at vaccination		
		Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum
1	10	21	22	23	0.90	1.7	2.10	0.60	1.2	1.65
2	10	35	36	37	1.35	1.8	2.25	0.45	0.9	1.50
3	10	49	50	51	0.90	1.7	2.25	<0.30	0.5	1.05
4	10	63	64	65	1.35	1.8	2.40	<0.30	0.1	0.60
5	10	NA	NA	NA	1.35	1.8	2.25	NA	NA	NA
6	10	20	21	21	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3

Mean titers are calculated considering <0.30 as 0.

TABLE 3 | Final selected linear model (M2) using the VNT titer at birth of the piglets ($n = 50$) as continuous response variable, the VNT titer of the dam and strains as possible explanatory variables.

	Estimate	SE	*t*-Value	*p*-Value
Intercept	0.6	0.1	5.5	$\ll$0.001
VNT titer of dam	1.6	0.2	8.3	$\ll$0.001
Strain A TUR/14/98	1.3	0.6	2.2	0.03
Strain O Manisa	0.6	0.2	3.5	0.0006
Interaction VNT titer dam and strain A TUR/14/98	−1.7	0.6	−2.9	0.005
Interaction VNT titer dam and strain O Manisa	−0.7	0.2	3.2	0.001

Strain Asia-1 was the baseline variable.

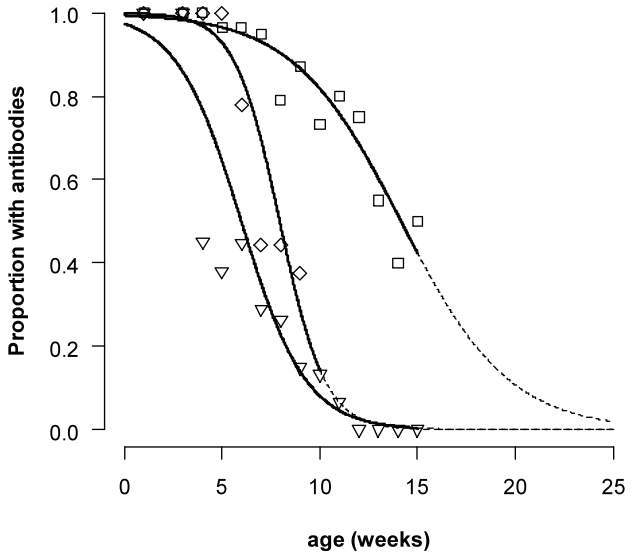

FIGURE 2 | Proportion of piglets with FMDV neutralizing antibody titers ≥0.3. For non-vaccinated piglets A TUR/14/98 (only Group 5, diamonds, central line). For all piglets with maternally derived antibodies (Group 1–5) for serotype O Manisa (squares, line on the right) and serotype Asia-1 Shamir (triangles, line on the left). The data were analyzed by a logistic mixed-effects model. The dashed line indicates the extrapolated part of the curve.

TABLE 4 | Final selected logistic mixed-effects model (M3) using the presence of a VNT titer (<0.3 is negative, 0.3 or higher is positive) in the piglets ($n = 50$) as binary response variable, the age (days), the VNT titer at birth, and strains as possible explanatory variables.

Random effects	Variance	SD		
Piglet	2.7	1.7		
Fixed effects	**Estimate**	**SE**	**z-Value**	**p-Value**
Intercept	−0.2	0.9	−4.5	0.8
VNT titer at birth	6.5	0.8	8.1	$\ll$0.001
Age (days)	−0.2	0.02	−8.8	$\ll$0.001
Strain A TUR/14/98	2.7	2.6	1.0	0.3
Strain O Manisa	−1.2	1.2	−0.9	0.3
Interaction age and strain A TUR/14/98	−0.4	0.05	−0.8	0.4
Interaction age and strain O Manisa	0.1	0.02	3.6	0.0003

The piglet was included as random variable. Strain Asia-1 was the baseline variable.

TABLE 5 | Final selected linear mixed-effects model (M4) using the VNT titer in the piglets ($n = 50$) as continuous response variable, the age, VNT titer at birth, VNT titer of the sows, and strains as possible explanatory variables.

Random effects	Variance	SD	
Piglet	0.01	0.10	
Residual	0.04	0.20	
Fixed effects	**Estimate**	**SE**	**t-Value**
Intercept	0.2	0.07	2.8
Age (days)	−0.02	0.002	−8.3
VNT titer at birth	0.8	0.06	12
VNT titer dam	0.4	0.1	3.9
Strain A TUR/14/98	−0.8	0.4	−2.0
Strain O Manisa	−0.05	7	−0.8
Interaction age and strain A TUR/14/98	−0.002	0.002	−0.8
Interaction age and strain O Manisa	0.006	0.001	6.4
Interaction age and VNT titer at birth	−0.005	0.001	−5.3
Interaction VNT titer dam and strain A TUR/14/98	0.8	0.4	1.9
Interaction VNT titer dam and strain O Manisa	−0.1	0.09	−1.6

The piglet was included as random variable. Strain Asia-1 was the baseline variable.

Figure 3 shows the response of the piglets to homologous vaccination with a monovalent FMDV serotype A TUR/14/98 vaccine. The curves show that all groups of piglets respond to vaccination; after vaccination, the curve does not follow the decrease of maternally derived antibodies observed in non-vaccinated piglets. However, piglets vaccinated 4 or 5 weeks after birth did not have a response to the vaccine up to the level seen in a previous study (horizontal dotted line in **Figure 3**) in which pigs were

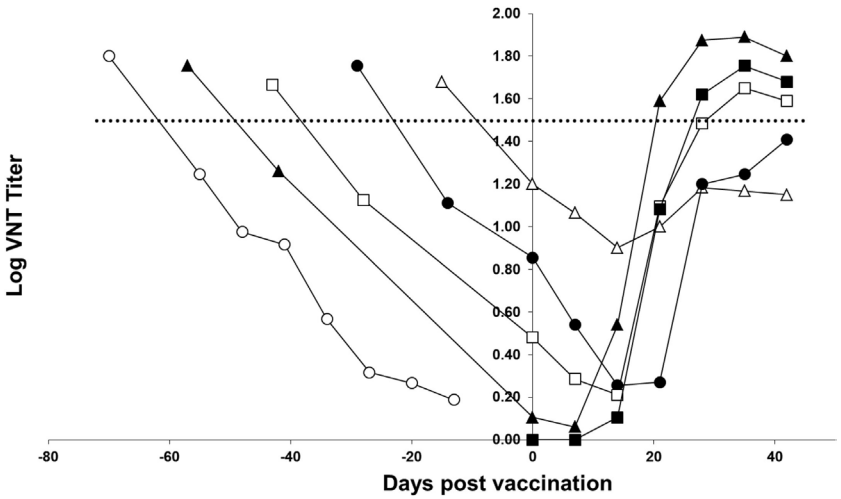

FIGURE 3 | FMD type A TUR/14/98 serology profile following homologous vaccination of piglets with maternally derived antibodies. Piglets were either vaccinated at 3 (open triangle), 5 (solid circle), 7 (open box), or 9 (solid triangle) weeks of age. One group of piglets (open circle) did not receive a vaccination and one group did not have maternally derived antibodies (solid box). The horizontal line indicates the mean virus neutralization titer observed 28 days postvaccination in a previous study in pigs vaccinated with a vaccine containing six PD_{50} per dose.

vaccinated with an FMD vaccine containing six PD_{50} per dose. The response in the piglets vaccinated 7 and 9 weeks after birth was similar to the response observed in pigs vaccinated with an FMD vaccine containing six PD_{50} per dose. Based on this six PD_{50} per dose threshold value, the response in the piglets vaccinated 7 and 9 weeks after birth was deemed sufficient, and the piglets vaccinated 9 weeks after birth had an earlier and initially a higher response than the piglets born from non-vaccinated sows.

DISCUSSION

The objective of this study was to determine the optimal age for FMD vaccination of piglets based on measurement of serological titers following vaccination and the corresponding level of protection that can be expected. In contrast to an earlier publication (4), we observed a response in piglets with maternally derived antibodies vaccinated 3 weeks after birth; following vaccination, the curve did not follow the decrease of maternally derived antibodies observed in non-vaccinated piglets. In our experiment, when considering the six PD_{50} per dose threshold value (shown in **Figure 3**) the response to vaccination was sufficient when piglets were vaccinated 7–9 weeks after birth. Piglets can respond to vaccination in the presence of maternally derived antibodies, as has been shown before for FMDV vaccines (14), as well as for influenza vaccines (6). Our experiment confirms the earlier finding that the higher the maternally derived antibody titer, the lower the response to vaccination. To induce a neutralizing antibody titer likely to confer protection, piglets should ideally be vaccinated when maternally derived antibodies are at very low level. The median time that maternally derived antibodies are present in piglets depends on the titer at birth and the serotype. The titer at birth depends on the titer of the sows and the serotype of the vaccine. We observed a large variation in antibody titers between seroptypes in the sows, which, in turn, resulted in differences between serotypes in maternal antibody titers and, consequently, the optimal time for vaccination of the piglets. The antibody response for Asia-1 Shamir in pigs was known to be lower in comparison to A TUR/14/98 and O Manisa (15). It is difficult to extrapolate our findings to other FMD vaccines since different vaccine formulations might induce higher or lower responses in sows. The vaccination protocol of the sows can also influence the outcome. In our case, the sows were vaccinated three times during pregnancy, because the study was a repeat-dose safety study for a vaccine marketing authorization application. In a field situation, it is probably easier to vaccinate sows one or two times per year, which will probably result in higher variation in titers in the piglets compared with a scheme suggested earlier (16), where sows are vaccinated before pregnancy and boosted once during pregnancy to obtain the highest titers in the piglets. Therefore, it is important that authorities responsible for vaccination monitor the response and study the optimal time for vaccination on a regular basis, as different FMD vaccines used in sows can influence the immune status of the mother.

A remarkable finding in both the logistic mixed-effects model and the linear mixed-effects model was the interaction effect between age and serotype. In the linear mixed-effects model, this interaction could be explained in the difference in censoring for the different serotypes; for serotype Asia-1, there were more observations with a VNT titer <0.3. In the logistic mixed-effects model, there was no censoring. This indicates that the decrease of maternally derived antibodies was different between the different serotypes. Such an interaction effect was not observed when we studied the duration of maternally derived antibodies in calves (5). The difference can be explained by the fact that decrease of maternally derived antibodies is not

only due to antibody metabolism but also due to the growth of the piglet (3), in combination with the fact that the titers at birth were not the same. The biggest change in weight (relatively) is in the beginning, when piglets grow from 1–2 kg to approximately 10 kg within a month. This is almost a 10-fold increase in weight. The next 10-fold increase takes more than 5 months. In the profile of the O Manisa, maternally derived antibody titers, approximately a 1 $\log_{10}$ decrease is seen in the first 4 weeks, then the decrease becomes less steep. For serotype Asia-1 and the non-vaccinated piglets for A TUR/14/98, a 1 $\log_{10}$ decrease is also observed in the same period. But for serotype Asia-1, most piglets have titers below the detection level of the assay after 4 weeks (**Figure 2**), so it is not possible to assess the reduction in slope. It is unlikely that the metabolization of maternally derived antibodies is different between the different serotypes.

The antibody response in this study was only followed for 6 weeks after vaccination, but fattening pigs should be protected for at least 6 months. Therefore, it is advisable that further studies in countries using vaccination are carried out, also to obtain data on antibody response in a field situation.

The most important result from our study was the observation that large differences arise in the duration of the maternally derived antibodies, which mainly depend on the titer at birth, which, in turn, depends on the titer in the sows. Therefore, every country that uses vaccination to control FMD in swine populations should determine the optimal vaccination strategy for the vaccine they are using monitoring titers in sows. A reassessment of the strategy is warranted when new FMD vaccines are introduced.

ETHICS STATEMENT

Study approved by the Central Veterinary Institute, Dier experimenten commissie (Animal Ethics Committee).

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the experiment and analysis of the data, and reporting was performed by the first two authors.

FUNDING

This study was funded by the Ministry of Economic Affairs, project WOT-01-003-11, the Netherlands.

REFERENCES

1. *Council Directive 2003/85/EC of 29 September 2003 on Community Measures for the Control of Foot-and-Mouth Disease Repealing Directive 85/511/EEC and Decisions 89/531/EEC and 91/665/EEC and Amending Directive 92/46/EEC*. (2003). Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:306:0001:0087:en:PDF
2. Graves JH. Transfer of neutralizing antibody by colostrum to calves born of foot-and-mouth disease vaccinated dams. *J Immunol* (1963) 91(2):251–6.
3. Francis MJ, Black L. Effect of the sow vaccination regimen on the decay rate of maternally derived foot-and-mouth disease antibodies in piglets. *Res Vet Sci* (1984) 37(1):72–6.
4. Francis MJ, Black L. Response of young pigs to foot-and-mouth disease oil emulsion vaccination in the presence and absence of maternally derived neutralising antibodies. *Res Vet Sci* (1986) 41(1):33–9.
5. Dekker A, Eble P, Stockhofe N, Chenard G. Intratypic heterologous vaccination of calves can induce an antibody response in presence of maternal antibodies against foot-and-mouth disease virus. *BMC Vet Res* (2014) 10(1):127. doi:10.1186/1746-6148-10-127
6. Markowska-Daniel I, Pomorska-Mól M, Pejsak Z. The influence of age and maternal antibodies on the postvaccinal response against swine influenza viruses in pigs. *Vet Immunol Immunopathol* (2011) 142(1–2):81–6. doi:10.1016/j.vetimm.2011.03.019
7. Barteling S, Meloen R. A simple method for the quantification of 140S particles of foot-and-mouth disease virus (FMDV). *Arch Virol* (1974) 45(4):362–4.
8. Barteling SJ, Vreeswijk J. Developments in foot-and-mouth disease vaccines. *Vaccine* (1991) 9:75–88. doi:10.1016/0264-410X(91)90261-4
9. OIE. Foot-and-mouth disease (infection with foot-and-mouth disease virus). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees)*. (2012). 31 p. Available from: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.08_FMD.pdf
10. Bates D, Maechler M, Bolker B. *lme4: Linear Mixed-Effects Models Using Eigen and S4. R Package Version 1.0-5*. (2013). Available from: https://cloud.r-project.org/web/packages/lme4/index.html
11. Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* (2015) 67(1):1–48. doi:10.18637/jss.v067.i01
12. Bates DM. *lme4: Mixed-Effects Modeling with R*. (2010). Available from: http://lme4.r-forge.r-project.org/book/
13. Vaida F, Blanchard S. Conditional Akaike information for mixed-effects models. *Biometrika* (2005) 92(2):351–70. doi:10.1093/biomet/92.2.351
14. Liao PC, Lin YL, Jong MH, Chung WB. Efficacy of foot-and-mouth disease vaccine in pigs with single dose immunization. *Vaccine* (2003) 21(17–18):1807–10. doi:10.1016/S0264-410X(03)00030-6
15. Selman P, Chénard G, Dekker A. Cedivac-FMD; duration of immunity in cattle, sheep and pigs. *European Commission for the Control of FMD, Session of the Research Group of the Standing Technical Committee*. Paphos, Cyprus (2006).
16. Panjevic D, Valcic M. Humoral immunity of neonatal swine after FMD vaccination. *Zentralbl Veterinarmed B* (1989) 36(2):119–22.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 21 November 2016
doi: 10.3389/fvets.2016.00105

Transmission of Foot-and-Mouth Disease Virus during the Incubation Period in Pigs

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *06 October 2016*
Accepted: *07 November 2016*
Published: *21 November 2016*

Citation:
Stenfeldt C, Pacheco JM, Brito BP, Moreno-Torres KI, Branan MA, Delgado AH, Rodriguez LL and Arzt J (2016) Transmission of Foot-and-Mouth Disease Virus during the Incubation Period in Pigs. Front. Vet. Sci. 3:105. doi: 10.3389/fvets.2016.00105

Understanding the quantitative characteristics of a pathogen's capability to transmit during distinct phases of infection is important to enable accurate predictions of the spread and impact of a disease outbreak. In the current investigation, the potential for transmission of foot-and-mouth disease virus (FMDV) during the incubation (preclinical) period of infection was investigated in seven groups of pigs that were sequentially exposed to a group of donor pigs that were infected by simulated-natural inoculation. Contact-exposed pigs were comingled with infected donors through successive 8-h time slots spanning from 8 to 64 h post-inoculation (hpi) of the donor pigs. The transition from latent to infectious periods in the donor pigs was clearly defined by successful transmission of foot-and-mouth disease (FMD) to all contact pigs that were exposed to the donors from 24 hpi and later. This onset of infectiousness occurred concurrent with detection of viremia, but approximately 24 h prior to the first appearance of clinical signs of FMD in the donors. Thus, the latent period of infection ended approximately 24 h before the end of the incubation period. There were significant differences between contact-exposed groups in the time elapsed from virus exposure to the first detection of FMDV shedding, viremia, and clinical lesions. Specifically, the onset and progression of clinical FMD were more rapid in pigs that had been exposed to the donor pigs during more advanced phases of disease, suggesting that these animals had received a higher effective challenge dose. These results demonstrate transmission and dissemination of FMD within groups of pigs during the incubation period of infection. Furthermore, these findings suggest that under current conditions, shedding of FMDV in oropharyngeal fluids is a more precise proxy for FMDV infectiousness than clinical signs of infection. These findings may impact modeling of the propagation of FMD outbreaks that initiate in pig holdings and should be considered when designing FMD control strategies.

Keywords: foot-and-mouth disease, foot-and-mouth disease virus, virus diseases, pigs, transmission, incubation, subclinical, preclinical

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease that affects cloven-hoofed animal species. The infectious agent, foot-and-mouth disease virus (FMDV; genus *Aphthovirus*, family *Picornaviridae*), is infectious at low doses and capable of rapid dissemination within susceptible animal populations (1). Clinical FMD is characterized by fever, lameness, and ptyalism concurrent with the occurrence of vesicular lesions in the oral cavity and on the feet. However, the clinical manifestations of FMD may vary greatly depending both on biological properties of the different virus strains and on the host species affected (2–4).

Although FMD-associated mortality rates among adult animals are generally low, the intensive countermeasures enacted to combat disease outbreaks in FMD-free countries often result in depopulation and destruction of large numbers of infected and susceptible animals (5–7). Large regions of the world, including Europe, Australia, North America, and parts of South America, are kept free of FMD by means of strict regulations on import of animals and agricultural products. Animal populations within these regions where prophylactic vaccination is not practiced are highly vulnerable to potential FMDV incursions due to the lack of herd immunity. As access to international trade markets for agricultural products is largely dictated by a country's official FMD status, introduction of the disease into these regions will have massive financial and logistical implications for the agricultural sectors (8). Additionally, there are substantial ethical and environmental concerns associated with depopulation of large numbers of animals for the purpose of controlling potential FMD outbreaks.

The ability to efficiently combat FMD outbreaks in regions with highly susceptible animal populations is dependent on early detection of the incursion as well as the ability to efficiently trace and identify animals, herds, and premises that may have been exposed to the source of infection (9–12). The time elapsed from the first infection until the first case has been detected is generally referred to as the "high risk period" (13). As was seen during the extensive FMD outbreak in the UK in 2001, inability to detect infection during this early phase of an outbreak can result in substantial dissemination before appropriate countermeasures, such as animal movement restrictions, are enforced (14, 15). Two critical factors that complicate control of the early phase of an outbreak are potentially subtle or unapparent clinical signs of infection as well as disease transmission occurring during the incubation period, prior to the development of detectable clinical signs of FMD.

In a study by Charleston et al. (16), it was concluded that cattle exposed to FMDV by direct contact to infected cattle, were not infectious until, on average, 0.5 days after the appearance of clinical signs of FMD. Thus, the conclusion of this study was that transmission of FMDV during the incubation phase would not be likely to have a significant influence on disease dissemination in an outbreak situation. In contrast to this, a previous publication by Orsel et al. (17) concluded that substantial FMDV transmission may occur prior to onset of clinical signs in groups of cattle or pigs that are housed together. However, the latter publication also reported substantial differences in the occurrence of preclinical FMDV transmission depending on host species (cattle, pigs, or sheep) as well as on the age of the animals. While the occurrence of preclinical transmission was low within groups of young calves and lambs, it was substantially higher within groups of multiparous dairy cows as well as among 10–12 weeks old pigs (17).

Orsel's study design allowed for an approximation of the extent of preclinical transmission that had occurred. However, it was not possible to determine at which specific times transmission had occurred or to estimate the onset of infectiousness in the donor animals. These limitations resulted from the utilization of data from previous experiments that were originally designed for other purposes (18–21). The ratios of preclinical transmission were estimated by determining the number of new infections [defined by detection of FMDV shedding in oropharyngeal fluid (OPF)] that occurred before the donors developed clinical signs of FMD.

Multiple investigations have demonstrated rapid and efficient transmission of FMDV within groups of pigs that are housed together during the clinical phase of infection (18, 22–29). However, to the best of our knowledge, there are no published works characterizing the onset or continuous progression of infectiousness in FMDV-infected pigs. The current investigation was designed to determine the onset of infectiousness in relation to the development of clinical disease in pigs infected with FMDV. This work provides a novel and detailed characterization of the time-dependent progression of FMDV transmission dynamics within groups of pigs. The demonstration of substantial preclinical transmission of FMDV may influence modeling of FMDV outbreak scenarios involving this host species and is highly relevant to the development of outbreak response strategies.

MATERIALS AND METHODS

Virus

The virus used for this study was a cattle-derived strain of FMDV A24 Cruzeiro that had been passed once in pigs. Details of the generation and titration of the virus stock has been published previously (23).

Animal Experiments

All animal studies were performed within the BSL-3Ag containment facility at the Plum Island Animal Disease Center. Experimental protocols were approved by the facility's Institutional Animal Care and Use Committee, which functions to ensure ethical and humane treatment of experimental animals. All animals were castrated male Yorkshire pigs, weighing approximately 30 kg upon delivery that were obtained from a certified vendor (Animal Biotech Industries Inc., Danboro, PA, USA). Pigs were allowed 2 weeks of acclimation in the facility before the start of the experiment.

Preliminary Studies

In order to determine appropriate design of FMD transmission studies in pigs, a series of experiments were performed to establish

the antemortem infection dynamics subsequent to simulated-natural inoculation of donor pigs. These experiments have been described in detail in other publications (30–32). A system of intra-oropharyngeal (IOP) deposition of 100 50% infectious doses titrated in pig heel bulbs [50% PHID; (29)] was selected based on consistent, synchronous FMD in inoculated pigs and close simulation of natural infection. A total of 15 pigs were inoculated with FMDV A24 using this dose and route combination in order to determine the duration of the incubation period (i.e., onset of clinical signs) and to estimate the inferred latent period (i.e., onset of FMDV shedding as proxy for contagiousness). The duration of these experiments ranged from 12 h to 60 days depending on study objectives. An intensive schedule of sample collection was utilized through the early phase of infection to enable detailed characterization of infection dynamics in infected pigs. In brief, samples consisting of serum and oropharyngeal (OP) swabs (see below) were collected at 4- to 6-h intervals until 24 h post-inoculation (hpi), and at 12- to 24-h intervals subsequently, with some variation in sampling time points between study cohorts.

Contact Transmission Trials

The contact transmission trial included 40 pigs that were divided into 8 groups of 5 pigs per group (**Figure 1**). All groups were housed in separate isolation rooms and one group (the donor pigs) was infected with FMDV A24 Cruzeiro using the optimized IOP-inoculation system (30).

Starting at 8 hpi of the donor pigs, the remaining seven groups of pigs (contact groups 1–7) were sequentially comingled with the donor pigs for periods of 8 h each (**Figure 1**). The donor pigs were kept in the same isolation room throughout the study, while contact groups were moved from a clean pre-exposure room into the donor room, and subsequently transferred to a different, clean post-exposure room before the subsequent contact group was moved into the donor room (**Figure 1**). Thus, the first contact group was exposed to the donor pigs from 8 to 16 hpi, the second group was exposed from 16 to 24 hpi, continuing similarly, until the seventh and final contact group had been exposed to the donors, at 64 hpi (**Figure 1**). Water was available *ad libitum* throughout the experiment. A small amount of feed was distributed on the floor of the donor's room at the start of each contact period. Physical handling and sample collection was standardized to avoid passive transfer of virus between rooms. Animal handlers moved from clean to contaminated areas with showers and changes of clothes between rooms.

Clinical Evaluation and Sample Collection

Samples consisted of whole blood collected in serum separation tubes from the jugular vein and OP swabs obtained through direct swabbing of the tonsil of the soft palate using a large cotton swab. Swabs were immersed in 2-ml minimal essential media containing 25mM HEPES directly upon collection. Blood samples and OP swabs were centrifuged to extract serum and

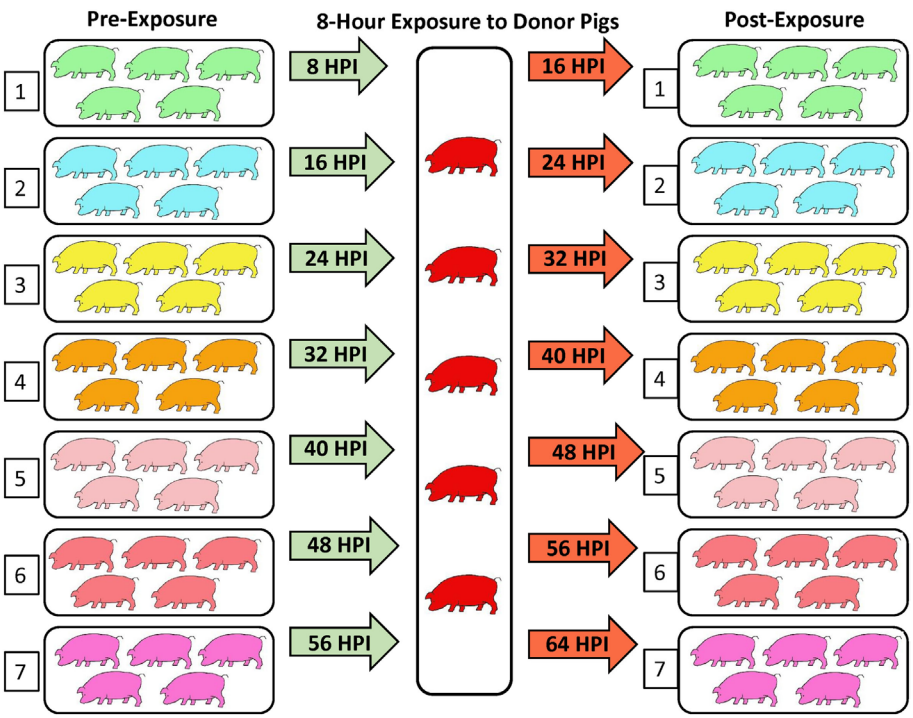

FIGURE 1 | Experimental design. Seven groups of five pigs were comingled with five FMDV-infected donor pigs through successive 8-h time slots. The first contact group was housed together with the donor pigs from 8 to 16 h post-infection (hpi) of the donors. Contact groups were sequentially shifted in and out of the isolation room housing the donor pigs at 8-h intervals until all contact groups had been exposed to the donors at 64 hpi. Contact groups were housed in separate isolation rooms before and after exposure and were monitored for the development of FMD.

OPF, respectively. All samples were immediately frozen at −70°C until further processing.

The onset and progression of the pigs' clinical status (lesion distribution) were quantitated using a previously described scoring system (29, 30, 33). In brief, each of 16 digits having a characteristic FMDV lesion contributed one point toward a cumulative score, with 4 additional single points added for lesions within the oral cavity, on the snout, on the lower lip, and on carpal/tarsal skin, respectively, thus resulting in an initial maximum score of 20. Individual animals' scores were subsequently converted to a 0–5 scale to facilitate statistical comparison to other investigations performed within our laboratory. This was achieved by dividing each animal daily score by the maximum score and multiplying the fractional value by 5.

Serum and OP swabs were collected from all pigs prior to inoculation or exposure. Subsequent sampling of donor pigs consisted of collection of blood at 16, 24, 48, and 64 hpi, and OP swabs directly after inoculation and at 8-h intervals subsequently, corresponding to the time points when contact groups were moved in/out of the donor's room. Clinical observations of donor pigs were likewise performed at 8-h intervals.

Post-exposure OP swabs were collected from contact pigs as they were transferred out of the donor's room [8 h post-exposure (hpe)] and again at 16 and 24 hpe. Serum and OP swabs were subsequently collected at 24-h intervals (corresponding to once per day) from 24 hpe until the pigs had developed fulminant clinical FMD and were removed from the study (72–120 hpe). Pigs that did not develop any signs of FMD were sampled once daily for 10 days, with additional samples collected at 14 and 21 days post-exposure (dpe). Clinical examinations of contact pigs were performed each time samples were collected.

FMDV RNA Detection in Serum and Swabs

Serum and OPF were analyzed using qRT-PCR, targeting the 3D region of the FMDV genome (34), as described previously (31, 32, 35, 36). Cycle threshold values were converted into FMDV genome copy numbers (GCN) per milliliter by use of a standard curve derived from analysis of 10-fold dilutions of *in vitro* synthesized FMDV RNA. The equation of the curve of GCN versus Ct values was further adjusted for dilutions used during processing of samples. Results reported in **Figures 2** and **3** represent the geometric group mean ($\log_{10}$ GCN/ml ± SEM) for each time point.

Virus Isolation

Oropharyngeal swab samples collected from contact groups 1 and 2 were cleared from debris and potential bacterial contamination by centrifugation through Spin-X® filter columns (pore size 0.45 μm, Sigma-Aldrich) and were subsequently analyzed for infectious FMDV through virus isolation (VI) on LFBK αvβ6 cells (37, 38), following a protocol previously described (36). Absence of FMDV was further confirmed by qRT-PCR analysis of VI cell culture supernatants.

Definitions

Successful transmission was determined by the detection of clinical FMD in contact-exposed pigs concurrent with viral dynamics consistent with infection. Pigs that did not develop clinical signs of FMD were kept through 21 days for the assessment of seroconversion to rule out the possibility of subclinical infection. The onset of FMDV shedding was determined as the time of the first detection of FMDV RNA in OPF that led to sustained subsequent detection. Using this definition, a single occurrence of FMDV RNA detection in OPF was not considered as virus shedding unless detection occurred in the subsequent sample. Viremia was defined by detection of FMDV RNA in serum. The onset of clinical FMD was determined as the first observed vesicular lesion in the oral cavity, on the snout or on the feet. All observations were made at individual animal level. But, transmission events could not be attributed to individual animals as contacts ($n = 5$) and donors ($n = 5$) cohabitated in the same containment unit during exposure.

Statistical Analysis

The time to first detection of clinical FMD lesions, viremia (defined by detection of FMDV RNA in serum), and continuous shedding of FMDV RNA in OPF was compared across groups using log-rank tests with Kaplan–Meier estimated survival curves (39, 40). Computations were carried out in the statistical program R using the survival package (41). p-Values of ≤ 0.05 were considered indicative of significant differences between groups.

RESULTS

Preliminary Studies

Fifteen pigs were inoculated with FMDV A24 Cruzeiro using a simulated-natural system of IOP deposition in order to estimate the parameters of incubation and latency, by determining onset of clinical disease and FMDV shedding. The selected dose and challenge route generated consistent clinical FMD and highly synchronous progression of infection in inoculated pigs (**Figures 2A,C,E**). Abundant but declining levels of FMDV RNA detected in OP swabs directly following inoculation were interpreted as residual inoculum. From 8 hpi, FMDV RNA detection in OPF increased, suggesting *de novo* replication of virus in the oropharynx (**Figure 2A**). The average detection level of FMDV RNA at the time of first detection of *de novo* shedding was 5.8 GCN/ml. Virus shedding peaked at approximately 72 hpi before gradually declining. Viremia, defined by detection of FMDV RNA in serum, was first detected at 18 hpi and peaked at 72 hpi (**Figure 2C**). A single vesicular lesion was detected at 36 hpi in one animal, with lesions appearing between 48 and 72 hpi in the remaining pigs (**Figure 2E**). Based on these data, it was determined that under these conditions, the transition from preshedding to shedding (inferred infectious) phases of infection occurred at 8 hpi (**Figure 2A**), while the transition from incubation to clinical phase occurred at 36–48 hpi (**Figure 2E**). On the basis of these preliminary experiments, it was determined that, in order to dissect incubation and latent periods under the current experimental conditions, a transmission experiment would have to span from a minimum of 8–48 hpi.

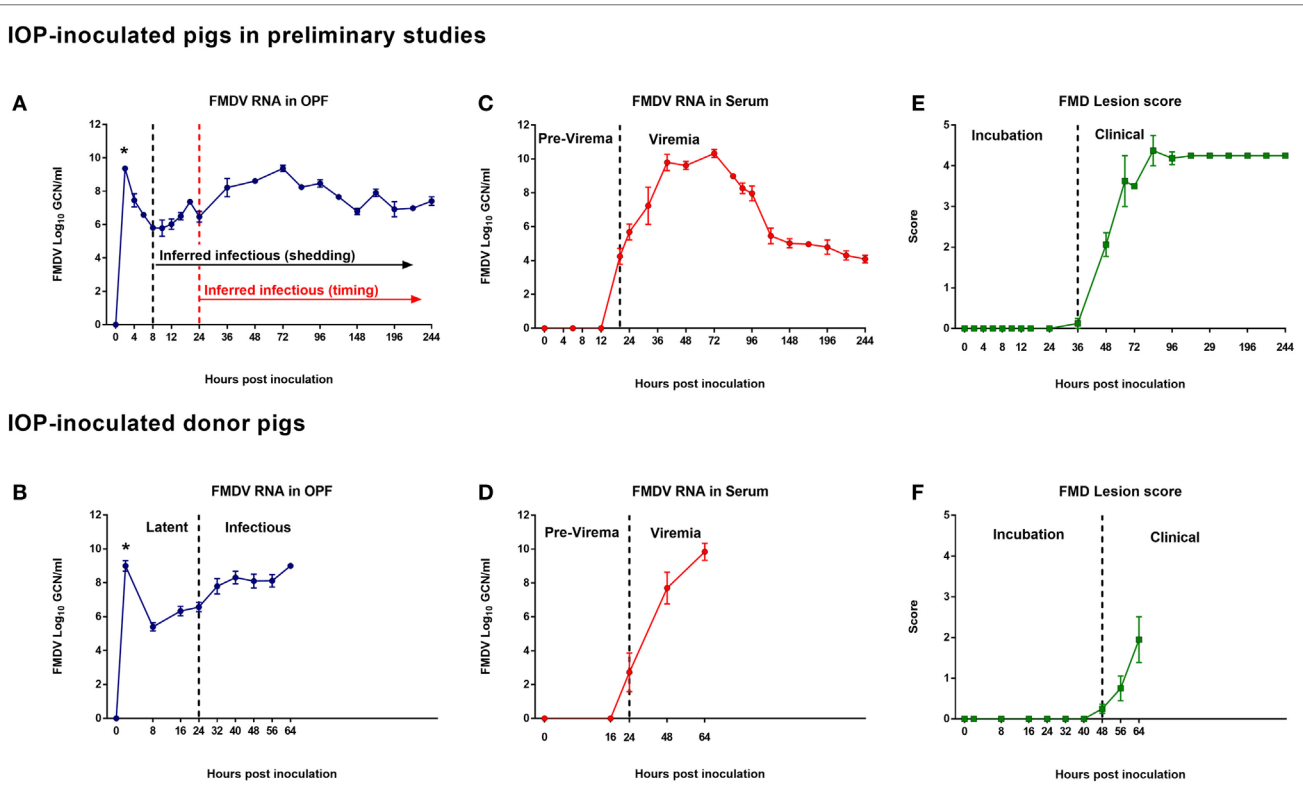

FIGURE 2 | FMDV infection dynamics in pigs inoculated *via* intra-oropharyngeal (IOP) route. Infection dynamics in preliminary investigations (top row; $n = 15$) and subsequent transmission trial (bottom row; $n = 5$). **(A,B)** FMDV RNA in OPF determined by qRT-PCR and expressed as $\log_{10}$ genome copy numbers (GCN)/ml (mean values ± SEM). Abundant FMDV RNA measured directly after inoculation was interpreted as residual inoculum indicated by (*). Infectiousness was not determined in the preliminary experiments which had no contact exposure groups. Rather, inferred infectiousness was estimated based on onset of shedding which occurred at 8 hpi [**(A)** black dashed line; Inferred Infectiousness (shedding)]. Inferred infectiousness was also retrospectively extrapolated from the measured time of onset of infectiousness in the subsequent transmission trial [**(A)** red dashed line; Inferred Infectiousness (timing)]. The donors' transition from latent to infectious phases of infection occurred at 24 hpi [**(B)** black dashed line] as determined by successful contact transmission of FMDV. **(C,D)** FMDV RNA in serum determined by qRT-PCR and expressed as $\log_{10}$ genome copy numbers (GCN)/ml (mean values ± SEM). Viremia was detected at 18 hpi in preliminary studies **(C)** and at 24 hpi in the donors of the transmission trial **(D)**. **(E,F)** Cumulative lesion score in preliminary investigations **(E)** and in the donors of the transmission trial **(F)**. The transition from incubation to clinical phases of infection (vertical dashed lines) occurred at 36 and 48 hpi, respectively. Assay detection limits: serum = 2.68 $\log_{10}$ GCN/ml; OPF = 3.08 $\log_{10}$ GCN/ml.

Contact Transmission Trial

Infection Dynamics in IOP-Inoculated Donor Pigs

Five pigs designated as virus donors were inoculated with FMDV A24 Cruzeiro by the IOP route, as described previously (30, 31). Overall, infection dynamics were highly similar to the preliminary experiments (**Figure 2**). Adequate deposition of inoculum was confirmed by detection of abundant quantities of virus measured in OP swabs directly following inoculation ("*" in **Figure 2B**). After clearance of residual inoculum, FMDV shedding was detected in OPF of all five donor pigs at 8 hpi, with a mean value of 5.22 $\log_{10}$ GCN/ml. FMDV shedding in OPF increased continuously reaching a maximum level of 9.0 $\log_{10}$ GCN/ml at 64 hpi at which time the donor pigs were euthanized (**Figure 2B**). FMDV RNA was detected in serum at 24 hpi in three out of five donor pigs, and at 48 hpi in the remaining two donor pigs. The maximum mean serum concentration of 9.85 $\log_{10}$ GCN/ml was measured at the time of euthanasia (64 hpi; **Figure 2D**). Early clinical signs of FMDV, including subtle blanching and vesiculation at coronary bands and tongue, were observed in three donor pigs at 48 hpi, and one pig at 56 hpi; cumulative lesion scores in these four pigs progressed gradually until the final assessment at 64 hpi (**Figure 2F**). One pig did not develop FMD lesions within the study period; however, successful infection of this pig was confirmed by detection FMDV RNA in OPF and blood at 8 and 48 hpi, respectively.

Contact Groups 1 and 2

Contact group 1 cohabitated with the donor pigs from 8 to 16 hpi, whereas contact group 2 was subsequently exposed to the donors from 16 to 24 hpi (**Figure 1**). Detection of FMDV RNA in OPF of donor pigs was 5.22 $\log_{10}$ GCN/ml at the start of contact group 1 exposure, and 6.51 $\log_{10}$ GCN/ml at the end of contact group 2 exposure (**Table 1**). None of the 10 pigs in contact groups 1 or 2 developed any signs of FMDV infection (**Figures 3–6**; **Table 1**). Low quantities of FMDV RNA were detected in OP swabs of one pig in contact group 2 at 8 hpe, corresponding to the end of the contact exposure. FMDV RNA was not detected in any subsequent OPF or serum samples collected from the pigs in

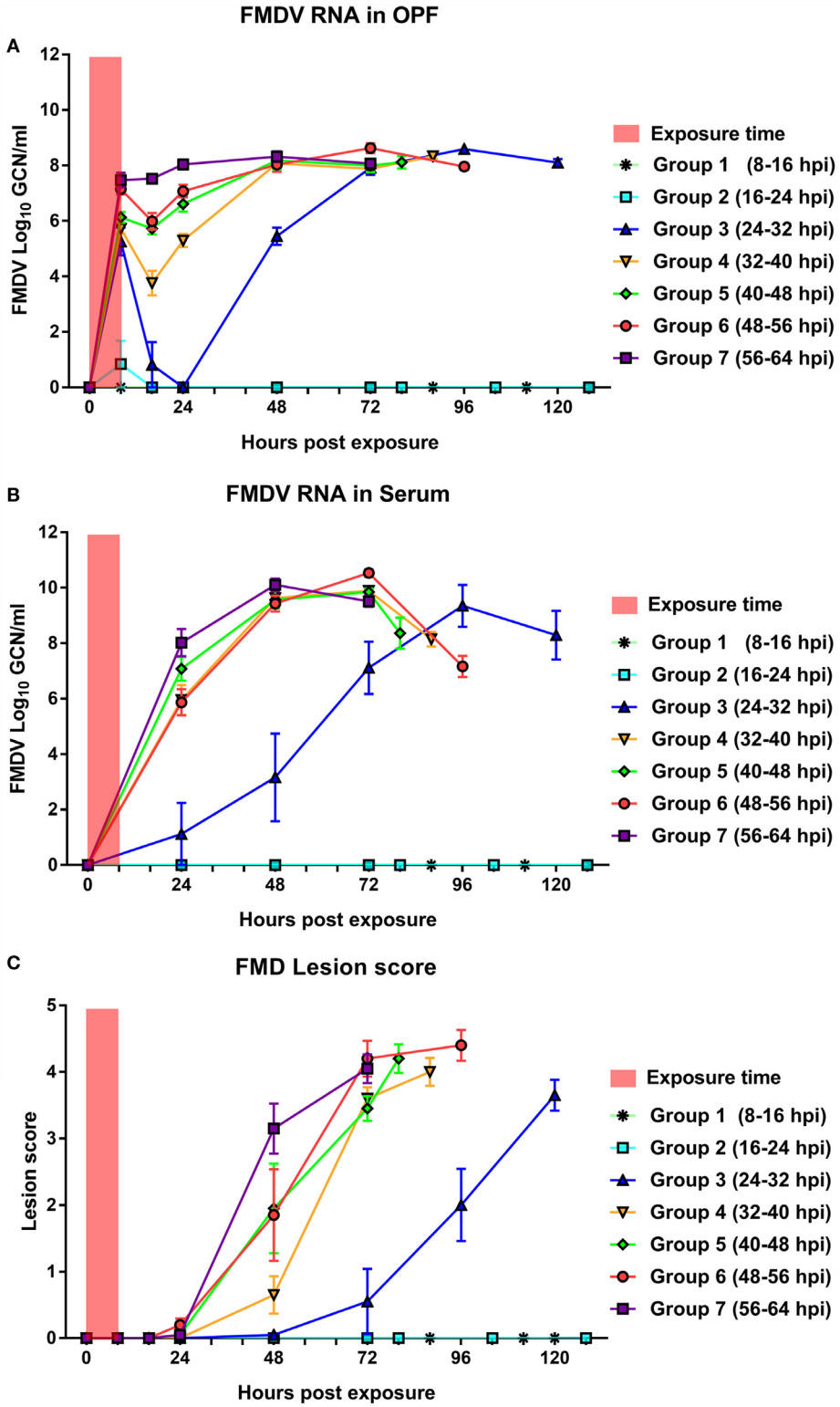

FIGURE 3 | **Infection dynamics in sequentially exposed contact groups**. Quantities of FMDV RNA in OP swabs **(A)** and serum **(B)**, as well as cumulative lesion score **(C)** in seven groups of pigs that were exposed to FMDV-infected donor pigs during successive 8-h slots. Group means and SEM. There was no detection of infection in contact groups 1 and 2, which were exposed to the donors from 8 to 16 and 16 to 24 hpi, respectively. The onset and progression of infection in contact group 3 (exposed from 24 to 32 hpi) were delayed compared to subsequent groups. FMDV RNA in serum and OPF determined by qRT-PCR and expressed as $\log_{10}$ genome copy numbers (GCN)/ml (mean values ± SEM). Assay detection limits: serum = 2.68 $\log_{10}$ GCN/ml; OPF = 3.08 $\log_{10}$ GCN/ml.

TABLE 1 | Progression of infectiousness in pigs inoculated with FMDV *via* intra-oropharyngeal (IOP) route.

Contact group	Exposure time[a] (hpi)	Donor pig characteristics during exposure				Infected contact pigs (infected/not infected)	Infectious phase of donors
		Viremia	Clinical FMD	FMDV RNA in OPF[b]			
				Start exposure	End exposure		
1	8–16	No	No	5.22	6.45	0/5	Latent
2	16–24	No	No	6.45	6.51	0/5	Latent
3	24–32	Yes	No	6.51	7.22	5/0	Infectious (*incubation*)
4	32–40	Yes	No	7.22	8.31	5/0	Infectious (*incubation*)
5	40–48	Yes	Yes	8.31	8.10	5/0	Infectious (*clinical*)
6	48–52	Yes	Yes	8.10	8.10	5/0	Infectious (*clinical*)
7	52–64	Yes	Yes	8.10	9.00	5/0	Infectious (*clinical*)

[a]*Expressed as hours post-infection of donors.*
[b]*Expressed as log_{10} GCN/ml.*

either of these two groups (**Figure 3**). Pigs in groups 1 and 2 were monitored through 21 dpe. Serum samples collected at 21 dpe did not contain neutralizing antibodies against FMDV, confirming that the pigs had not been subclinically infected (not shown).

Contact Group 3

The five pigs in contact group 3 were exposed to the donor pigs from 24 to 32 hpi. During this period, donor pigs were viremic, but had not yet developed fever (not shown) or vesicular FMD lesions (**Figure 2**). FMDV RNA detection in OPF of the donors was 6.51 log_{10} GCN/ml at the start of exposure, and 7.22 log_{10} GCN/ml when contact group 3 was removed from the room (**Table 1**). FMDV RNA was detected in OPF of all five pigs in groups 3 at the end of the exposure period (8 hpe; **Figure 3A**), with a mean value of 5.25 log_{10} GCN/ml. However, FMDV RNA was only detected in OPF from one out of the five pigs at the subsequent time point (16 hpe), and OPF from all five pigs were below the limit of detection for FMDV RNA at 24 hpe (**Figure 3A**). Viral shedding in OPF was again detected in all pigs at 48 hpe and gradually increased to a peak mean value of 8.59 log_{10} GCN/ml measured at 96 hpe (**Figure 3A**). FMDV RNA in serum was detected at 24 hpe in one pig, at 48 hpe in the second pig, and at 72 hpe in the remaining three pigs (**Figure 3B**). Vesicular lesions were detected in all five pigs between 48 and 96 hpe (24 h after the first detection of FMDV RNA in serum). There was a continuous increase in cumulative lesion scores until the pigs were euthanized at 120 hpe (**Figure 3C**).

Contact Group 4

The five pigs of contact group 4 cohabitated with the donor pigs from 32 to 40 hpi. Similar to the previous exposure period, the donor pigs were viremic during the contact period, but without any clinical signs of FMD (**Figure 2**; **Table 1**). Mean detection of FMDV RNA in OPF of the donors was 7.22 log_{10} GCN/ml at the initiation of exposure, and 8.31 log_{10} GCN/ml at the end of exposure (**Table 1**; **Figure 2D**). FMDV RNA was detected in OPF from all five pigs in contact group 4 at the end of the exposure period (8 hpe), with a mean value of 5.72 log_{10} GCN/ml (**Figure 3A**). Virus detection in OPF was negative in three out of the five pigs at 16 hpe, while shedding was continuous in two pigs. There was subsequently a consistent increase in FMDV RNA levels in OPF, from 24 hpe until a maximum mean value of 8.33 log_{10} GCN/ml was measured before the pigs were euthanized at 88 hpe (**Figure 3A**). FMDV RNA was detected in serum of all five pigs at 24 hpe, with serum levels reaching a maximum mean value of 9.89 log_{10} GCN/ml at 72 hpe (**Figure 3B**). Vesicular lesions were detected at 48 hpe in all five pigs, with cumulative lesions scores consistently increasing until the time of euthanasia (**Figure 3C**).

Contact Group 5

The five pigs in contact group five were exposed to the donor pigs from 40 to 48 hpi which corresponded to the end of the incubation period and transition to the clinical phase of infection for the donor group. There were no clinical signs of FMD in the donor pigs at the start of the contact group 5 exposure slot (40 hpi), but three out of the five donor pigs had developed vesicular lesions by 48 hpi (**Figure 2F**). Mean shedding of FMDV RNA in OPF of the donors was 8.31 log_{10} GCN/ml at the start of group 5 exposure, with a marginal decrease to 8.10 log_{10} GCN/ml at the end of exposure (**Table 1**; **Figure 2D**). Mean FMDV RNA detection in OPF from contact group 5 at the end of the exposure (8 hpe) was 6.13 log_{10} GCN/ml (**Figure 3A**). This quantity had decreased marginally to 5.72 log_{10} GCN/ml at 16 hpe, but shedding was continuous in all five pigs. Peak shedding (8.18 log_{10} GCN/ml) was detected at 48 hpe, and virus shedding was sustained close to that level until the pigs were euthanized at 80 hpe (**Figure 3A**). All five pigs were viremic at 24 hpe, with peak serum concentration of virus (9.84 log_{10} GCN/ml) measured at 72 hpe (**Figure 3B**). One pig had a clearly demarcated coronary band vesicle at 24 hpe, and clinical lesions were detected at 48 hpe in the other four pigs. Cumulative lesion scores progressed rapidly, and all pigs had severe clinical FMD and were unwilling to stand and/or move at the time of euthanasia (80 hpe) (**Figure 3C**).

Contact Group 6

Contact group 6 was exposed to the donors from 48 to 56 hpe. Four out of the five donor pigs had early signs of clinical FMD during this time frame. The mean quantities of FMDV RNA detected in OPF of the donors were 8.10 log_{10} GCN/ml at the beginning and at the end of group 6 exposure. Mean OPF detection in the contact pigs at the end of the exposure period (8 hpe) was 7.15 log_{10} GCN/ml. Similar to contact group 5, there was a modest drop in OPF detection in contact group 6 pigs by 16 hpe (5.99 log_{10} GCN/ml). Shedding was continuous in all five pigs, with a peak mean value

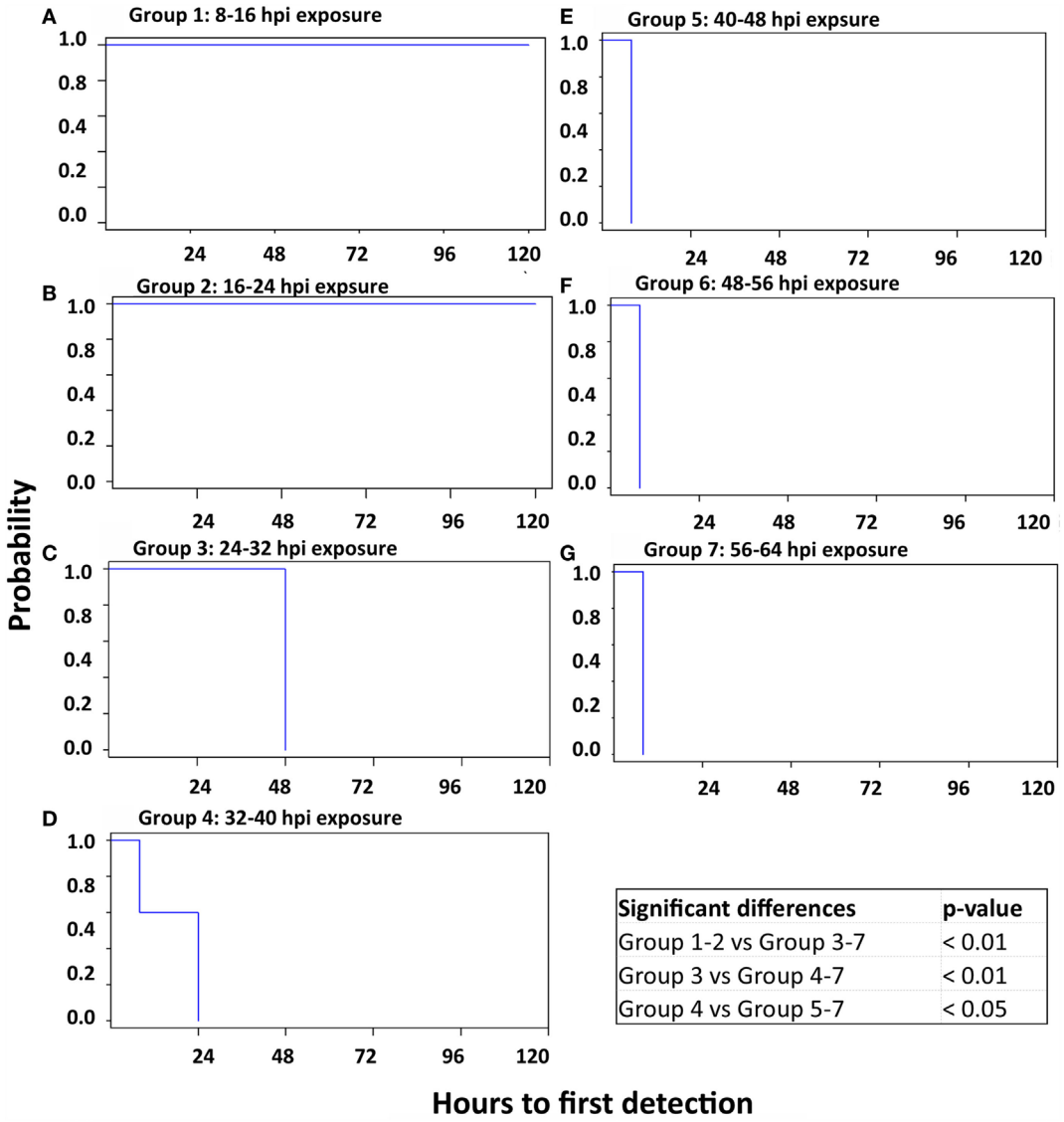

FIGURE 4 | Kaplan–Meier survival curves; time to detection of OP shedding of FMDV. Survival curves delineating the time to onset of oropharyngeal shedding of FMDV RNA in seven sequentially exposed groups of pigs. There was no shedding of FMDV in groups 1 and 2 (A and B), which were thus significantly different from all other groups. Additionally, the onset of FMDV shedding was significantly delayed in groups 3 and 4 compared to all subsequent groups. **(A)** Group 1: 8–16 hpi exposure. **(B)** Group 2: 16–24 hpi exposure. **(C)** Group 3: 24–32 hpi exposure. **(D)** Group 4: 32–40 hpi exposure. **(E)** Group 5: 40–48 hpi exposure. **(F)** Group 6: 48–56 hpi exposure. **(G)** Group 7: 56–64 hpi exposure.

of 8.63 $\log_{10}$ GCN/ml at 72 hpe (**Figure 3A**). All pigs were viremic at 24 hpe, with peak mean serum concentration of virus (10.53 $\log_{10}$ GCN/ml) measured at 72 hpe (**Figure 3B**). Clinical lesions appeared at 24 hpe (three pigs), 48 hpe (one pig), or 72 hpe (one pig) (**Figure 3C**). Similar to the preceding contact group, all pigs were severely affected by the clinical disease starting at 48–72 hpe and were euthanized at 96 hpe.

Contact Group 7

Contact group 7 was exposed to the donors from 56 to 64 hpi. The clinical status of the donor group was similar to the previous exposure period, with vesicular lesions in four out of the five pigs (**Figure 2F**). Mean shedding in OPF of donor pigs was 8.10 $\log_{10}$ GCN/ml at the beginning of the exposure and 9.00 $\log_{10}$ GCN/ml at the end of the contact period (**Table 1**; **Figure 2B**). The mean OPF detection of FMDV RNA in contact pigs at the end of exposure was 7.47 $\log_{10}$ GCN/ml, and OPF shedding increased continuously until maximum values of 8.32 $\log_{10}$ GCN/ml were measured at 48 hpe (**Figure 3A**). The mean serum concentration of FMDV RNA at 24 hpe was 8.02 $\log_{10}$ GCN/ml, with an increase to a peak average value of 10.10 at 48 hpe (**Figure 3B**). Clinical lesions were detected at 24 hpe in one pig and 48 hpe in four pigs (**Figure 3C**). The pigs in group 7 were euthanized at 72 hpe due to the severity of clinical FMD.

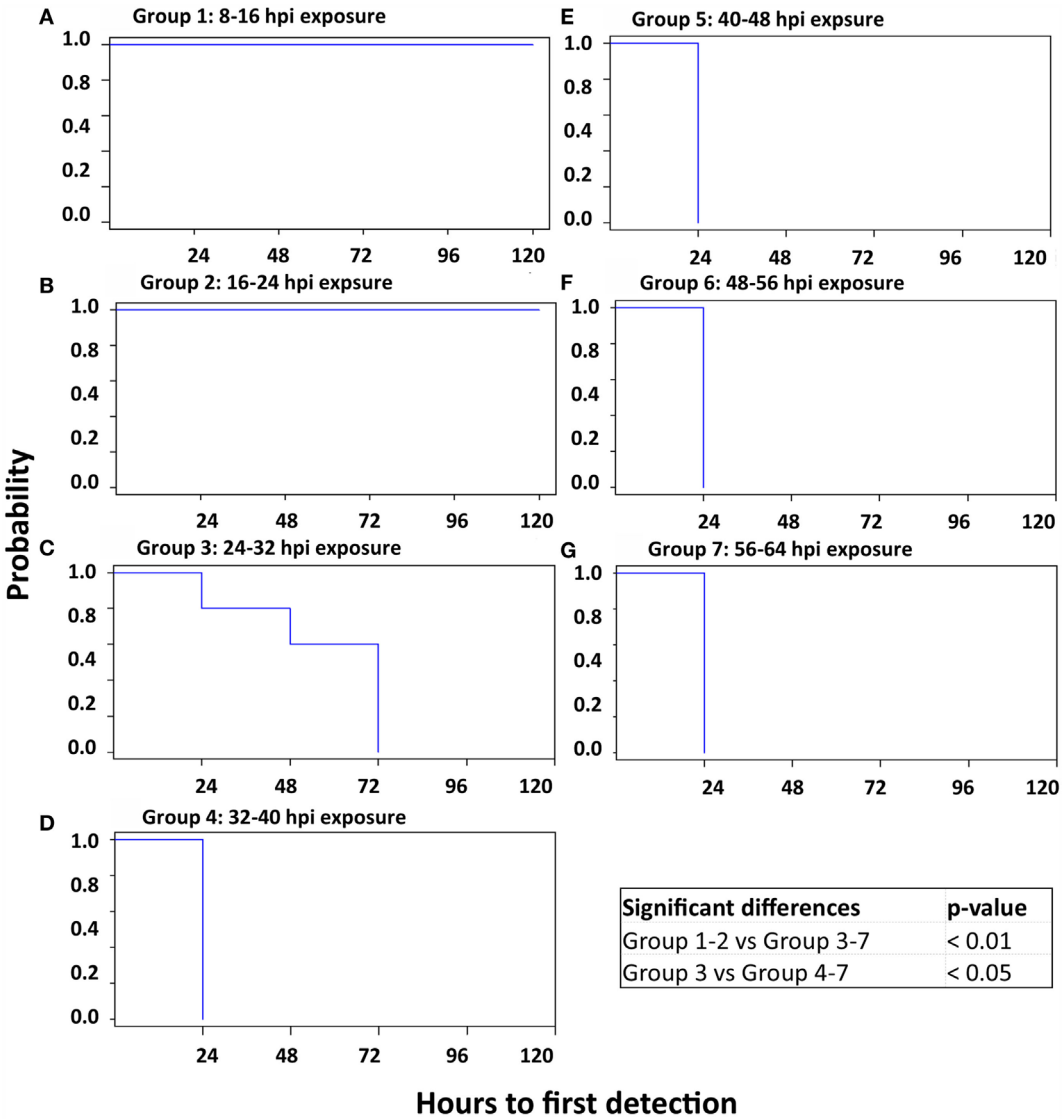

FIGURE 5 | Kaplan–Meier survival curves; time to detection of viremia. Survival curves delineating the time to onset of viremia, defined by detection of FMDV RNA in serum. Seven groups of pigs were sequentially exposed to FMDV-infected donor pigs. There was no detection of FMDV in serum in groups 1 and 2 **(A,B)**, which were thus significantly different from all other groups. Additionally, the onset of FMDV viremia was significantly delayed in group 3 compared to subsequent groups. **(A)** Group 1: 8–16 hpi exposure. **(B)** Group 2: 16–24 hpi exposure. **(C)** Group 3: 24–32 hpi exposure. **(D)** Group 4: 32–40 hpi exposure. **(E)** Group 5: 40–48 hpi exposure. **(F)** Group 6: 48–56 hpi exposure. **(G)** Group 7: 56–64 hpi exposure.

Statistical Comparison of Time to Onset of Viremia, Shedding, and Clinical Disease across Contact Groups

The time to event for onset of important disease dynamic parameters were compared across contact groups in order to characterize differences associated with the conditions of contact exposure. An omnibus test indicated that there were significant differences among the Kaplan–Meier estimated survival curves for the seven contact groups at the 0.05 significance level in the elapsed times from contact exposure until the first detection of FMDV shedding in OPF, onset of viremia, and appearance of clinical FMD lesions across contact groups. Specifically, the onset and progression of these indicators of infection were more rapid in contact groups that had been exposed to the donors during later stages of infection. Contact groups 1 and 2, which did not become infected with FMDV through contact were, as expected, significantly different pairwise from all other groups in all three parameters evaluated (**Figures 4–6**). Contact group 3, which had been exposed to the donors from 24 to 32 hpi, was also significantly different compared to groups 4–7, pairwise, for all parameters as FMDV shedding in OPF (**Figure 4**), viremia (**Figure 5**), and clinical lesions (**Figure 6**) were delayed relative to subsequent contact groups. Additionally, detection of FMDV shedding in OPF occurred significantly later in contact group 4 (32–40 hpi exposure) relative to later groups (**Figure 4**), whereas

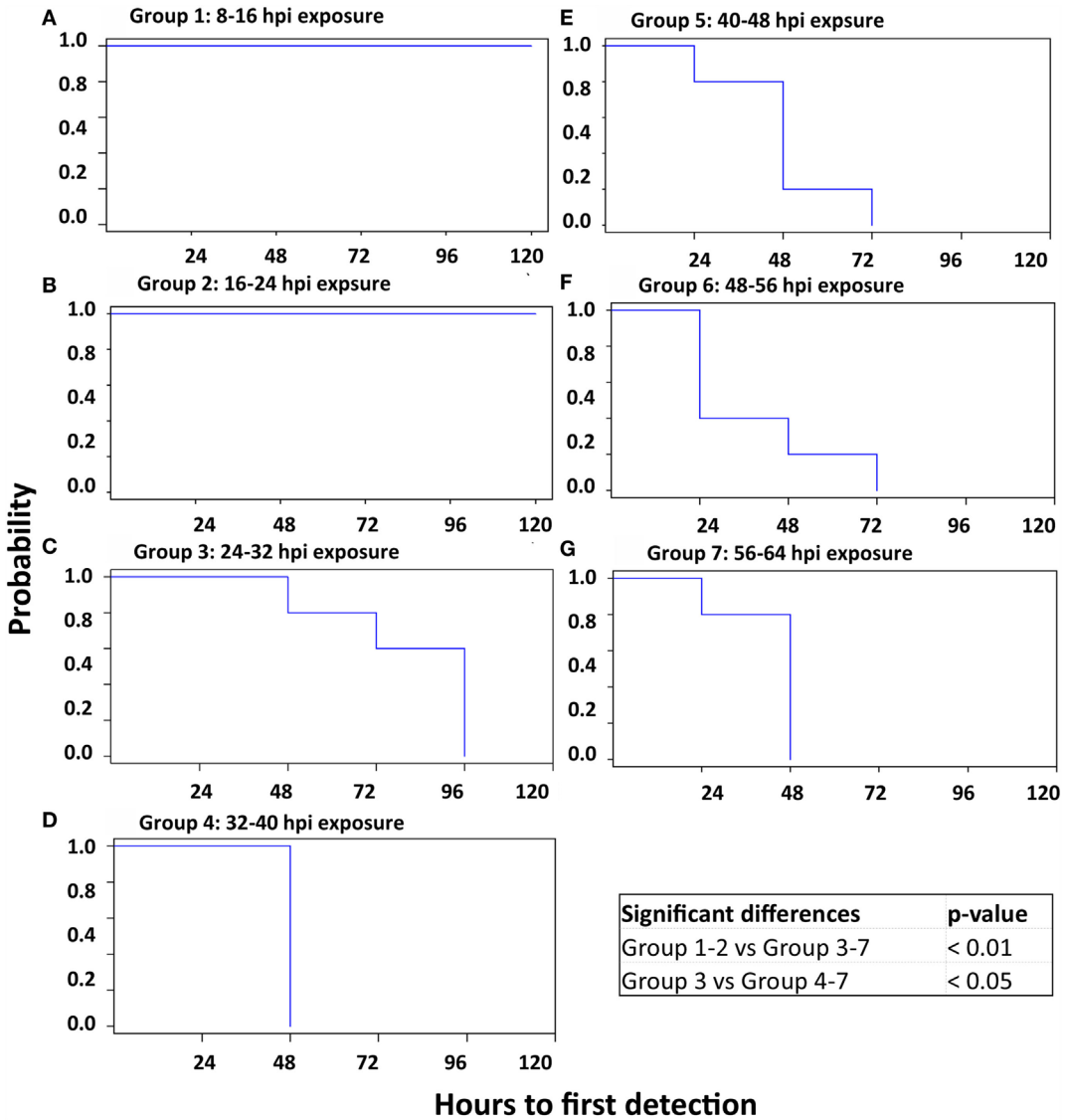

FIGURE 6 | Kaplan–Meier survival curves; time to detection of FMD lesions. Survival curves delineating the time to detection of vesicular FMD lesions in seven sequentially exposed groups of pigs. There were no FMD lesions in groups 1 and 2 **(A,B)**, which were thus significantly different from all other groups. Additionally, the onset of clinical FMD was significantly delayed in group 3 compared to subsequent groups. **(A)** Group 1: 8–16 hpi exposure. **(B)** Group 2: 16–24 hpi exposure. **(C)** Group 3: 24–32 hpi exposure. **(D)** Group 4: 32–40 hpi exposure. **(E)** Group 5: 40–48 hpi exposure. **(F)** Group 6: 48–56 hpi exposure. **(G)** Group 7: 56–64 hpi exposure.

this group was not different from later groups with regard to detection of viremia or clinical lesions (**Figures 5** and **6**).

DISCUSSION

The highly contagious nature of FMDV can be attributed to a combination of factors including broad host range, low infectious dose, and shedding of large quantities of virus by infected animals (3, 4, 42, 43). Even though FMDV transmission may occur *via* both direct (animal to animal) and indirect (mechanical transfer and airborne spread) mechanisms, the most significant risk for dissemination of disease during the early phase of an outbreak is transport of infected animals (44). The extent of spread during the early, high risk period of an FMD outbreak can be influenced by the occurrence of mild or unrecognized clinical symptoms of disease, as well as the potential for disease transmission from infected animals that have not yet developed clinical disease (13). The current investigation was designed for the purpose of determining the onset of infectiousness (end of latent period) in relation to the appearance of clinical signs (end of incubation period) in pigs infected with FMDV. Different characteristics of infection dynamics were considered for appropriateness as proxies for contagiousness for modeling purposes. An intensive schedule of sampling and clinical observations in combination

with continuous, sequential exposure of contact pigs to donors enabled detailed characterization of the progressive transmission dynamics of FMDV in groups of pigs.

The experimental design was based on previous experience of characterizing infection dynamics of this specific FMDV strain in pigs (23, 30–32). The donor pigs were infected using a simulated-natural system of IOP inoculation (30, 31) and optimal timing of contact exposure was determined based on data obtained from a series of preliminary experiments utilizing the same virus and challenge system. The IOP-inoculation system has the advantage of utilizing a natural route of FMDV exposure for pigs while also facilitating precise control of the timing and dose of virus challenge. Additionally, as this is a needle-free inoculation system, there are no primary vesicles at injection sites that could constitute an additional (artificial) source of virus exposure for contact animals.

Foot-and-mouth disease virus shedding was continuously detected in OPF from all pigs in the donor group from the time of inoculation until termination of their monitoring period. Despite this continuous detection, there was no transmission of FMDV to any of the pigs in the first two contact groups, which were exposed to the donors from 8 to 16 and 16 to 24 hpi, respectively. On this basis, the transition from latency to infectiousness within the donor group was determined to have occurred between 24 and 32 hpi as all contact pigs that were exposed to the donors from this time and onward developed severe clinical FMD within 1–3 days after exposure. There were, however, no clinical signs of FMD detected in any of the donor pigs before 48 hpi. Thus, pigs in contact groups 3 and 4 (exposed from 24 to 32 and 32 to 40 hpi, respectively) were infected, while all of the donor pigs were still within the incubation (preclinical) phase of infection. The difference between detection of shedding versus confirmed infectiousness indicated that presence of FMDV in donor pigs was not sufficient to define infectiousness and suggests that a threshold quantity of shedding is required for FMDV to be transmitted.

Successful transmission of FMDV occurred concurrently with the first detection of viremia in the donor pigs. Even though the presence of virus in the blood can be assumed to not have any causal relationship to transmission of infection, the current and previous investigations have demonstrated that occurrence of viremia is associated with a concurrent surge in virus shedding *via* the OP route (23, 31, 45). These data suggest that onset of viremia and threshold-defined shedding of FMDV are better proxies for infectiousness than onset of clinical disease (end of incubation period).

There was moderate variation in the onset of viremia and the first detection of clinical lesions among pigs within the earliest contact groups to get infected (group 3), whereas the variation in infection dynamics was lower within later groups (groups 4 through 7). FMDV shedding in group 3 was low but largely consistent across animals through the early time points after exposure. Specifically, FMDV shedding in OPF was below the limit of detection at 24 hpe in all five pigs of contact group 3. This modest and relatively synchronous FMDV shedding in OPF through 8–48 hpe suggests that all five pigs in group 3 did likely get infected directly by the donors. However, due to limitations of the study design, it was not possible to rule out the possibility of within-group transmission in this group. Contrastingly, the highly synchronous infection dynamics within subsequent contact groups strongly suggested direct transmission from donors to contact pigs.

All pigs in contact groups 3 through 7, which were exposed to donors from 24 hpi and later, developed similarly severe FMD characterized by high-titer viremia and vesicular lesions on all four feet as well as in the oral cavity or on the snout. Thus, there was no difference in disease severity between the pigs in any of these groups as they reached the pre-determined end point of the study. There were, however, more pronounced differences between these (infected) contact groups at earlier time points after exposure as was reflected in the significant differences in the time to event analyses. While the later contact groups (groups 5 through 7) had a very rapid onset of severe FMD, the progression of clinical disease was slower and more gradual in contact group 3. This finding is consistent with previous investigations which have described similar associations between increased challenge dose and a shorter time to onset of viremia and clinical lesions in FMDV-exposed pigs (25, 28, 30). In the current study, the effective challenge dose of successive contact groups was reflected by the quantity of FMDV detected in OPF from the donor pigs, which steadily increased from 8 to 40 hpi. The increase in FMDV shedding by donors through subsequent exposure periods (40–64 hpi) was less pronounced. However, the appearance of vesicular lesions, containing high loads of amplifying virus, by 48 hpi would have contributed to a progressively higher challenge dose for the later challenge groups. It is noteworthy that the progression of viremia and clinical FMD in contact groups 5 through 7 was faster than in the directly inoculated donors, suggesting that the contact challenge received by these groups was greater than the IOP-delivered dose. This is consistent with the concept that direct contact exposure, albeit of limited duration, is a highly stringent challenge system for FMDV studies in pigs [reviewed in Ref. (46)].

A previous study by Quan et al. (26) concluded that although there was a progressive increase in infectiousness and FMDV transmission over time when pigs were exposed in groups, this was not the case when contact pigs were individually exposed to infected donors. Specifically, there was very limited transmission of FMDV when one contact pig was exposed to one donor pig, regardless of the infectious state of the donor pig. Similarly, a previous investigation from our laboratory demonstrated that the duration of contact exposure had substantial influence on FMDV transmission within groups of pigs, and that the effect of altered exposure duration was strain specific (23). The combined conclusions of these previous works accentuate the critical influence of experimental design on the outcome and interpretation of transmission experiments. The lack of evaluation of individual (one-on-one) exposure of contact pigs in the current study limits the ability to attribute FMDV transmission to specific individuals or to precisely measurable shedding parameters. However, pigs are generally not housed individually, or in pairs, under commercial production conditions; therefore, estimation of transmission proxies based on isolated individuals could underestimate parameters for modeling of disease spread in natural settings.

The results of this study suggest that FMDV shedding parameters may be better proxies for FMDV transmission than clinical signs of disease under these specific experimental conditions. This finding differs from the conclusions published by Charleston et al. in 2011, which were based on an experimental design investigating transmission of serotype O FMDV between calves that were housed in pairs (one-on-one exposure) (16). Furthermore, our findings suggest that FMDV transmission occurred when the mean levels of FMDV shedding in OPF within the donor group exceeded a distinct threshold (6.50 $\log_{10}$ GCN/ml $\pm$ 0.58). Thus, OP shedding of FMDV in pigs should not be treated as a categorical variable indicative of infectiousness. This is specifically relevant to meta-analyses conducted to obtain infection parameters (i.e., estimation of latent and infectious periods) that feed mathematical modeling, which have not traditionally incorporated this concept.

The current investigation demonstrated transmission of FMDV during the incubation period of pigs housed in groups. The transition from latent to infectious phases of disease occurred approximately 24 h prior to the appearance of clinical signs of disease. There was a progressive increase in infectiousness of donor pigs through the acute phase of disease as the onset and progression of clinical FMD in contact pigs were faster in groups that were exposed to the donors during advanced stages of clinical FMD, which is consistent with an increased effective challenge dose. These findings should be considered for modeling of FMDV outbreaks involving pigs. Similar studies performed in other susceptible species may provide additional insights to the relationships between incubation and latency of FMDV infection.

AUTHOR CONTRIBUTIONS

CS contributed to study design, coordinated and executed the animal experiments, and drafted the manuscript. JP contributed to study design and execution of the animal experiments, and oversaw laboratory analyses. BB performed statistical analyses and interpretation of data. K-MT performed statistical analyses and interpretation of data. MB performed statistical analyses and interpretation of data. AD coordinated and oversaw data analyses and contributed scientific content. LR contributed to study design and scientific content. JA conceived and coordinated the work, contributed to writing the manuscript, and promulgated addition of vertical lines in **Figure 2**. All the authors have critically reviewed and revised the manuscript and approved the final product.

ACKNOWLEDGMENTS

Drs. Helena C. de Carvalho Ferreira, Steven I. Rekant, Lauro Velazquez-Salinas, Erin B. Howey, Brenton Sanford, and Jolene Carlson are thanked for contributing essential practical support during the transmission study. Elizabeth Bishop, Ethan Hartwig, George Smoliga and Steve Pauszek are thanked for excellent support with laboratory analyses.

FUNDING

This project was funded through an interagency agreement with the Science and Technology Directorate of the U.S. Department of Homeland Security under Award Number HSHQDC-11-X-00189 with additional funding from ARS-CRIS Project 1940-32000-057-00D. CS, KM-T, and BB are recipients of a Plum Island Animal Disease Center Research Participation Program fellowship, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement with the U.S. Department of Energy. None of the funding sources had influence upon design or performance of experimental study, interpretation of results, or writing of the manuscript.

REFERENCES

1. Grubman MJ, Baxt B. Foot-and-mouth disease. *Clin Microbiol Rev* (2004) 17(2):465–93. doi:10.1128/CMR.17.2.465-493.2004
2. Alexandersen S, Mowat N. Foot-and-mouth disease: host range and pathogenesis. *Curr Top Microbiol Immunol* (2005) 288:9–42.
3. Arzt J, Baxt B, Grubman MJ, Jackson T, Juleff N, Rhyan J, et al. The pathogenesis of foot-and-mouth disease II: viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virus-host interactions. *Transbound Emerg Dis* (2011) 58(4):305–26. doi:10.1111/j.1865-1682.2011.01236.x
4. Arzt J, Juleff N, Zhang Z, Rodriguez LL. The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. *Transbound Emerg Dis* (2011) 58(4):291–304. doi:10.1111/j.1865-1682.2011.01204.x
5. Mansley LM, Donaldson AI, Thrusfield MV, Honhold N. Destructive tension: mathematics versus experience – the progress and control of the 2001 foot and mouth disease epidemic in Great Britain. *Rev Sci Tech* (2011) 30(2):483–98.
6. Yang PC, Chu RM, Chung WB, Sung HT. Epidemiological characteristics and financial costs of the 1997 foot-and-mouth disease epidemic in Taiwan. *Vet Rec* (1999) 145(25):731–4.
7. Bouma A, Elbers AR, Dekker A, de Koeijer A, Bartels C, Vellema P, et al. The foot-and-mouth disease epidemic in The Netherlands in 2001. *Prev Vet Med* (2003) 57(3):155–66. doi:10.1016/S0167-5877(02)00217-9
8. Junker F, Ilicic-Komorowska J, van Tongeren F. *Impact of Animal Disease Outbreaks and Alternative Control Practices on Agricultural Markets and Trade: The Case of FMD*. OECD Food, Agriculture and Fisheries Working Papers, No. 19. Paris: OECD Publishing (2009).
9. Boklund A, Halasa T, Christiansen LE, Enoe C. Comparing control strategies against foot-and-mouth disease: will vaccination be cost-effective in Denmark? *Prev Vet Med* (2013) 111(3–4):206–19. doi:10.1016/j.prevetmed.2013.05.008
10. Halasa T, Willeberg P, Christiansen LE, Boklund A, Alkhamis M, Perez A, et al. Decisions on control of foot-and-mouth disease informed using model predictions. *Prev Vet Med* (2013) 112(3–4):194–202. doi:10.1016/j.prevetmed.2013.09.003
11. Carpenter TE, Thurmond MC, Bates TW. A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. *J Vet Diagn Invest* (2004) 16(1):11–6. doi:10.1177/104063870401600103
12. Backer JA, Hagenaars TJ, Nodelijk G, van Roermund HJ. Vaccination against foot-and-mouth disease I: epidemiological consequences. *Prev Vet Med* (2012) 107(1–2):27–40. doi:10.1016/j.prevetmed.2012.05.012
13. Tomassen FH, de Koeijer A, Mourits MC, Dekker A, Bouma A, Huirne RB. A decision-tree to optimise control measures during the early stage of a foot-and-mouth disease epidemic. *Prev Vet Med* (2002) 54(4):301–24. doi:10.1016/S0167-5877(02)00053-3
14. Mansley LM, Dunlop PJ, Whiteside SM, Smith RG. Early dissemination of foot-and-mouth disease virus through sheep marketing in February 2001. *Vet Rec* (2003) 153(2):43–50. doi:10.1136/vr.153.2.43
15. Gibbens JC, Sharpe CE, Wilesmith JW, Mansley LM, Michalopoulou E, Ryan JB, et al. Descriptive epidemiology of the 2001 foot-and-mouth

disease epidemic in Great Britain: the first five months. *Vet Rec* (2001) 149(24):729–43.
16. Charleston B, Bankowski BM, Gubbins S, Chase-Topping ME, Schley D, Howey R, et al. Relationship between clinical signs and transmission of an infectious disease and the implications for control. *Science* (2011) 332(6030):726–9. doi:10.1126/science.1199884
17. Orsel K, Bouma A, Dekker A, Stegeman JA, de Jong MC. Foot and mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows. *Prev Vet Med* (2009) 88(2):158–63. doi:10.1016/j.prevetmed.2008.09.001
18. Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* (2007) 25(34):6381–91. doi:10.1016/j.vaccine.2007.06.010
19. Orsel K, de Jong MC, Bouma A, Stegeman JA, Dekker A. The effect of vaccination on foot and mouth disease virus transmission among dairy cows. *Vaccine* (2007) 25(2):327–35. doi:10.1016/j.vaccine.2006.07.030
20. Orsel K, Dekker A, Bouma A, Stegeman JA, de Jong MC. Quantification of foot and mouth disease virus excretion and transmission within groups of lambs with and without vaccination. *Vaccine* (2007) 25(14):2673–9. doi:10.1016/j.vaccine.2006.11.048
21. Orsel K, Dekker A, Bouma A, Stegeman JA, de Jong MC. Vaccination against foot and mouth disease reduces virus transmission in groups of calves. *Vaccine* (2005) 23(41):4887–94. doi:10.1016/j.vaccine.2005.05.014
22. Pacheco JM, Lee KN, Eschbaumer M, Bishop EA, Hartwig EJ, Pauszek SJ, et al. Evaluation of infectivity, virulence and transmission of FDMV field strains of serotypes O and A isolated in 2010 from outbreaks in the Republic of Korea. *PLoS One* (2016) 11(1):e0146445. doi:10.1371/journal.pone.0146445
23. Pacheco JM, Tucker M, Hartwig E, Bishop E, Arzt J, Rodriguez LL. Direct contact transmission of three different foot-and-mouth disease virus strains in swine demonstrates important strain-specific differences. *Vet J* (2012) 193(2):456–63. doi:10.1016/j.tvjl.2012.01.012
24. Parida S, Fleming L, Oh Y, Mahapatra M, Hamblin P, Gloster J, et al. Reduction of foot-and-mouth disease (FMD) virus load in nasal excretions, saliva and exhaled air of vaccinated pigs following direct contact challenge. *Vaccine* (2007) 25(45):7806–17. doi:10.1016/j.vaccine.2007.08.058
25. Quan M, Murphy CM, Zhang Z, Alexandersen S. Determinants of early foot-and-mouth disease virus dynamics in pigs. *J Comp Pathol* (2004) 131(4):294–307. doi:10.1016/j.jcpa.2004.05.002
26. Quan M, Murphy CM, Zhang Z, Durand S, Esteves I, Doel C, et al. Influence of exposure intensity on the efficiency and speed of transmission of Foot-and-mouth disease. *J Comp Pathol* (2009) 140(4):225–37. doi:10.1016/j.jcpa.2008.12.002
27. Eble P, de Koeijer A, Bouma A, Stegeman A, Dekker A. Quantification of within- and between-pen transmission of foot-and-mouth disease virus in pigs. *Vet Res* (2006) 37(5):647–54. doi:10.1051/vetres:2006026
28. Alexandersen S, Quan M, Murphy C, Knight J, Zhang Z. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J Comp Pathol* (2003) 129(4):268–82. doi:10.1016/S0021-9975(03)00045-8
29. Pacheco JM, Mason PW. Evaluation of infectivity and transmission of different Asian foot-and-mouth disease viruses in swine. *J Vet Sci* (2010) 11(2):133–42. doi:10.4142/jvs.2010.11.2.133
30. Stenfeldt C, Pacheco JM, Rodriguez LL, Arzt J. Infection dynamics of foot-and-mouth disease virus in pigs using two novel simulated-natural inoculation methods. *Res Vet Sci* (2014) 96(2):396–405. doi:10.1016/j.rvsc.2014.01.009
31. Stenfeldt C, Pacheco JM, Rodriguez LL, Arzt J. Early events in the pathogenesis of foot-and-mouth disease in pigs; identification of oropharyngeal tonsils as sites of primary and sustained viral replication. *PLoS One* (2014) 9(9):e106859. doi:10.1371/journal.pone.0106859
32. Stenfeldt C, Pacheco JM, Smoliga GR, Bishop E, Pauszek SJ, Hartwig EJ, et al. Detection of foot-and-mouth disease virus RNA and capsid protein in lymphoid tissues of convalescent pigs does not indicate existence of a carrier state. *Transbound Emerg Dis* (2016) 63(2):152–64. doi:10.1111/tbed.12235
33. Pacheco JM, Henry TM, O'Donnell VK, Gregory JB, Mason PW. Role of nonstructural proteins 3A and 3B in host range and pathogenicity of foot-and-mouth disease virus. *J Virol* (2003) 77(24):13017–27. doi:10.1128/JVI.77.24.13017-13027.2003
34. Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, et al. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. *J Am Vet Med Assoc* (2002) 220(11):1636–42. doi:10.2460/javma.2002.220.1636
35. Arzt J, Pacheco JM, Rodriguez LL. The early pathogenesis of foot-and-mouth disease in cattle after aerosol inoculation: identification of the nasopharynx as the primary site of infection. *Vet Pathol* (2010) 47(6):1048–63. doi:10.1177/0300985810372509
36. Pacheco JM, Arzt J, Rodriguez LL. Early events in the pathogenesis of foot-and-mouth disease in cattle after controlled aerosol exposure. *Vet J* (2010) 183(1):46–53. doi:10.1016/j.tvjl.2008.08.023
37. LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, et al. A continuous bovine kidney cell line constitutively expressing bovine alphavbeta6 integrin has increased susceptibility to foot-and-mouth disease virus. *J Clin Microbiol* (2013) 51(6):1714–20. doi:10.1128/JCM.03370-12
38. Swaney LM. A continuous bovine kidney cell line for routine assays of foot-and-mouth disease virus. *Vet Microbiol* (1988) 18(1):1–14. doi:10.1016/0378-1135(88)90111-3
39. Kaplan EL, Meier P. Nonparametric-estimation from incomplete observations. *J Am Stat Assoc* (1958) 53(282):457–81. doi:10.2307/2281868
40. Harrington DP, Fleming TR. A class of rank test procedures for censored survival-data. *Biometrika* (1982) 69(3):553–66. doi:10.1093/biomet/69.3.553
41. R-CoreTeam. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: The R Foundation for Statistical Computing (2016).
42. Sellers RF. Quantitative aspects of the spread of foot and mouth disease. *Vet Bull* (1971) 41(6):431–9.
43. Alexandersen S, Zhang Z, Donaldson AI, Garland AJM. The pathogenesis and diagnosis of foot-and-mouth disease. *J Comp Pathol* (2003) 129(1):1–36. doi:10.1016/S0021-9975(03)00041-0
44. Donaldson AI, Alexandersen S, Sorensen JH, Mikkelsen T. Relative risks of the uncontrollable (airborne) spread of FMD by different species. *Vet Rec* (2001) 148(19):602–4. doi:10.1136/vr.148.19.602
45. Fukai K, Yamada M, Morioka K, Ohashi S, Yoshida K, Kitano R, et al. Dose-dependent responses of pigs infected with foot-and-mouth disease virus O/JPN/2010 by the intranasal and intraoral routes. *Arch Virol* (2015) 160(1):129–39. doi:10.1007/s00705-014-2239-4
46. Stenfeldt C, Diaz-San Segundo F, de Los Santos T, Rodriguez LL, Arzt J. The pathogenesis of foot-and-mouth disease in pigs. *Front Vet Sci* (2016) 3:41. doi:10.3389/fvets.2016.00041

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer LL and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 25 April 2016
doi: 10.3389/fvets.2016.00032

Seroprevalence of Foot-and-Mouth Disease in Susceptible Wildlife in Israel

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Foot-and-mouth disease (FMD) epidemics recur in Israel almost every year. Wild even-toed ungulates are seldom affected during these epidemics. The seroprevalence of FMD in wild ungulates during 2000 and 2005–2013 was estimated using anti-non-structural proteins ELISA. Overall, 209 samples were tested, comprising sera of 120 wild boar (*Sus scrofa lybicus*), 64 mountain gazelles (*Gazella gazella gazella*), 6 water buffaloes (*Bubalus bubalis*), and 19 Persian fallow deer (*Dama dama mesopotamica*). None of the tested animals presented clinical signs of FMD during blood collection. Sixteen samples [7.7% (95% confidence interval ($CI_{95\%}$) = 4.4–12.1%)] were found to be seropositive. Fifteen out of 120 samples (12.5%) from wild boar were seropositive, compared with only 1 out of 89 samples (1.1%) from all other species combined (Fisher's exact test: $p = 0.003$). Most of the positive samples obtained from wild boar [13/15 (86.7%)] were collected during 2007, and analysis was restricted to that year and species only. The seroprevalence of FMD in this species during 2007 was estimated at 54.2% ($CI_{95\%}$ = 32.8–74.5%; $n = 24$). A significant infection cluster, comprising nine seropositive samples collected in three different locations, was identified in the north-eastern part of Israel. These findings indicate that wild boar was affected during the 2007 FMD epidemic, even though wild boar presenting FMD typical clinical signs were not observed during that year. The actual role of wild boar in the spread of FMD virus in this epidemic, however, could not be determined. The negligible seroprevalence of FMD found for all other surveillance years indicates that ongoing circulation of FMD among wildlife in Israel is unlikely. It is concluded that while the role of wildlife species in the dynamics of FMD in Israel is usually limited, there might be occasions, in which wildlife plays a part in the spread of the virus.

Keywords: FMD, wildlife, wild boar, NSP, prevalence

OPEN ACCESS

Edited by:
Andres M. Perez, University of Minnesota, USA

Reviewed by:
Mariana Boadella, SABIOtec Spin-Off SL, Spain
Jose Angel Barasona, University of Castilla – La Mancha, Spain

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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *09 February 2016*
Accepted: *05 April 2016*
Published: *25 April 2016*

Citation:
Elnekave E, King R, van Maanen K, Shilo H, Gelman B, Storm N and Klement E (2016) Seroprevalence of Foot-and-Mouth Disease in Susceptible Wildlife in Israel. Front. Vet. Sci. 3:32. doi: 10.3389/fvets.2016.00032

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease, affecting cloven-hoofed ungulates (1) and causing major economic damage (2). Many wildlife species have been found to be susceptible to FMD infection, such as species of buffalo, deer, and wild boar (*Sus scrofa*) (3). Although wildlife species have been suggested as having contributed to FMD dynamics in several outbreaks (4, 5), their actual role in FMD dynamics was estimated to be of only limited significance (3, 6).

Foot-and-mouth disease epidemics have recurred, apart from in 2010, every year in Israel in the past decade. However, two out of 109 (1.8%) of the outbreaks that occurred during these epidemics affected wildlife: during 2007 in "Ramot Yissakhar" (mainly) in the Lower Galilee (north-eastern part of Israel); and next to the "Tzur Natan" settlement in the Sharon plain (the northern coastal plain of Israel). Both were caused by FMD virus of serotype O, affecting mountain gazelles (*Gazella g. gazella*) and resulting in severe clinical manifestations and even mortality (7, 8). A similar presentation, but with a higher percentage of mortality, was reported following the FMD outbreaks during 1985 among mountain gazelles in "Ramot Yissakhar" and the southern Golan Heights in the north of Israel (9).

Incursions of the FMD virus from surrounding countries into Israel have been previously demonstrated (10, 11). A possible role of wild ungulates in the spread of the disease was suggested, especially through the wild boar and mountain gazelles that are abundant in the northern part of Israel. Wild boar could also play a role in introducing the disease when crossing the borders with the surrounding countries. However, to date, the seroprevalence of FMD among wildlife species in Israel had never been estimated and published in the peer-reviewed literature. We have recently estimated the seroprevalence of FMD in small ruminants (12) and in cattle (Elnekave, personal communication) in Israel. The aim of this study was to expand the knowledge on FMD dynamics in Israel by (i) estimating the seroprevalence of FMD infection among wildlife in Israel and (ii) discussing its importance in the dynamics of FMD in Israel.

MATERIALS AND METHODS

Study Population

Wild even-toed ungulate serum samples were collected by one of the authors (Roni King) during 2000 and 2005–2013. Overall, 244 samples were available, of which 35 samples were of poor quality for laboratory testing (i.e., hemolytic) and were therefore excluded. Consequently, 209 samples were tested, comprising 120 wild boar (*Sus scrofa lybicus*), 64 mountain gazelles, 6 water buffaloes (*Bubalus bubalis*), and 19 Persian fallow deer (*Dama dama mesopotamica*). The number of samples collected from each species and the year are provided in **Table 1**. Samples from mountain gazelle, Persian fallow deer, and water buffalo were collected either from injured wild animals or during immobilization performed to enable translocation of these animals. Samples from wild boar were mostly collected from hunted or severely injured wild animals that were euthanized.

Prevalence Estimation

Presence of antibodies specific to non-structural proteins (NSP) was detected using PrioCHECK® FMD virus NS-blocking ELISA [Prionics Lelystad B.V., The Netherlands (currently owned by Thermo Fisher Scientific, Inc.)]. Tests were performed according to the manufacturer's guidelines (http://www.fao.org/ag/againfo/commissions/docs/Workshop/nakuru_2010/PrioCHECK_FMDV-NS7610440_v1.2.pdf). The percentage of inhibition (PI) of each sample was calculated using the following formula:

In our study, serial testing, previously suggested by Paton et al. (13), was used in order to increase the test specificity. Seropositive samples (i.e., PI $\geq$ 50%) were therefore retested, and only samples found positive in two repeated tests were considered positive. FMD prevalence was thus calculated twice (i) using all positive samples found for the first test and (ii) using only positive results found for both tests.

In order to avoid over-estimation of FMD prevalence, we based the analysis only on the results that were positive in both tests.

Data Analysis

Data obtained for the collected samples comprise the host species, sampling date, approximate location of sample collection, and also sex where possible. Although the age of the animals was not documented properly in all cases, the majority of samples were collected from animals older than 1 year, including all the samples that were eventually found to be seropositive. Data on FMD outbreak occurrence were obtained from the Israeli veterinary services (IVS) annual reports and from reports submitted to the OIE [based on the data published on the World Animal Health Information Database (WAHID)].

Using ArcGIS 10.0 (ESRI, Redlands, CA, USA), the samples' approximate locations and the locations of outbreaks in both domestic species (during 2006–2007) and wildlife (in "Ramot Yissakhar," see above) were mapped. Additionally, the Euclidean distances to the nearest FMD outbreak during 2006–2007 were calculated for wild boar samples collected during 2007. Disease clusters in wild boar collected during 2007 were identified using SatScan™ software (14).

Data were summarized using Microsoft Excel® data spreadsheet. Data analysis was restricted to wild boar samples collected during 2007 (see below). The associations between the different variables and seropositivity were estimated. Fisher's exact tests were performed to assess statistical significance of the association of seroprevalence with discrete variables, and a logistic regression model was fitted for continuous variables. Statistical analysis was performed using WinPEPI™ statistical package (15) and SPSS™ statistics version 21.0 (IBM Corp., Armonk, NY, USA). A significance level of $\alpha = 0.05$ was applied.

RESULTS

None of the sampled animals presented clinical signs of FMD. A total of 17/209 animals [8.1% (95% confidence interval ($CI_{95\%}$) = 4.8–12.7%)] and 16/209 animals [7.7% ($CI_{95\%}$ = 4.4–12.1%)] were found to be seropositive using all positive results from the first test and only positive results on both tests, respectively. Most of the positive samples were collected in the northern part of Israel (**Figure 1**).

Fifteen out of 120 samples (12.5%) collected from wild boar were seropositive, compared to only one out of 89 samples (1.1%) obtained from all other species combined (**Table 1**; Fisher's exact

TABLE 1 | Samples collected from wild ungulate species in Israel during 2000 and 2005–2013.

Species	Collection years [# of samples (# of positive)]											
	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013	Unknown	All
Wild boar (*Sus scrofa lybicus*)	1 (0)	–	1 (0)	24 (13)	7 (0)	46 (0)	8 (0)	9 (0)	15 (2)	8 (0)	1 (0)	120 (15)
Palestine mountain gazelle (*Gazella gazella gazella*)	–	4 (0)	5 (0)	8 (0)	11 (0)	6 (0)	12 (0)	4 (0)	7 (0)	7 (0)	–	64 (0)
Water buffalo (*Bubalus bubalis*)	–	–	–	4 (1)	–	–	–	2 (0)	–	–	–	6 (1)
Persian fallow deer (*Dama dama mesopotamica*)	–	–	–	2 (0)	3 (0)	6 (0)	6 (0)	1 (0)	1 (0)	–	–	19 (0)
Total	1 (0)	4 (0)	6 (0)	38 (14)	21 (0)	58 (0)	26 (0)	16 (0)	23 (2)	15 (0)	1 (0)	209 (16)

The number of samples collected for each year and the number of positive samples, given in brackets, are indicated for each species.

test: $p = 0.003$). Most of the positive samples obtained from wild boar [13/15 (86.7%)] were collected during 2007 (**Table 1**). Therefore, further analysis was restricted to wild boar samples collected during that year.

Thirteen out of 24 samples collected from wild boar during 2007 were positive (**Table 1**), and the FMD seroprevalence in wild boar during 2007 was estimated at 54.2% ($CI_{95\%} = 32.8–74.5\%$). A significant positive association was found between proximity to an outbreak and seropositivity (OR = 2.13, $CI_{95\%} = 1.06–4.27$, p-value = 0.03, logistic regression).

Data on wild boar sex (female/male) were not available for 22 samples, and analysis of this variable was therefore based on a small data set. No significant association of sex with infection was found when only samples collected in 2007 were analyzed ($n = 18$; p-value = 0.304, Fisher's exact test), or when samples collected from all years were analyzed ($n = 98$; p-value = 0.310, Fisher's exact test).

A significant infection cluster (coordinates: 32.612485 N, 35.535678 E; radius = 19.7km; and p-value = 0.002) was detected in wild boar samples collected during 2007. The cluster comprised nine seropositive samples from three different locations adjacent to FMD outbreaks (**Figure 2**).

DISCUSSION

The seroprevalence of FMD in different wildlife species in Israel sampled during 2000 and 2005–2013 is presented for the first time.

Fifty-seven percent and 31% of the samples were collected from wild boar and mountain gazelles, respectively. These two species are significantly more abundant in Israel than the Persian fallow deer and the water buffalo, which have been re-introduced into the wild in restricted locations in Israel. Thus, the present sampling provides a good representation of the wild even-toed ungulates that might play an important role in FMD dynamics in Israel.

Most of the seropositive samples were of wild boar collected during 2007. The seroprevalence in wild boar during this year was estimated at 54.2% ($CI_{95\%} = 32.8–74.5\%$). The infection cluster detected in the north-eastern part of Israel comprised nine positive samples collected from three locations, adjacent to the FMD outbreaks that occurred during 2007, and a positive association was found between the proximity to an FMD outbreak and seropositivity. A similar association between seropositive results and proximity to outbreak centers was reported in Bulgaria, following the FMD epidemic there in 2011 (16). However, a lower seroprevalence (of 6.9 and 11.5%) was estimated in wild boar in Bulgaria and the adjacent area in Turkey, respectively (16, 17). This might indicate of differences in the virus transmission to wildlife during those outbreaks (e.g., higher infectiousness). The high seroprevalence in wild boar in Israel indicates that these animals were probably infected during the 2007 FMD epidemic in Israel, even though none of the sampled animals presented clinical signs of FMD during sampling, and there was no other evidence (i.e., reports on lameness in wild boar or animals displaying poor body condition) that indicated clinical signs of FMD in wild boar during this epidemic. Additionally, seropositive samples were collected only from wild boar older than 1 year, making it possible that these animals had been infected before 2007 and remained seropositive due to the longevity of antibodies to NSP (18). However, this scenario is less likely, as FMD infection had not been detected at all in wildlife in the few years prior to 2007.

The transmission of FMD from wild to domestic even-toed ungulates has been suggested in several studies, such as in antelopes (impala or kudu) infecting cattle in Zimbabwe (5) and the FMD outbreak in Bulgaria, where the index case was a wild boar with clinical signs of FMD (4, 16). Additionally, experimental studies have demonstrated the transmission of several FMD serotypes from wild boar to other wild boar and to domestic pigs, despite the variable levels of clinical presentation in the infected wild boar (19, 20). These findings, combined with the high seroprevalence found in wild boar in Israel during 2007, especially in the north-eastern part of Israel, may suggest that wild boar could have played a role in the disease transmission during that year.

The almost complete absence of seropositive samples in all years, but 2007, indicates that ongoing circulation of FMD virus among wildlife species in Israel is unlikely. This is corroborated by the absence of clinical infections in wildlife in Israel throughout those years (based on the data published on the WAHID interface and in the IVS yearly reports). These results are in accordance with previous studies suggesting that, apart from the African buffalo (*Syncerus caffer*) that was found to be an infective carrier of FMD virus (6), other wildlife species are not capable of carrying the FMD virus for long periods (3, 6).

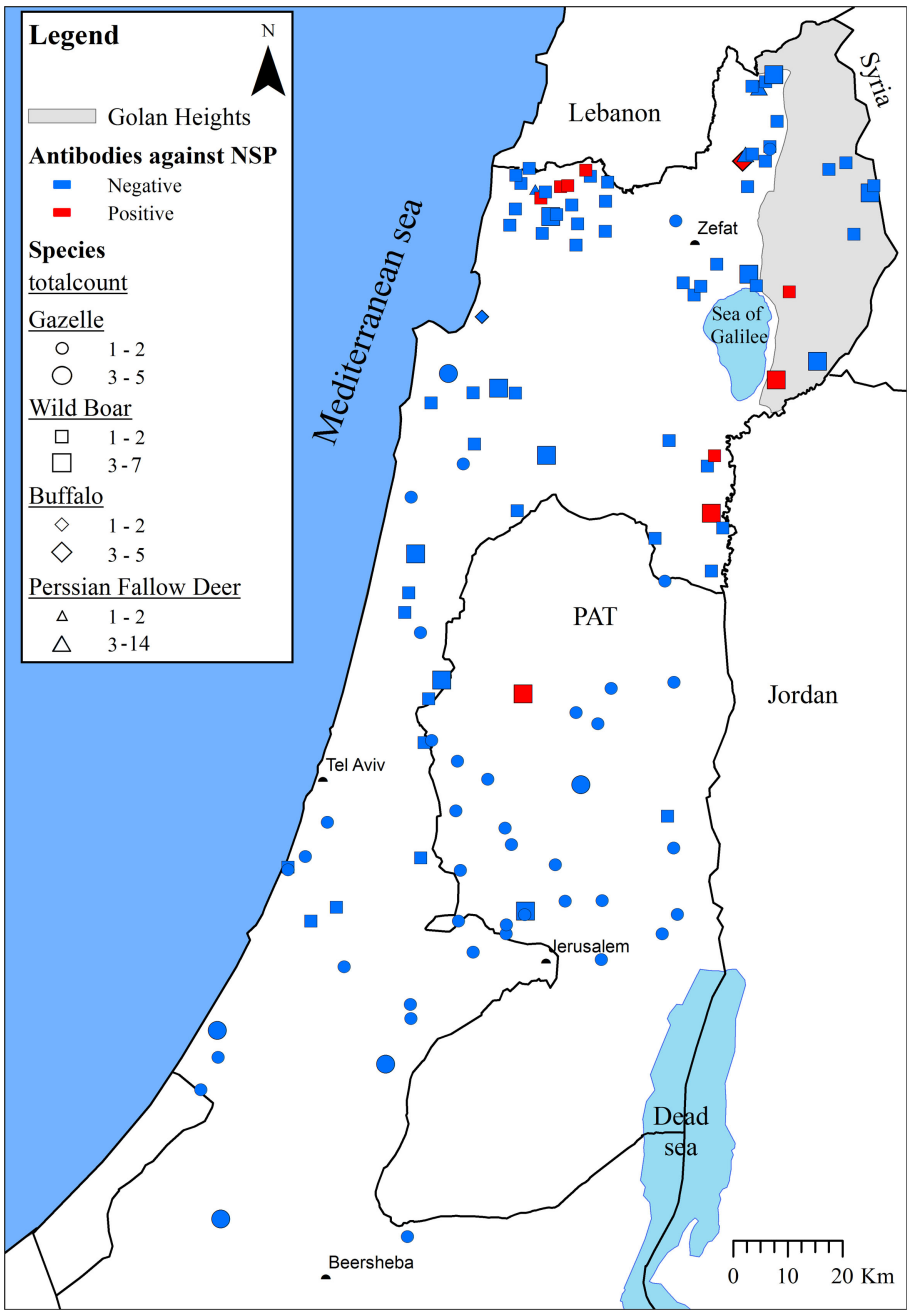

FIGURE 1 | The seroprevalence of foot-and-mouth disease virus in wildlife species in Israel during 2000 and 2005–13. The approximate locations from which samples were collected are indicated. Wildlife species and the number of collected samples are illustrated by different shapes and sizes, respectively. A collection location was considered positive (marked red) if at least one of the species samples was found to be positive. Otherwise, the location was considered negative (marked blue).

While the wild boar population in Israel continues to increase (21), the size of the two main mountain gazelle populations in Israel (i.e., in "Ramot Yissakhar" and southern Golan Heights) has significantly decreased since 1985, especially in the southern Golan Heights (22). This decrease, leading to lower densities of mountain gazelles, can partially explain the rare FMD occurrence in this wildlife species, while adjacent livestock populations are more frequently affected. Morgan et al. (23) demonstrated that small-size wildlife populations will fail to propagate an FMD epidemic. Several additional explanations may also be suggested, such as (i) variability in the virulence of different FMD serotype and subtypes can lead to higher infection and transmission rates of the wildlife species (3, 6); (ii) variability in the susceptibility of different wildlife

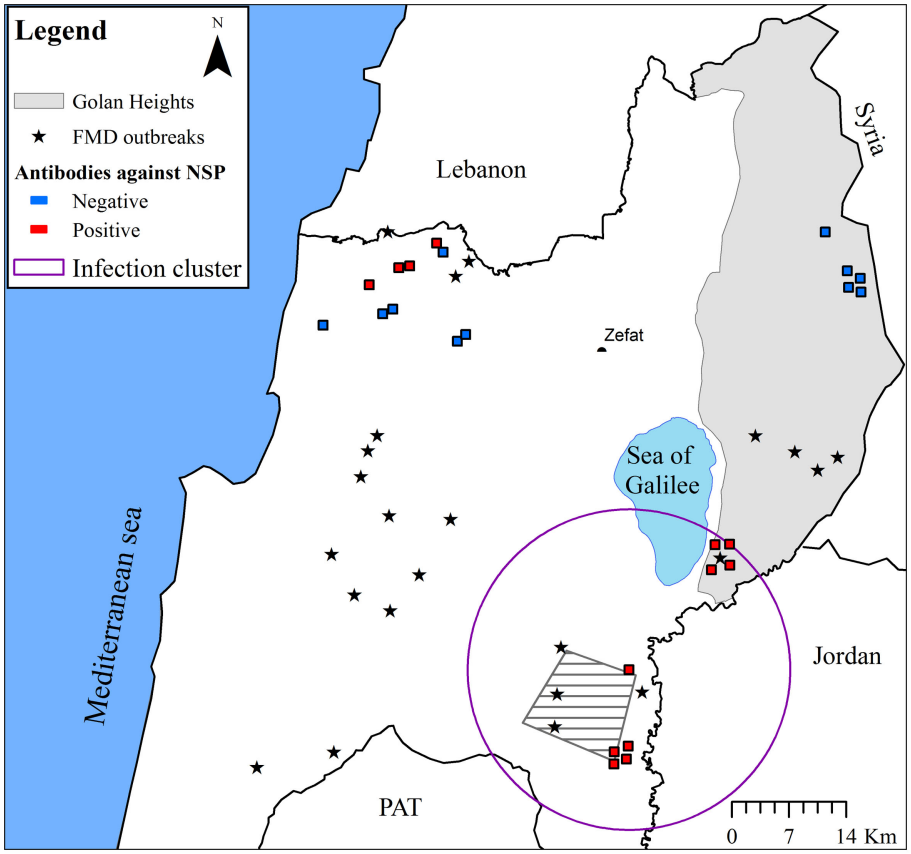

FIGURE 2 | The seroprevalence of FMD in wild boar in Israel during 2007. The approximate locations from which samples were collected are indicated (samples collected from the same location were manually scattered around the location in order to allow better visualization). Positive samples are marked red and negative samples marked blue. FMD outbreak locations (during 2006–2007) are indicated by stars. Additionally, the approximate area of the main mountain gazelle population that was affected by FMD during 2007 ("Ramot Yissakhar") is indicated by a gray polygon filled with diagonal lines. Significant prevalence cluster is indicated by a purple circle.

species to infection (for most species of wild ungulates the susceptibility is unknown) (3, 6); and (iii) fluctuations in the wildlife population densities in certain locations throughout the year (e.g., as a result of food or water abundance) may influence the risk of disease transmission within the population and between wildlife and livestock (6).

CONCLUSION

A negligible seroprevalence of FMD was found in the wildlife in Israel for all surveillance years but 2007. During 2007, wildlife species were clinically and subclinically affected by FMD. These findings indicate that an ongoing circulation of FMD among wildlife in Israel is unlikely, and that the wildlife species' role in the dynamics of FMD in Israel is probably limited during most years. However, in certain years, infected wildlife species might play a role in contributing to the virus dynamics in Israel.

AUTHOR CONTRIBUTIONS

EE (equal contributor) – conception and design, analysis and interpretation of data, drafting of manuscript, critical revision, and statistical analysis. RK (equal contributor) – conception and design, acquisition of data, analysis and interpretation of data, and critical revision. KM – analysis and interpretation of data, and critical revision. HS, BG, and NS – analysis and interpretation of data. EK (advisor) – conception and design, analysis and interpretation of data, critical revision, and statistical analysis.

ACKNOWLEDGMENTS

The research was funded by the Israeli Dairy Board (grant number 705 0057). ELISA kits were kindly donated by the European Commission for the Control of Foot-and-Mouth Disease (EuFMD). Many rangers of the INPA helped in obtaining the samples.

REFERENCES

1. Grubman MJ, Baxt B. Foot-and-mouth disease. *Clin Microbiol Rev* (2004) **17**(2):465–93. doi:10.1128/CMR.17.2.465-493.2004
2. Knight-Jones TJ, Rushton J. The economic impacts of foot and mouth disease – what are they, how big are they and where do they occur? *Prev Vet Med* (2013) **112**(3–4):161–73. doi:10.1016/j.prevetmed.2013.07.013
3. Weaver GV, Domenech J, Thiermann AR, Karesh WB. Foot and mouth disease: a look from the wild side. *J Wildl Dis* (2013) **49**(4):759–85. doi:10.7589/2012-11-276
4. Valdazo-Gonzalez B, Polihronova L, Alexandrov T, Normann P, Knowles NJ, Hammond JM, et al. Reconstruction of the transmission history of RNA virus outbreaks using full genome sequences: foot-and-mouth disease virus in Bulgaria in 2011. *PLoS One* (2012) **7**(11):e49650. doi:10.1371/journal.pone.0049650
5. Hargreaves SK, Foggin CM, Anderson EC, Bastos AD, Thomson GR, Ferris NP, et al. An investigation into the source and spread of foot and mouth disease virus from a wildlife conservancy in Zimbabwe. *Rev Sci Tech* (2004) **23**(3):783–90.
6. Thomson GR, Vosloo W, Bastos AD. Foot and mouth disease in wildlife. *Virus Res* (2003) **91**(1):145–61. doi:10.1016/S0168-1702(02)00263-0
7. OIE. *World Organisation for Animal Health. Foot and Mouth Disease*. Israel (2007). Follow-up Report No. 9. Available from: http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=5094
8. Berkowitz A, Waner T, King R, Yadin H, Perle S. Description of the pathology of a gazelle that died during a major outbreak of foot-and-mouth disease in Israel. *J S Afr Vet Assoc* (2010) **81**(1):62–4. doi:10.4102/jsava.v81i1.99
9. Shimshony A. Foot and mouth disease in the mountain gazelle in Israel. *Rev Sci Tech Off Int Epiz* (1988) **7**:917–23.
10. Stram Y, Engel O, Rubinstein M, Kuznetzova L, Balaish M, Yadin H, et al. Multiple invasions of O1 FMDV serotype into Israel revealed by genetic analysis of VP1 genes of Israeli's isolates from 1989 to 2007. *Vet Microbiol* (2011) **147**(3–4):398–402. doi:10.1016/j.vetmic.2010.07.004
11. Alkhamis MA, Perez AM, Yadin H, Knowles NJ. Temporospatial clustering of foot-and-mouth disease outbreaks in Israel and Palestine, 2006-2007. *Transbound Emerg Dis* (2009) **56**(3):99–107. doi:10.1111/j.1865-1682.2009.01066.x
12. Elnekave E, van Maanen K, Shilo H, Gelman B, Storm N, Berdenstain S, et al. Prevalence and risk factors for foot and mouth disease infection in small ruminants in Israel. *Prev Vet Med* (2016) **125**:82–8. doi:10.1016/j.prevetmed.2015.12.019
13. Paton DJ, de Clercq K, Greiner M, Dekker A, Brocchi E, Bergmann I, et al. Application of non-structural protein antibody tests in substantiating freedom from foot-and-mouth disease virus infection after emergency vaccination of cattle. *Vaccine* (2006) **24**(42–43):6503–12. doi:10.1016/j.vaccine.2006.06.032
14. Kulldorff M, Nagarwalla N. Spatial disease clusters: detection and inference. *Stat Med* (1995) **14**(8):799–810. doi:10.1002/sim.4780140809
15. Abramson JH. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. *Epidemiol Perspect Innov* (2011) **8**(1):1. doi:10.1186/1742-5573-8-1
16. Alexandrov T, Stefanov D, Kamenov P, Miteva A, Khomenko S, Sumption K, et al. Surveillance of foot-and-mouth disease (FMD) in susceptible wildlife and domestic ungulates in Southeast of Bulgaria following a FMD case in wild boar. *Vet Microbiol* (2013) **166**(1–2):84–90. doi:10.1016/j.vetmic.2013.05.016
17. EFSA, AHAW Panel (EFSA Panel on Animal Health and Welfare). Scientific opinion on foot-and-mouth disease in Thrace. *EFSA J* (2012) **10**(4):91; 2635. doi:10.2903/j.efsa.2012.2635
18. Elnekave E, Shilo H, Gelman B, Klement E. The longevity of anti NSP antibodies and the sensitivity of a 3ABC ELISA – A 3 years follow up of repeatedly vaccinated dairy cattle infected by foot and mouth disease virus. *Vet Microbiol* (2015) **178**(1–2):14–8. doi:10.1016/j.vetmic.2015.04.003
19. Mohamed F, Swafford S, Petrowski H, Bracht A, Schmit B, Fabian A, et al. Foot-and-mouth disease in feral swine: susceptibility and transmission. *Transbound Emerg Dis* (2011) **58**(4):358–71. doi:10.1111/j.1865-1682.2011.01213.x
20. Breithaupt A, Depner K, Haas B, Alexandrov T, Polihronova L, Georgiev G, et al. Experimental infection of wild boar and domestic pigs with a foot and mouth disease virus strain detected in the southeast of Bulgaria in December of 2010. *Vet Microbiol* (2012) **159**(1–2):33–9. doi:10.1016/j.vetmic.2012.03.021
21. Mendelssohn H, Yom-Tov Y. A report of birds and mammals which have increased their distribution and abundance in Israel due to human activity. *Isr J Zool* (1999) **45**(1):35–47.
22. Kaplan D. Long-term mountain gazelle (*Gazella gazella gazella*) population fluctuations in Northern Israel. *Z Jagdwiss* (2002) **48**(1):167–71. doi:10.1007/BF02192405
23. Morgan E, Lundervold M, Medley G, Shaikenov B, Torgerson P, Milner-Gulland E. Assessing risks of disease transmission between wildlife and livestock: the Saiga antelope as a case study. *Biol Conserv* (2006) **131**(2):244–54. doi:10.1016/j.biocon.2006.04.012

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

REVIEW
published: 30 November 2016
doi: 10.3389/fvets.2016.00102

Challenges of Generating and Maintaining Protective Vaccine-Induced Immune Responses for Foot-and-Mouth Disease Virus in Pigs

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Vaccination can play a central role in the control of outbreaks of foot-and-mouth disease (FMD) by reducing both the impact of clinical disease and the extent of virus transmission between susceptible animals. Recent incursions of exotic FMD virus lineages into several East Asian countries have highlighted the difficulties of generating and maintaining an adequate immune response in vaccinated pigs. Factors that impact vaccine performance include (i) the potency, antigenic payload, and formulation of a vaccine; (ii) the antigenic match between the vaccine and the heterologous circulating field strain; and (iii) the regime (timing, frequency, and herd-level coverage) used to administer the vaccine. This review collates data from studies that have evaluated the performance of foot-and-mouth disease virus vaccines at the individual and population level in pigs and identifies research priorities that could provide new insights to improve vaccination in the future.

Keywords: foot-and-mouth disease, pigs, vaccination, immunity

OPEN ACCESS

Edited by:
Andres M. Perez, University of Minnesota, USA

Reviewed by:
Danilo Bucafusco, National Agricultural Technology Institute, Argentina
Carolina Stenfeldt, Agricultural Research Service, United States Department of Agriculture, USA

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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *02 September 2016*
Accepted: *01 November 2016*
Published: *30 November 2016*

Citation:
Lyons NA, Lyoo YS, King DP and Paton DJ (2016) Challenges of Generating and Maintaining Protective Vaccine-Induced Immune Responses for Foot-and-Mouth Disease Virus in Pigs. Front. Vet. Sci. 3:102. doi: 10.3389/fvets.2016.00102

INTRODUCTION

Foot-and-mouth disease (FMD) is a viral disease of cloven-hooved animals causing severe economic impacts (1). The disease circulates widely in sub-Saharan Africa and Asia, but has been largely eradicated from South America as well as much of the developed world. It is caused by a Picornavirus (FMD virus: FMDV) that exists as seven immunologically distinct serotypes. Global FMD control efforts are focused at reducing the burden of disease, with the longer-term goal to sequentially eliminate the virus from livestock populations. Vaccination can be a highly effective tool to control FMD, especially when it is implemented together with effective zoo-sanitary measures (farm biosecurity and quarantine) and culling of infected animals. During the 1980s, vaccines were used to effectively eradicate FMD from continental Europe (2), and, more recently, FMD control in South America has employed extensive use of vaccination (3).

In attempts to maximize the impact of limited vaccine resources, most FMD control programs emphasize the use of FMDV vaccines in cattle. As a consequence, many of the published studies that evaluate FMDV vaccine performance have also focused exclusively on their use in cattle. However, some countries have large pig populations that are a major target for FMDV vaccination. The impact of FMD in pigs has recently become particularly important in many Asian countries, such as China

and the Republic of Korea, where there have been extensive and sustained FMD outbreaks due to serotype O and A lineages that have emerged from mainland Southeast Asia (4, 5). The continued occurrence of FMD cases in countries that have large pig populations despite extensive vaccination has raised questions about the effectiveness of vaccination in pigs, but published field studies that analyze this issue appear to be lacking. This review highlights the difficulties of FMDV vaccination in pigs at the individual and population level and summarizes the studies that have evaluated the performance of FMDV vaccines in this important domesticated livestock species.

GENERAL CONSIDERATIONS FOR FMD VACCINATION

Types of Vaccines in Commercial Use Today in Pigs

Foot-and-mouth disease vaccines have been produced on a large scale since the 1940s (6) and are currently manufactured by at least 56 commercial and governmental institutions around the world (O Mezzer, Vallée SA, Personal Communication, 2014). In all FMDV susceptible species, there are many types of vaccine available, not just by virtue of the serotypes and strains included, but also the adjuvant (aluminum hydroxide/saponin or oil adjuvants as: oil in water, water in oil, and double water emulsion) and inactivation method (binary ethyleneimine or rarely formaldehyde) (7). For pigs, currently available vaccines are formulated with an oil adjuvant, due to poor immunogenicity with the aqueous equivalents, and contain either killed/inactivated FMD virus or a synthetic viral peptide (8, 9).

Reasons for Vaccine Failure

There are a number of problems with current FMD vaccines that limit their effective use. These include: imperfect antigenic match between the field virus and vaccine strain; variable antigenic payload; antigen instability (principally the 146S virus particles); requirement for a cold-chain; poor adaptation of certain strains for vaccine production; short duration of protection and requirements for repeat boosting; non-sterile immunity with clinically protected animals sometimes becoming infected; high levels of coverage required for herd immunity; and interference by maternally derived antibody (10, 11). Despite these problems, FMD vaccines can play a vital role in disease control and are very widely used, with over two billion doses estimated to be used globally each year (12). The general reasons for vaccination failure have been helpfully summarized by Heininger et al. (13). "Vaccine failure" may be related to the recipient (pig) or the actual vaccine. "Failure to vaccinate" can be due to errors in vaccine use by the user and program-related problems. In the context of porcine FMD vaccines, these key issues are summarized in **Figure 1**.

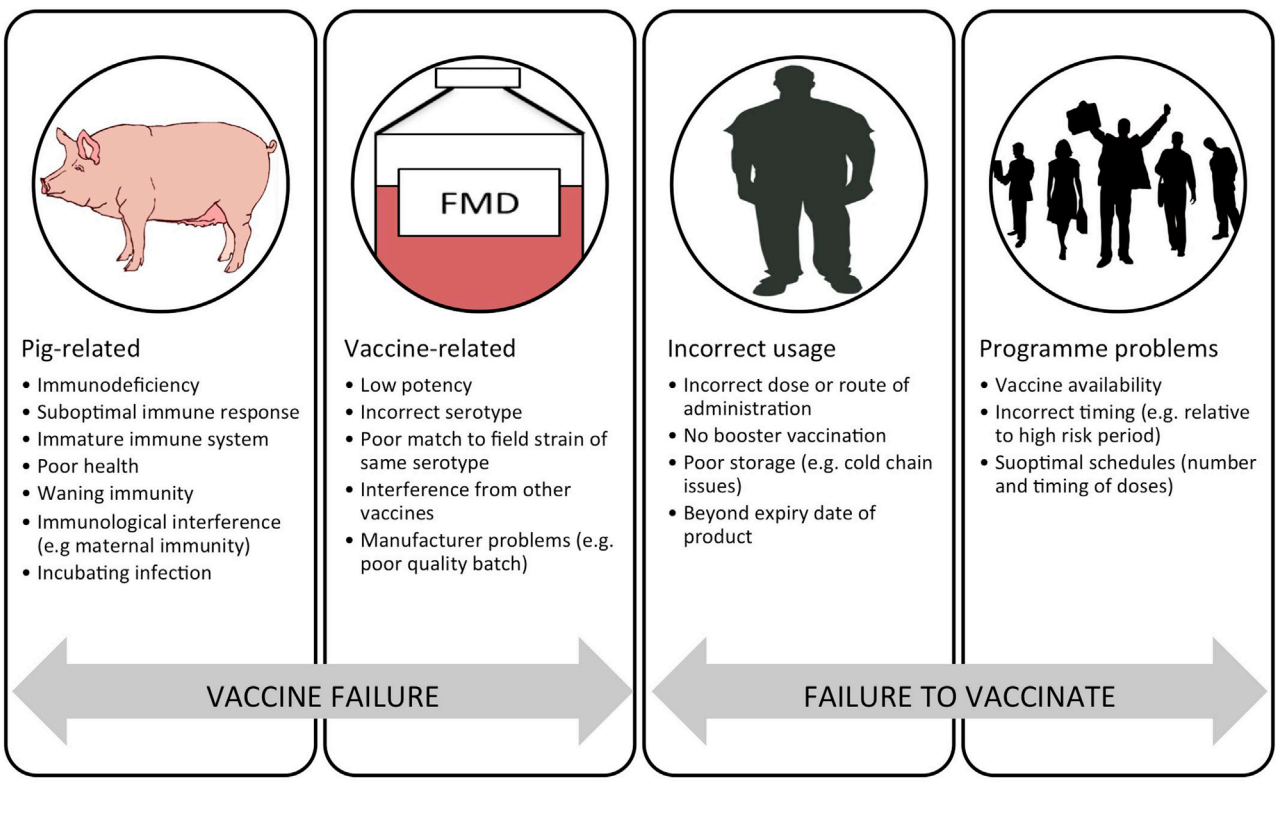

FIGURE 1 | Schematic representation of the reasons for a failure in vaccination divided into "vaccine failure" and "failure to vaccinate." Adapted from Ref. (13).

IMMUNITY AND IMMUNOGENICITY

Comparative interpretation of reports on the evaluation of FMD vaccines are often complicated by significant differences in the potency and other characteristics (e.g., different adjuvants and oil emulsions) of the different vaccines under study, as well as different methods and severity of challenge models (mainly direct or indirect contact with infected unvaccinated or vaccinated donors or intramuscular or intradermal inoculation). The immune responses of pigs to FMD vaccines are less well studied than those of cattle (e.g., details of antibody isotypes, of local immunity, of breadth of antigenic protection, and of the correlation between antibody responses and protection), and there are few field study reports on vaccinated pigs (14). As for cattle and other species, establishing reliable correlates of serological protection for easy interpretation of field studies on vaccine-induced immunity in pigs are hampered by their dependence on specific attributes of the tests, vaccines, and challenge viruses involved.

Immune Response to FMD Vaccines in Pigs

Inactivated oil-adjuvanted FMD vaccines elicit antibody responses in pigs, and the extent of seroconversion measured by virus neutralization and liquid phase blocking ELISA (LPBE) tests can help to predict clinical protection (15–17). Eblé et al. (18) showed that reduced virus shedding was also correlated to neutralizing antibody levels induced by vaccination and that vaccine-induced mucosal IgA was associated with reduced susceptibility to infection. Cox et al. (19) showed that pigs immunized with high-potency vaccines could be protected against challenge 7 months later, associated with sustained levels of neutralizing antibody and a sustained increase in some cytokine levels in serum (IL-6, IL-8, and in some pigs IL-12). Compared to unvaccinated pigs, vaccinated animals that became infected had lower and shorter lived antibody responses to FMDV non-structural proteins (18, 20).

High potency vaccines can protect pigs by ~4 days after vaccination, before the development of appreciable antibodies (21) and, as for cattle, there appears to be a gray zone where the protection afforded by low levels of antibody is unpredictable (17). This suggests that other factors are involved in protective immunity. Systemic levels of some cytokines have been shown to increase following FMD vaccination in pigs (22, 23), and Rigden et al. (24) showed enhanced chemotaxis of cells of the innate immune defenses. Furthermore, the induction of both cellular and humoral arms of the immune system postvaccination has been demonstrated by measuring Th1 [interferon (IFN) gamma] and Th2 (IL-10) responses (25). Zhang et al. (17) studied cell-mediated immunity in 30 vaccinated and 3 unvaccinated pigs given three different doses of vaccine and challenged intramuscularly with 1000 pig ID50 at 28 days post vaccination (dpv). Twenty-five pigs had antibody levels measured by LPBE that could be associated with protection or not (the gray zone). Protection was associated with vaccine-induced increases in cytotoxic T cell numbers and in levels of IFN gamma, IL-12, and IL-15 in serum. Garcia-Briones et al. (26) reported that a recombinant vaccinia virus expressing the FMDV 3D protein could partially protect pigs through a cell-mediated mechanism in the absence of a humoral antibody response to FMDV.

VACCINE POTENCY AND PROTECTION

Potency is defined by the OIE as the "concentration of the immunologically active component" (27). Potency according to this definition is often measured by vaccine manufacturers through the quantification of antigen so that a dose of a vaccine delivers a known antigen "payload." The conventional method of evaluating the effectiveness of FMD vaccines is by experimentally challenging vaccinated and unvaccinated control animals. Although inconsistent with the OIE definition of potency, these evaluations are commonly known as "potency tests." The first of these tests estimates the 50% protective dose (PD_{50}) value and is also the recommended European Pharmacopeia (EP) test. The PD_{50} value is defined as the dose that protects 50% of those under the particular challenge regimen (28). The second OIE-approved test is the "Protection against Podal Generalisation" (PPG) method, which is commonly used in South America.

In the 2009 OIE guidelines, there are descriptions of protocols for calculation of the PD_{50} and PPG based on challenge experiments in pigs which are very similar to those described in cattle. For the PD_{50}, three groups of five pigs, no younger than 2 months of age and free of FMD serum antibody, are given either a full dose, quarter dose, or 1/16th dose. They are challenged 28 days later by intradermal inoculation of 10,000 $TCID_{50}$ of the vaccine strain into one of the heel bulbs of the foot. Two unvaccinated control pigs are included for comparison and to demonstrate a consistent phenotype of the challenge strain. For the PD_{50} test, the main difference with the pig protocol is the route of inoculation, as cattle are challenged *via* the intradermolingual route. A PPG equivalent, whereby 16 animals are challenged after receiving a full dose is also described. These descriptions were not included in the 2015 version of this document that states "In general, a successful test in cattle is considered to be sufficient evidence of the quality of a vaccine to endorse its use in other species. Under circumstances where a vaccine is produced for use primarily in a species other than cattle, it may be more appropriate to potency test the vaccine in that same species" (27). Li et al. (29) have proposed an easier approach to inoculation by challenging intramuscularly behind the ear although a suckling mice passaged strain was needed over a conventional cell passaged version. In China, intramuscular inoculation of 1000 pig ID50 of challenge virus is widely used, as described in studies to evaluate novel vaccines (see section below).

Transmission Studies

There are numerous examples of challenge studies in pigs in the scientific literature to either evaluate the clinical protection afforded by vaccines or their potential role in reducing transmission. Salt et al. (21) evaluated a high potency, oil-based, monovalent serotype C vaccine (strain Oberbayern) by exposing groups of three non-vaccinated or vaccinated pigs to infected animals at 4, 8, 12, 16, and 21 dpv. The challenge virus was homologous to the vaccine strain. Contact was indirect to simulate airborne transmission and looked at both "water-in-oil-in-water" and

"oil-in-water" vaccines. All unvaccinated controls showed generalized disease, but all vaccinated animals were protected from clinical disease. Li et al. (29) reported the findings of a homologous PPG test for a serotype O strain using 16 vaccinated pigs and 3 unvaccinated controls that were challenged intramuscularly behind the ear, 28 dpv. All vaccinated animals were protected from clinical disease, and the authors stated that two of the three controls had to show clinical disease for the test to be valid.

Eblé et al. (30) used challenge studies to estimate the impact of vaccination on transmission within pens in a high containment unit using a serotype O Taiwan strain. The vaccine was a double oil emulsion containing 3 μg of 146S antigen per dose. A single animal in a group of six was challenged by intradermal inoculation in the heel bulb, 7 or 14 dpv. Transmission to the in-contact pigs was evaluated by observing clinical signs, seroconversion to NSP antibodies, and detecting virus in oral swabs and serum. Three of the five contact animals in the 7-day group showed generalized clinical disease compared to none of those in the 14-day group. Additionally, no virus could be detected in the 14-day group providing evidence that vaccination can reduce transmission at 14 dpv in this setting. In contrast, a study performed by Parida et al. (20), evaluated transmission and protection at 10 and 29 dpv. The oil-adjuvanted vaccine used was of high potency ($>18PD_{50}$ based on cattle experiments) and contained the O Manisa strain. Challenge was through exposure by direct contact with pigs with clinical disease caused by the O UKG 34/2001 strain of serotype O. Of animals challenged at 10 dpv, 13/16 (81%) were clinically diseased, while in the 29-day group, 2/8 (25%) were affected. In both groups, disease was reported to be milder and associated with reduced virus shedding compared to the unvaccinated control animals. Similar studies by Orsel et al. (31) aimed to assess transmission from infected, vaccinated pigs that had received O Manisa vaccine 14 days before challenge with O/NET/2001. They showed that vaccinated pigs could transmit infection to other vaccinated pigs as readily as to non-vaccinated controls. However, further work by the same group demonstrated that vaccination was able to reduce the transmission between pens (32). The differences reported in these studies could be attributed to different exposure methods, strains, or the small numbers of animals used.

Challenge studies were also performed to evaluate protection from an O Manisa vaccine to a strain from the O Mya98 lineage (33). Vaccines were double oil adjuvanted and $>6.0PD_{50}$ (presumably based on bovine challenge studies although this is not stated). Groups of five pigs were vaccinated and intradermally challenged at either 4 or 7 dpv. A non-vaccinated control group of five animals was also challenged for comparison. All control animals showed generalized disease. Four out of five (80%) animals challenged at 7 dpv were protected compared with three (60%) animals challenged at 4 dpv indicating animals may be protected soon after vaccination. Virus shedding was significantly lower in vaccinated animals compared to controls. Each group was in indirect contact (not physical but shared air handling unit) with five unvaccinated pigs to assess transmission in a controlled environment. No clinical signs or seroconversion was seen in pigs that were in contact with the vaccinated groups despite live virus being detected in the blood. This is in contrast to pigs that were in contact with the unvaccinated control animals although a breach in biosecurity may have explained this contrast. A similar study was performed by the same group using a serotype A Malaysia 97 vaccine and a serotype A/ASIA/Sea-97 lineage challenge strain (relationship value, r_1, around 0.5). Protection from generalized clinical disease was seen in all animals vaccinated 4 and 7 days pre challenge. No disease, FMD antibodies, or live virus was seen in the contact groups, although some animals in contact with the 4-day group were PCR positive on nasal swab (34).

In response to an FMD epidemic in Southeast Asia where there was only a moderate match between field and O Manisa vaccine strains (r_1 around 0.3), Park et al. (35) performed homologous and heterologous challenge studies to evaluate a new vaccine seed strain (O/Andon/SKR/2010). Groups of five, FMD antibody-free, 3-month-old pigs received one of three different antigen payloads (7.5, 10, and 15 μg) in an oil-adjuvanted vaccine and were intradermally challenged 30 dpv with the homologous O/Andon/SKR/2010 strain. Two placebo injected pigs were challenged for comparison. All vaccinated animals were protected from clinical disease, ignoring any lesions seen at the inoculation site. Both control animals had generalized disease. The 10 μg group was subsequently challenged with a heterologous strain of the ME-SA topotype (r_1 value around 0.5), and all animals were protected from clinical disease.

Challenge studies like those described can provide useful information on the potential role of vaccines in FMD control. There is evidence that protection may occur as early as 4 days, and vaccination may reduce transmission. Great care must be taken when extrapolating such results to a population level due to several factors including: variability in effective contact rates and virus shedding (quantity and duration) in the field; exposure routes and doses that have unclear relevance to field conditions; small sample sizes leading to uncertainty in the results from random error; and likely reduced responses to vaccination under program conditions. Therefore, these studies should be complimented by field-based epidemiological studies. Nowadays, decision making on how and when to use vaccination is greatly influenced by simulation studies with computer models. Unfortunately, it is not yet clear how to parameterize such models to make use of the results of potency tests.

VACCINATION PROGRAMMES

The level of immunity required to control disease at a population level depends in large part on the basic reproduction number (R_0) defined as the average number of secondary cases for each primary case in a completely susceptible population. The "effective" reproduction number is the same calculation but in a population with a proportion of immune individuals. If the effective reproduction number is less than one, on average, the circulation of infection will tend to reduce and ultimately cease. On this basis, the herd immunity required to bring the reproduction number to this level (called the "herd immunity threshold" or HIT) can be calculated by

$$\mathrm{HIT} = 1 - \frac{1}{R_0}$$

The basic reproduction number depends on the effective contact rate (i.e., contact between individuals sufficient for transmission per unit time, also known as the transmission parameter), duration of infectiousness, and population size (36). It is possible to estimate the duration of infectiousness from transmission studies although there is likely variation between viral strains and hosts (37). The effective contact rate is likely to be variable depending on environmental factors such as population or stocking density, production systems, season, and nature of any biosecurity practices. There is also the added complexity of population structures and the consideration of transmission at both the within and between herd level (38). R_0 and the HIT can be estimated using mathematical models, although these should be parameterized as much as possible from field data and tailored to a specific country or region. Small-scale transmission studies can be used to parameterize models, but these should be validated from field-derived data to give greater confidence in model predictions.

The HIT is useful in giving a theoretical target for vaccination coverage (39). In pigs, maintaining sufficient population immunity through vaccination for FMD is a major challenge. Virus transmissibility is potentially high due to higher levels of virus excretion in this species (40), the intensive nature of modern pig production, and a rapid population turnover (particularly in fattening pigs typically slaughtered at 6–7 months old). Additionally, maternal antibodies interfere with the response to vaccines, and there is need for repeated doses of vaccine (discussed in detail in the following section). In some sub-populations with a high transmission risk, a relatively higher vaccination coverage is likely to be required making the case for risk-based vaccination targeting areas of high transmission identified using repeatable epidemiological methods.

Vaccination Regimes

Table 1 gives two proposed schedules for FMD vaccination in pigs both of which acknowledge the potential impact of maternally derived antibodies (MDA). Experiments have tried to address the issue of MDA interference with vaccination. Francis and Black (41) found that pigs as young as 1 week of age could mount a neutralizing antibody response to vaccine in the absence of MDA. They compared these responses to piglets with MDA from vaccinated sows and found that piglets aged between 1–4 weeks did not show any response with antibodies continuing to decline. An increase was seen in piglets vaccinated at 8 weeks old but was lower in the presence of higher levels of MDA. A recent study by Dekker et al. (42) assessed the serological response to vaccination in piglets at different ages (3–9 weeks) in the presence of MDA. Based on receiving a single dose and neutralizing titers 6 weeks post vaccination, the authors found that vaccination at 7–9 weeks old was optimal. Increases in titers were seen in all age categories although the responses were heavily dependent on the MDA level, which in turn was heavily influenced by the titer in the sow.

Two published studies from Taiwan have attempted to establish the optimal times and schedules for vaccination in pigs using field-derived serological evidence. Chung et al. (43) performed serological surveys as part of an active surveillance strategy on commercial pig farms with a herd size ≥5000. Farms were using an oil-based, $>6.0PD_{50}$ serotype O vaccine. Two dose primary course schedules of 8 and 12 weeks, 10 and 14 weeks, and 12 and 16 weeks were compared through homologous neutralization tests on sera from 97 farms. This suggested that animals vaccinated at 12 and 16 weeks of age had the highest titers and there were significant differences between the vaccine products. This analysis was univariable and did not account for possible confounders and the time between vaccination and sampling. Liao et al. (44) performed a study whereby groups of between 6 and 15 piglets were vaccinated between 2 and 16 weeks old (some groups receiving a booster 4 weeks later). Based on neutralizing titers and homologous challenge studies, both performed at 24 weeks old, the authors suggested the optimum time for the first dose to be 8 weeks of age and titers were not significantly different if the piglet received a second dose at 12 weeks of age. This latter evidence for not needing a second dose based on antibody titers is contrary to the suggested schedules in **Table 1**.

Routes of Administration

Both of the vaccines listed in **Table 1** are licensed for intramuscular administration in the neck region. Granulomas have been reported to occur in pigs at injection sites post vaccination with water-in-oil adjuvants (45). Although according to McKercher and Gailiunas (45) these were barely visible 6–12 months after vaccination, this could still be a problem in fattening pigs

TABLE 1 | Recommended schedules for commercially available oil-adjuvanted FMD vaccines licensed for use in pigs.

Product/ Company	Schedules	Source
AFTOPOR (Merial Animal Health)	Once at 2.5 mo (if sporadic FMD cases in area) Twice at 2 and 3 mo (Epizootics or highly virulent strain) >2 wo if unvaccinated herd	"Guidance for Foot and Mouth Disease Vaccination," Merial Animal Health Limited
DECIVAC (MSD Animal Health)	Young animals with no maternal antibodies: primary dose >2 wo, second dose 6 weeks later in endemic areas. Revaccination 4–6 months later Young animals with maternal antibodies: primary dose 4–8 wo onward, second dose 6 weeks later in endemic areas, with revaccination 4–6 months later Adults: every 6 months	http://www.msd-animal-health.ph/products/131_118551/ProductDetails_131_118625.aspx

Based on manufacturers listed at http://www.cfsph.iastate.edu/Vaccines/ (accessed August 9, 2016), where the company website states the schedule in English language and specifically for pigs. Both vaccines are licensed for intramuscular injection in the neck region.
wo, weeks old; mo, months old.

slaughtered at 6–7 months of age where the neck region can have significant value. Such lesions have been reported to occur in 15–20% of pigs but could be easily removed by dissection at slaughter (46). Basarab et al. (47) reported 5/32 (16%) pig carcases had large residual lesions after using a water-in-oil emulsion FMD vaccine requiring extensive dissection. These animals were vaccinated as weaners and the lesions were present at the end of the fattening period although the exact length of time between vaccination and slaughter is not reported. This same study found that intraperitoneal vaccination was equally efficacious to pigs vaccinated intramuscularly based on challenge studies but without the local tissue reaction. An experimental study by Eblé et al. (48) demonstrated that intradermal vaccination at 1/10th the dose of a normal killed vaccine was equally as effective based on challenge studies and neutralizing titers. The small numbers of animals in both of these studies may mean they were statistically underpowered, although both intraperitoneal and intradermal vaccination may offer significant advantages by reducing tissue lesions in fattening animals.

The issue of injection-site granulomas post vaccination has been particularly highlighted in the Republic of Korea and has been proposed as an important factor that has contributed to a reduced uptake of vaccination that has compromised coverage. As an example, a recent unpublished survey of 470 fattening cross-bred pigs from four commercial farms found visually observable lesions in 87, 80, and 80% at 1, 2, and 3 months post vaccination, respectively. These were visible in live pigs, and all had received a two-dose primary course with the first dose given at 6–8 weeks of age and the second dose 2 weeks later. The injection site was in the neck approximately 2.5 cm caudal to the base of the ear. A subset of animals were slaughtered to demonstrate the gross pathology present as shown in **Figure 2**.

Although maintaining effective levels of coverage are challenging, a good understanding of the epidemiology will inform targeted vaccination strategies and more effective use of resources. The optimal vaccination schedules will vary depending on the antibody levels in the sow, which in turn will depend on vaccine type and schedules, natural exposure, and other sow- or piglet-related factors. Therefore, it is clear that countries embarking on vaccination programs should perform their own studies to establish optimal vaccination strategies as also suggested by Dekker et al. (42).

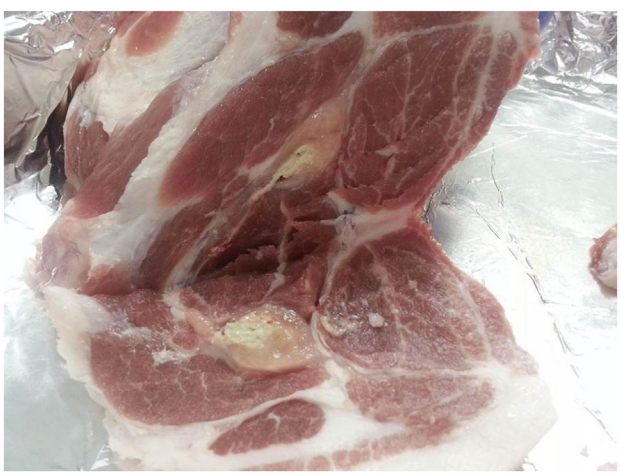

FIGURE 2 | Gross pathology lesion of an injection-site granuloma in the neck region of a pig from the Republic of Korea.

NOVEL APPROACHES TO VACCINES AND VACCINATION

Recent years have seen encouraging results with novel FMD vaccines and adjuvants. Those tested in pigs are considered briefly in this review and **Table 2** summarizes some of the most promising challenge studies. Peptide vaccines for type O FMDV have been used in China for vaccination of pigs and continue to be improved. More data are needed on the breadth of cross-protection afforded by these vaccines against heterologous virus strains of the same serotype as used for peptide design. New vaccines have been designed, modified, and evaluated based upon FMD virus-like particles (VLP) generated *in vitro* or in the vaccinated pig through expression by virus vectors, especially adenoviruses. Specific methods of attenuating live FMDV now show considerable promise for overcoming the problem of combining inocuity with immunogenicity and can provide protection within 2 days. Data on duration of protection are awaited. IFNs and IFN inducers can not only provide extremely rapid and serotype non-specific protection against FMDV but they can also enhance the protection afforded by specific FMDV antigens and reduce the doses of adenovirus-vectored vaccines required for protection. New adjuvants have mostly been tested as additional incipients for oil-based vaccines and properly controlled and powered comparative studies of different adjuvants have not been published. There have been few recent studies of mucosal vaccine targeting or to evaluate DNA vaccines.

Adenovirus-Vectored Vaccines

Adenovirus-vectored FMD vaccines conditionally licensed in the USA in 2012 for use in cattle, have also shown efficacy in pigs. A replication-defective human serotype 5 adenovirus expressing the capsid encoding genes and the 3C protease needed for their cleavage and incorporating genetic material from the A24 strain of FMDV was given to pigs at a dose of 5×10^9 pfu, resulting in complete clinical protection against homologous FMDV by contact challenge at 7, 14, and 42 dpv (56). It was later shown that a modified vector insert also expressing the FMDV 2B gene improved the early antibody response to the FMDV capsid (57).

The same adenovirus vector when administered at high doses can deliver IFNs to provide early protection against FMDV and types I, II, and III IFN given this way can all provide protection to pigs for up to 5 days with evidence of synergistic action between different IFN types [reviewed by Stenfeldt et al. (58)]. Patch et al. (59) explored the possibility of selecting for a cytotoxic T cell response to FMDV in pigs vaccinated with an adenovirus expressing an inefficiently cleaved capsid precursor, but the protective value of this was not reported.

TABLE 2 | Selected pig challenge study results with promising outcomes for novel vaccines.

Vaccine	Vaccination[a]	Challenge	Protection	Reference
Live FMDV A12 attenuated by L^{pro} mutation (A12-SAP)	15 pigs vaccinated with 10^5, 10^6, or 10^7 pfu A12-SAP by subcutaneous injection	Intradermal heel bulb inoculation with 10^5 FMDV A12 at 21 dpv	All 15 pigs protected against clinical signs (fever or vesicles), viremia, and nasal shedding	(49)
	9 pigs vaccinated with 10^6 pfu A12-SAP by subcutaneous injection	Intradermal heel bulb inoculation with 5×10^5 FMDV A12 at 2, 7, or 14 dpv	8 of 9 pigs protected against clinical signs	
Adenovirus vector expressing FMDV A24 P1-2A, 2B, 3B, 3C with Poly ICLC adjuvant in PBS	6 pigs vaccinated with 2.5×10^6 vector plus 1 mg poly ICLC by subcutaneous injection of 2 ml dose at 2 sites (other vaccination schedules evaluated)	Intradermal heel bulb inoculation with 10^5 FMDV A24 at 7 or 21 dpv	All 3 pigs challenged at 21 dpv protected against clinical signs, viremia, and nasal shedding (partial protection when challenged at 7 dpv)	(50)
Adenovirus vectors, one expressing porcine alpha and gamma interferons and the other expressing 3 small interfering RNAs	15 minipigs vaccinated with 7.2×10^9 or $1.75\ 10^{10}$ $TCID_{50}$ of a combination of the adenovirus vectors (1:5 ratio of Ad-IFN titer to Ad-3siRNA titer) by intramuscular injection (other vaccination schedules evaluated)	Direct contact of 5 groups of 3 "vaccinated" minipigs at 2, 4, and 7 dpv, for 18 h with donor minipigs infected with FMDV strain O/Andong/SKR/2010	At the low "vaccine" dose, complete clinical protection in 2/3, 1/3, and 0/3 minipigs at 2, 4, and 7 dpv. At the high "vaccine" dose it was 3/3 and 1/3 at 4 and 7 dpv. Viremia and oral shedding also reduced or prevented in some minipigs	(51)
FMDV multi-epitope (B and T cell) from 4 FMDV O topotype viruses with poly IC adjuvant. VP1 epitopes from O/Mya/98, O/HN/CHA/09, O/Tibet/99, O/IRN/2010. Two universal (non-FMDV) T cell epitopes	45 pigs vaccinated in three groups of 15 pigs, each group consisting of 3 subgroups of 5 pigs receiving different doses: full (2 ml), 1/3, or 1/9 dose by volume intramuscularly. The full dose contained 300 μg of epitope protein and 300 μg poly IC	Three potency tests involving challenge at 28 dpv by intramuscular inoculation with 1000 50% infectious doses of one of three FMDV O strains: O/Mya/98, O/HN/CHA/93, O/Tibet/99	PD_{50} results were 15.6 (O/Mya/98 challenge), 15.6 (O/HN/CHA/93), and 7.0 (O/Tibet/99)	(52)
Pseudorabies virus expressing P1-2A, 3C from FMDV O/ES/2001 (PRV-P12A3C)	5 pigs vaccinated with 10^6 $TCID_{50}$ PRV-P12A3C in 2 ml by intramuscular injection with identical booster at 21 dpv	1000 50% infectious doses of FMDV O/OR/80 by intramuscular inoculation at 15 days after booster vaccination	3 of 5 vaccinated pigs fully protected against clinical signs	(53)
FMDV Asia1/Jiangsu/China/2005 VLP produced in *E coli* as SUMO-VP0/VP1/VP3 fusion proteins, subsequently purified and cleaved	5 pigs vaccinated with 50 μg VLP in oil adjuvant by intramuscular route	1000 50% infectious doses of FMDV Asia1/Jiangsu/China/2005 by intramuscular inoculation	All 5 vaccinated pigs fully protected against clinical signs	(54)
Dendrimeric B and T cell epitopes from FMDV O/UKG/11/2001	6 pigs vaccinated twice 21 days apart with 2 ml oil adjuvant containing 2 mg peptide by intramuscular route (other related vaccines evaluated)	1.6×10^4 FMDV O/UKG/11/2001 by heel bulb inoculation at 18 days after second vaccination	All 6 vaccinated pigs fully protected against clinical signs and for 5 of 6 pigs no virus shedding detected in pharyngeal or nasal swabs	(55)

[a]*All studies included control mock or unvaccinated pigs, and some studies included comparison with conventional vaccines, but details not given here.*

Kim et al. (51) developed recombinant adenoviruses for the simultaneous expression of porcine alpha and gamma IFNs as well as three small interfering RNAs targeting FMDV mRNAs encoding non-structural proteins. The antiviral effects of these vectors were synergistic in porcine cells, suckling mice, and minipigs. The vectors administered at high dose by the intramuscular route fully protected 3 pigs against an 18-h direct contact challenge 1 day later. Partial protection at challenge 2–4 days after administration was mostly lost at 7 days. *In vitro*, the combination treatment was effective against all serotypes of FMDV.

Other Vectored Vaccines

Canine adenovirus type 2 expressing VP1 elicited low levels of FMDV neutralizing antibody in pigs (60). A recombinant pseudorabies virus expressing the capsid and 3C encoding genes of FMDV serotype O partially protected (3 of 5) pigs against an intramuscular challenge with 1000 ID_{50} of a heterologous live type O FMDV [(53); **Table 2**]. An earlier pseudorabies virus vector expressing only VP1 of FMDV was less effective (61).

Yang et al. (62) reported the insertion of VP1 T and B cell epitopes of FMDV serotype O into a bamboo mosaic virus (BMV), resulting in expression of a fusion protein. Pigs inoculated intramuscularly with 5–10 mg of the recombinant BMV in a mineral oil adjuvant produced VP1-specific cell-mediated immunity and neutralizing antibodies. The protection of pigs against challenge with live FMDV was described after a double dose of the recombinant BMV, and protection was said to be possible after one dose.

Recombinant baculoviruses were used by Crisci et al. (63) to generate chimeric virus-like particles of rabbit haemmorhagic

disease virus fused to a FMDV T cell epitope from the 3A viral non-structural protein. Intramuscular inoculation of pigs with this chimera and an oil adjuvant generated FMDV-specific cell-mediated immunity and antibodies.

Interferons

Polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine and carboxymethyl cellulose (poly ICLC) is a synthetic double-stranded RNA (dsRNA) that is a viral mimic and activates multiple innate immune pathways through interaction with toll-like receptor 3 and MDA-5. It is a potent inducer of IFNs and can protect against FMD at 1 day after treatment (64). Its adjuvant affect on FMD vaccines in pigs was reported 40 years ago (65). Recently, it was shown to reduce, by 80-fold, the dose required for protection of a recombinant adenovirus expressing FMDV A24 capsids [(50); **Table 2**]. Another synthetic analog of dsRNA, polyinosinic-polycytidylic acid (poly IC), potentiated the protection afforded by a multi-epitope vaccine in pigs (66). This vaccine incorporated linked B cell epitopes (the G–H loop and C terminus of VP1) from four topotypes of serotype O flanked by two universal T cell epitopes. The final product in an oil adjuvant with poly IC protected pigs with 50% protection values of 7–16 against different challenge viruses [(52); **Table 2**].

Other Adjuvants

Barrette et al. (67) showed that intranasal immunization of pigs with detoxified *Escherichia coli* enterotoxins LTK63 and LTR72 linked to a peptide derived from the FMDV serotype O1-BFS VP1 G-H loop enhanced the antigen-specific mucosal and systemic immune responses to FMDV. Guo et al. (68) reported that a CpG-enriched plasmid enhanced the efficacy of a conventional FMD killed vaccine. Park et al. (35, 69) vaccinated groups of five pigs with a conventional FMD vaccine antigen plus either the oil adjuvant used in the Republic of Korea or with novel adjuvants (Carbigen, Emulsigen-D and ISA 201). In terms of immune response and post-challenge protection, the novel antigens were at least as good.

In a small field trial, administering 60 mg of poly gamma glutamic acid (PGA) 3 days before FMDV vaccination of young pigs resulted in slightly more animals with detectable levels of FMDV antibodies 2–6 weeks later (70). Li et al. (71) reported increased antibody responses of pigs to a conventional FMDV vaccine supplemented with ginseng stem and leaf saponins. Xiao et al. (72) showed that an extract of the seeds of *Momordica cochinchinensis* (Lour.) Spreng. (ECMS) had a synergistic effect in improving the immune response of pigs after vaccination with inactivated FMDV antigens in an oil emulsion vaccine.

Live Attenuated Vaccines

Deleting the Lpro gene of FMDV A12 gave rise to an attenuated virus that partially protected pigs against wild-type challenge (73). Meanwhile, FMDV A24 lacking Lpro but with a capsid substituted from serotype O was still somewhat virulent for pigs. Changing the capsid genes to those of a cell culture adapted virus eliminated the virulence, but the resulting virus did not protect pigs when used as a vaccine (74). In contrast, mutating a conserved protein domain within the Lpro gene of FMDV A12 gave rise to a virus that was avirulent in pigs at a dose of 10^7 but nevertheless elicited protection against FMDV challenge from 2 dpv (49).

Codon bias deoptimization of the FMDV capsid-coding region (P1) introduced 489 nucleotide changes (19%) but retained virus viability. The vaccine safety margin was ~1000-fold higher for pigs than for wild-type virus. Consistently, high levels of antibody titers were induced, even at the lowest dose tested (75).

Protein/Peptide Vaccines

Shao et al. (76) reported on the further development of a tandem repeat multiple-epitope recombinant vaccine against FMDV serotype O containing three copies of two VP1 epitopes of the O/China/99 strain of FMDV coupled with a porcine IgG heavy-chain constant region (77). This peptide vaccine elicited high titers of FMDV specific antibodies in pigs at 30 dpv and conferred complete protection against a challenge with 1000 50% infective doses of the O/China/99 strain. Trials of another B cell epitope vaccine (52) have already been described above under IFNs (**Table 2**). Dong et al. (78) inserted the coding sequences of a FMDV serotype O VP1 epitope into a coliphage, resulting in an epitope-phage recombinant protein that formed a virus-like particle (VLP). Challenge inoculation of twice vaccinated pigs with the live homologous virus resulted in three of five animals being clinically protected from FMD.

Building upon earlier work (79, 80), Blanco et al. (55) reported that a synthetic dendrimeric peptide vaccine comprising two copies of a FMDV VP1 B cell epitope linked to a FMDV 3A T cell epitope protected pigs against disease and virus shedding after two doses of vaccination followed by challenge inoculation with live homologous FMDV O UK 2001 (**Table 2**). Guo et al. (54) have developed a bacterial expression system to generate VLPs of the FMDV Asia 1 capsid proteins. The FMDV genes VP0, VP1, and VP3 were each expressed as fusion products with the small ubiquitin like modifier protein (SUMO) and after removal of the SUMO moiety, the FMDV proteins assembled into VLPs. Five pigs vaccinated with 50 μg of VLP emulsified in oil adjuvant were fully protected from challenge inoculation with live homologous FMDV (**Table 2**).

DNA Vaccines

DNA vaccines have not been completely effective in livestock despite promising results in mice. Multiple doses of plasmids expressing FMDV proteins or epitopes with coexpression of immunostimulants, and/or with conventional antigen boosters have been required to protect pigs against FMD (81–83). Most recently, Borrego et al. (84) reported partial protection of pigs after three immunizations with a DNA vaccine encoding FMDV B and T cell epitopes fused to the variable fragment of a mouse immunoglobulin against Class II swine leukocyte antigens.

Mucosal Vaccines

Although mucosal IgA may be elicited by parenteral immunization routes [e.g., Ref. (80)], mucosal vaccination might help to block FMDV entry. Barrette et al. (67) evaluated detoxified *Escherichia coli* enterotoxins LTK63 and LTR72 as mucosal adjuvants showing enhanced antigen-specific mucosal and systemic immunity for non-replicating antigens, including FMDV, upon intranasal

immunization in pigs. Song et al. (85) reported vaccination of pigs with a recombinant VP1 epitope complex of serotype O FMDV fused to the cholera toxin B subunit (hCTB). Eight of ten pigs that were given three intraperitoneal immunizations were protected from challenge by inoculation with 106.5 $TCID_{50}$ type O FMDV. Wang et al. (86) showed that intranasal delivery of cationic PLGA nano/microparticles loaded with various FMDV DNA vaccine formulations encoding IL-6 as a molecular adjuvant enhanced protective immunity against FMDV, particularly pc-IL2AP12A3C with the IL-6 gene located before the P12A3C gene. Nevertheless, only partial protection against challenge with FMDV was achieved in pigs.

Chimeric Killed Vaccines

Blignaut et al. (87) produced a killed vaccine from a chimeric virus in which the capsid encoding genes were replaced with those from a different serotype. The resulting SAT 2 FMDV with a SAT 1 capsid were used to make a conventional killed vaccine that was potency tested in 17 pigs (three groups of five pigs given different vaccine doses and two unvaccinated control pigs). After a SAT 1 challenge by heel bulb inoculation, the PD_{50} was found to be >6.4. Zheng et al. (88) substituted the capsid-encoding region of a serotype A virus vaccine for a more recent field isolate to update the antigenic match. The new vaccine was shown to protect against both the homologous strain and another semi-heterologous one.

RESEARCH PRIORITIES

This review summarizes studies that have been undertaken to evaluate the performance of FMD vaccines in pigs, as well as introduce novel vaccination strategies that might be employed for FMD control in the future. Collectively, these data provide a valuable body of evidence that are especially relevant in the parts of the world where pigs play a central role in the maintenance and spread of the virus. Although a number of these experimental studies have evaluated the performance of FMDV vaccines, it is apparent that field data for such evaluation in pigs are currently lacking. Furthermore, much of this work is dependent upon bovine reagents, such as antigenic profiling (vaccine-matching), or exploits *in vitro* measurements of "correlates of protection" derived from cattle studies. In view of this paucity of data, when using vaccines in these settings, it is important to consider the different factors that influence whether, or not, a vaccine is likely to be efficacious. These include the (i) regime used (timing and frequency of vaccination); (ii) potency and formulation of oil vaccines; and (iii) antigenic match between the vaccine and circulating field strain. Although these three points are often assessed (and discussed) separately, they have an intimate relationship that underpins the performance of a vaccine. For example, it is usually accepted that a less than perfect antigenic match can be compensated by administration of a high potency vaccine; however, the impact of vaccine regime (as well as the herd-level coverage) is often ignored. In order to improve vaccine-induced immune responses, additional areas that warrant further scientific investigation include more systematic research to evaluate alternative vaccine adjuvants for vaccination in pigs, and research to validate of alternative routes (IM, IP, SC, ID) and sites of vaccination (to minimize local tissue granulomas in valuable meat cuts) and even multiple sites (with a divided dose). Effective (improved) vaccination regimes are also necessary to generate optimum protection in pigs to accommodate maternal antibody responses (to reduce the immunity gap).

Data from recent field outbreaks in Asia highlight the challenges posed by the control of FMD in pigs. While initiatives to improve the quality of vaccines and coverage that are tailored for pigs have the potential to make a positive impact on FMD control, it should be remembered that vaccination-alone is not a magic panacea and that FMD control, especially in the face of high amounts of circulating virus, is often reliant upon the implementation of effective zoo-sanitary (bio-containment) measures, as well as the maintenance of adequate local veterinary resources so that new clinical cases are rapidly investigated and detected.

AUTHOR CONTRIBUTIONS

NL, DK, and DP wrote extensive sections of the manuscript. NL led the structuring and format of the review. YL provided unique insights and data from the FMD situation in the Republic of Korea (the section of granulomas with image). All the authors read, edited, and approved the final manuscript.

FUNDING

The authors acknowledge the positive contributions made to the discussion forum at the Global Foot-and-Mouth Disease Research Alliance (GFRA) meeting held in Hanoi, Vietnam, in October 2015, and funding from UK Department for Environment, Food and Rural Affairs (Defra) and the European Commission for the control of foot-and-mouth disease (EuFMD). The Pirbright Institute receives grant-aided support from Biotechnology and Biological Sciences Research Council of the United Kingdom (BBS/E/I/00001713). The work of the WRLFMD is supported with funding provided to the EuFMD from the European Union. The views expressed herein can in no way be taken to reflect the official opinion of the European Union.

REFERENCES

1. Knight-Jones TJD, Rushton J. The economic impacts of foot and mouth disease – what are they, how big are they and where do they occur? *Prev Vet Med* (2013) 112:161–73. doi:10.1016/j.prevetmed.2013.07.013
2. Leforban Y, Gerbier G. Review of the status of foot and mouth disease and approach to control/eradication in Europe and Central Asia. *Rev Sci Tech* (2002) 21(3):477–92. doi:10.20506/rst.21.3.1345
3. Clavijo A, Sanchez-Vazquez MJ, Buzanovsky LP, Martini M, Pompei JC, Cosivi O. Current status and future prospects to achieve foot-and-mouth disease eradication in South America. *Transbound Emerg Dis* (2015). doi:10.1111/tbed.12345
4. Knowles NJ, He J, Shang Y, Wadsworth J, Valdazo-González B, Onosato H, et al. Emergence of Southeast Asian foot-and-mouth disease viruses in East Asia. *Emerg Infect Dis* (2012) 18(3):499–501. doi:10.3201/eid1803.110908

5. Valdazo-González B, Timina A, Scherbakov A, Abdul-Hamid NF, Knowles NJ, King DP. Multiple introductions of serotype O foot-and-mouth disease viruses into East Asia in 2010-2011. *Vet Res* (2013) 44:76. doi:10.1186/1297-9716-44-76
6. Barteling SJ. Development and performance of inactivated vaccines against foot and mouth disease. *Rev Sci Tech* (2002) 21:577–88. doi:10.20506/rst.21.3.1361
7. Doel TR. FMD vaccines. *Virus Res* (2003) 91:81–99. doi:10.1016/S0168-1702(02)00261-7
8. Anderson EC, Masters RC, Mowat GN. Immune response of pigs to inactivated foot-and-mouth disease vaccines. Response to DEAE-dextran and saponin adjuvanted vaccines. *Res Vet Sci* (1971) 12:351–7.
9. Qiang Z, Huachun L. FMD vaccines and vaccination in China – production use and quality. *International Conference on Scientific Developments and Technical Challenges in the Progressive Control of FMD in South Asia*; 2011 Feb 13–15; New Delhi, India (2011).
10. Kitching P, Hammond J, Jeggo M, Charleston B, Paton D, Rodriguez L, et al. Global FMD control-Is it an option? *Vaccine* (2007) 25:5660–4. doi:10.1016/j.vaccine.2006.10.052
11. Paton DJ, Sumption KJ, Charleston B. Options for control of foot-and-mouth disease: knowledge, capability and policy. *Philos Trans R Soc Lond B Biol Sci* (2009) 364:2657–67. doi:10.1098/rstb.2009.0100
12. Sumption K. The global control of FMD. *Open Session of the EuFMD Standing Technical Committee; 2008 Oct 14–17*. Erice, Sicily (2008).
13. Heininger U, Bachtiar NS, Bahri P, Dana A, Dodoo A, Gidudu J, et al. The concept of vaccination failure. *Vaccine* (2012) 30:1265–8. doi:10.1016/j.vaccine.2011.12.048
14. Chen SP, Sun YF, Lee MC, Cheng IC, Yang PC, Huang TS, et al. Immune responses to foot-and-mouth disease virus in pig farms after the 1997 outbreak in Taiwan. *Vet Microbiol* (2008) 126:82–90. doi:10.1016/j.vetmic.2007.07.007
15. Black L, Francis MJ, Rweyemamu MM, Umebara O, Boge A. The relationship between serum antibody titres and protection from foot and mouth disease in pigs after oil emulsion vaccination. *J Biol Stand* (1984) 12:379–89. doi:10.1016/S0092-1157(84)80062-1
16. Cox SJ, Gubbins S, Barnett PV. IL-6 production following vaccination in pigs – an additional immune response parameter for assessing FMD vaccine efficacy? *Vaccine* (2011) 29:4704–8. doi:10.1016/j.vaccine.2011.04.100
17. Zhang L, Feng X, Jin Y, Ma J, Cai H, Zhang X. Immunoprotective mechanisms in swine within the "grey zone" in antibody response after immunization with foot-and-mouth disease vaccine. *Virus Res* (2016) 220:39–46. doi:10.1016/j.virusres.2016.04.008
18. Eblé PL, Bouma A, Weerdmeester K, Stegeman JA, Dekker A. Serological and mucosal immune responses after vaccination and infection with FMDV in pigs. *Vaccine* (2007) 25:1043–54. doi:10.1016/j.vaccine.2006.09.066
19. Cox SJ, Aggarwal N, Statham RJ, Barnett PV. Longevity of antibody and cytokine responses following vaccination with high potency emergency FMD vaccines. *Vaccine* (2003) 21:1336–47. doi:10.1016/S0264-410X(02)00691-6
20. Parida S, Fleming L, Oh Y, Mahapatra M, Hamblin P, Gloster J, et al. Reduction of foot-and-mouth disease (FMD) virus load in nasal excretions, saliva and exhaled air of vaccinated pigs following direct contact challenge. *Vaccine* (2007) 25:7806–17. doi:10.1016/j.vaccine.2007.08.058
21. Salt JS, Barnett PV, Dani P, Williams L. Emergency vaccination of pigs against foot-and-mouth disease: protection against disease and reduction in contact transmission. *Vaccine* (1998) 16:746–54. doi:10.1016/S0264-410X(97)86180-4
22. Barnard AL, Arriens A, Cox S, Barnett P, Kristensen B, Summerfield A, et al. Immune response characteristics following emergency vaccination of pigs against foot-and-mouth disease. *Vaccine* (2005) 23:1037–47. doi:10.1016/j.vaccine.2004.07.034
23. Barnett PV, Cox SJ, Aggarwal N, Gerber H, McCullough KC. Further studies on the early protective responses of pigs following immunisation with high potency foot and mouth disease vaccine. *Vaccine* (2002) 20:3197–208. doi:10.1016/S0264-410X(02)00242-6
24. Rigden RC, Carrasco CP, Barnett PV, Summerfield A, McCullough KC. Innate immune responses following emergency vaccination against foot-and-mouth disease virus in pigs. *Vaccine* (2003) 21:1466–77. doi:10.1016/S0264-410X(02)00663-1
25. Eblé PL, de Bruin MGM, Bouma A, van Hemert-Kluitenberg F, Dekker A. Comparison of immune responses after intra-typic heterologous and homologous vaccination against foot-and-mouth disease virus infection in pigs. *Vaccine* (2006) 24:1274–81. doi:10.1016/j.vaccine.2005.09.040
26. Garcia-Briones MM, Blanco E, Chiva C, Andreu D, Ley V, Sobrino F. Immunogenicity and T cell recognition in swine of foot-and-mouth disease virus polymerase 3D. *Virology* (2004) 322:264–75. doi:10.1016/j.virol.2004.01.027
27. World Organisations for Animal Health (OIE). Foot and mouth disease. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Version Adopted in May 2012)*. Paris: OIE (2015). p. 145–73.
28. European Directorate for the Quality of Medicines. Foot-and-mouth disease (ruminants) vaccine (inactivated). *European Pharmacopoeia. Version 5.5. 04/2005:0063*. Strasbourg, France: Council of Europe (2006).
29. Li D, Lu Z-J, Xie B-X, Sun P, Chen Y-L, Fu Y-F, et al. Alternative way to test the efficacy of swine FMD vaccines: measurement of pigs median infected dose (PID50) and regulation of live virus challenge dose. *Virol J* (2010) 7:215. doi:10.1186/1743-422X-7-215
30. Eblé PL, Bouma A, De Bruin MGM, Van Hemert-Kluitenberg F, Van Oirschot JT, Dekker A. Vaccination of pigs two weeks before infection significantly reduces transmission of foot-and-mouth disease virus. *Vaccine* (2004) 22:1372–8. doi:10.1016/j.vaccine.2003.11.003
31. Orsel K, de Jong MCM, Bouma A, Stegeman JA, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* (2007) 25:6381–91. doi:10.1016/j.vaccine.2007.06.010
32. van Roermund HJW, Eble PL, de Jong MCM, Dekker A. No between-pen transmission of foot-and-mouth disease virus in vaccinated pigs. *Vaccine* (2010) 28:4452–61. doi:10.1016/j.vaccine.2010.04.019
33. Vosloo W, Hong NTT, Geoffrey FT, Jacqueline MM, Jianning W, Van Phuc K, et al. Efficacy of a high potency O1 Manisa monovalent vaccine against heterologous challenge with a FMDV O Mya98 lineage virus in pigs 4 and 7 days post vaccination. *Vaccine* (2015) 33:2778–85. doi:10.1016/j.vaccine.2015.04.045
34. Nagendrakumar SB, Hong NTT, Geoffrey FT, Jacqueline MM, Andrew D, Michelle G, et al. A Malaysia 97 monovalent foot-and-mouth disease vaccine (>6PD50/dose) protects pigs against challenge with a variant FMDV A SEA-97 lineage virus, 4 and 7 days post vaccination. *Vaccine* (2015) 33:1–7. doi:10.1016/j.vaccine.2015.07.014
35. Park JN, Lee SY, Chu JQ, Lee YJ, Kim RH, Lee KN, et al. Protection to homologous and heterologous challenge in pigs immunized with vaccine against foot-and-mouth disease type O caused an epidemic in East Asia during 2010/2011. *Vaccine* (2014) 32:1882–9. doi:10.1016/j.vaccine.2014.01.067
36. Vynnycky E, White RG. How are models set up? *An Introduction to Infectious Disease Modelling*. New York: Oxford University Press (2010). p. 13–40.
37. Kinsley AC, Patterson G, VanderWaal KL, Craft ME, Perez AM. Parameter values for epidemiological models of foot-and-mouth disease in swine. *Front Vet Sci* (2016) 3:44. doi:10.3389/fvets.2016.00044
38. Hagenaars TJ, Dekker A, de Jong MCM, Eblé PL. Estimation of foot and mouth disease transmission parameters, using outbreak data and transmission experiments. *Rev Sci Tech* (2011) 30:467–77.
39. Fine PEM. The basics: infections, transmission and models. In: Vynnycky E, White R, editors. *An Introduction to Infectious Disease Modelling*. New York: Oxford University Press (2010). p. 1–12.
40. Alexandersen S, Quan M, Murphy C, Knight J, Zhang Z. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J Comp Pathol* (2003) 129:268–82. doi:10.1016/S0021-9975(03)00045-8
41. Francis MJ, Black L. Response of young pigs to foot-and-mouth disease oil emulsion vaccination in the presence and absence of maternally derived neutralising antibodies. *Res Vet Sci* (1986) 41:33–9.
42. Dekker A, Chénard G, Stockhofe N, Eblé PL. Proper timing of foot-and-mouth disease vaccination of piglets with maternally derived antibodies will maximize expected protection levels. *Front Vet Sci* (2016) 3:10–5. doi:10.3389/fvets.2016.00052
43. Chung WB, Liao PC, Chen SP, Yang PC, Lin YL, Jong MH, et al. Optimization of foot-and-mouth disease vaccination protocols by surveillance of neutralization antibodies. *Vaccine* (2002) 20:2665–70. doi:10.1016/S0264-410X(02)00201-3
44. Liao PC, Lin YL, Jong MH, Chung WB. Efficacy of foot-and-mouth disease vaccine in pigs with single dose immunization. *Vaccine* (2003) 21:1807–10. doi:10.1016/S0264-410X(03)00030-6

45. McKercher PD, Gailiunas P. Response of swine to inactivated foot-and-mouth disease vaccine. Duration of immunity and local tissue reaction. *Arch für die gesamte Virusforsch* (1969) 28:165–76. doi:10.1007/BF01249381
46. Nöckler A, Thalmann G, Haack P, Nehmzow P, Poewe R, Pietzsch E, et al. Riems foot-and-mouth disease oil emulsion vaccines for swine. 2. Use of foot-and-mouth 2-component oil emulsion vaccines in practice. *Arch für Exp Veterinärmedizin* (1990) 44:183–8.
47. Basarab O, Umehara O, Uribe L. The protection of fattening pigs against foot and mouth disease with an oil-adjuvant vaccine: studies on South American FMD virus strains. *Rev Sci Tech* (1982) 1:1147–54.
48. Eblé PL, Weerdmeester K, van Hemert-Kluitenberg F, Dekker A. Intradermal vaccination of pigs against FMD with 1/10 dose results in comparable vaccine efficacy as intramuscular vaccination with a full dose. *Vaccine* (2009) 27:1272–8. doi:10.1016/j.vaccine.2008.12.011
49. Díaz-San Segundo F, Weiss M, Pérez-Martín E, Dias CC, Grubman MJ, Santos TDL. Inoculation of swine with foot-and-mouth disease SAP-mutant virus induces early protection against disease. *J Virol* (2012) 86:1316–27. doi:10.1128/JVI.05941-11
50. Díaz-San Segundo F, Dias CC, Moraes MP, Weiss M, Perez-Martin E, Salazar AM, et al. Poly ICLC increases the potency of a replication-defective human adenovirus vectored foot-and-mouth disease vaccine. *Virology* (2014) 468:283–92. doi:10.1016/j.virol.2014.08.012
51. Kim S-M, Park J-H, Lee K-N, Kim S-K, You S-H, Kim T, et al. Robust protection against highly virulent foot-and-mouth disease virus in swine by combination treatment with recombinant adenoviruses expressing porcine alpha and gamma interferons and multiple small interfering RNAs. *J Virol* (2015) 89:8267–79. doi:10.1128/JVI.00766-15
52. Cao Y, Lu Z, Li D, Fan P, Sun P, Bao H, et al. Evaluation of cross-protection against three topotypes of serotype O foot-and-mouth disease virus in pigs vaccinated with multi-epitope protein vaccine incorporated with poly(I: C). *Vet Microbiol* (2014) 168:294–301. doi:10.1016/j.vetmic.2013.11.023
53. Zhang K, Huang J, Wang Q, He Y, Xu Z, Xiang M, et al. Recombinant pseudorabies virus expressing P12A and 3C of FMDV can partially protect piglets against FMDV challenge. *Res Vet Sci* (2011) 91:90–4. doi:10.1016/j.rvsc.2010.09.001
54. Guo HC, Sun SQ, Jin Y, Yang SL, Wei YQ, Sun DH, et al. Foot-and-mouth disease virus-like particles produced by a SUMO fusion protein system in *Escherichia coli* induce potent protective immune responses in guinea pigs, swine and cattle. *Vet Res* (2013) 44:1. doi:10.1186/1297-9716-44-48
55. Blanco E, Guerra B, de la Torre BG, Defaus S, Dekker A, Andreu D, et al. Full protection of swine against foot-and-mouth disease by a bivalent B-cell epitope dendrimer peptide. *Antiviral Res* (2016) 129:74–80. doi:10.1016/j.antiviral.2016.03.005
56. Moraes MP, Mayr GA, Mason PW, Grubman MJ. Early protection against homologous challenge after a single dose of replication-defective human adenovirus type 5 expressing capsid proteins of foot-and-mouth disease virus (FMDV) strain A24. *Vaccine* (2002) 20:1631–9. doi:10.1016/S0264-410X(01)00483-2
57. Pena L, Moraes MP, Koster M, Burrage T, Pacheco JM, Segundo FDS, et al. Delivery of a foot-and-mouth disease virus empty capsid subunit antigen with nonstructural protein 2B improves protection of swine. *Vaccine* (2008) 26:5689–99. doi:10.1016/j.vaccine.2008.08.022
58. Stenfeldt C, Diaz-San Segundo F, de Los Santos T, Rodriguez LL, Arzt J. The pathogenesis of foot-and-mouth disease in pigs. *Front Vet Sci* (2016) 3:41. doi:10.3389/fvets.2016.00041
59. Patch JR, Pedersen LE, Toka FN, Moraes M, Grubman MJ, Nielsen M, et al. Induction of foot-and-mouth disease virus-specific cytotoxic T cell killing by vaccination. *Clin Vaccine Immunol* (2011) 18:280–8. doi:10.1128/CVI.00417-10
60. Liu Y, Hu R, Zhang S, Zhang F, Li Z, Wei X, et al. Expression of the Foot-and-Mouth Disease Virus VP1 protein using a replication-competent recombinant canine adenovirus type 2 elicits a humoral antibody response in a porcine model. *Viral Immunol* (2006) 19:202–9. doi:10.1089/vim.2006.19.202
61. Qian P, Li XM, Jin ML, Peng GQ, Chen HC. An approach to a FMD vaccine based on genetic engineered attenuated pseudorabies virus: one experiment using VP1 gene alone generates an antibody responds on FMD and pseudorabies in swine. *Vaccine* (2004) 22:2129–36. doi:10.1016/j.vaccine.2003.12.005
62. Yang C-D, Liao J-T, Lai C-Y, Jong M-H, Liang C-M, Lin Y-L, et al. Induction of protective immunity in swine by recombinant bamboo mosaic virus expressing foot-and-mouth disease virus epitopes. *BMC Biotechnol* (2007) 7:62. doi:10.1186/1472-6750-7-62
63. Crisci E, Fraile L, Moreno N, Blanco E, Cabezón R, Costa C, et al. Chimeric calicivirus-like particles elicit specific immune responses in pigs. *Vaccine* (2012) 30:2427–39. doi:10.1016/j.vaccine.2012.01.069
64. Dias CC, Moraes MP, Weiss M, Diaz-San Segundo F, Pérez-Martín E, Salazar AM, et al. Novel antiviral therapeutics to control foot-and-mouth disease. *J Interferon Cytokine Res* (2012) 32:462–73. doi:10.1089/jir.2012.0012
65. Cunliffe HR, Richmond JY, Campbell CH. Interferon inducers and foot-and-mouth disease vaccines: influence of two synthetic polynucleotides on antibody response and immunity in guinea pigs and swine. *Can J Comp Med* (1977) 41:117–21.
66. Cao Y, Lu Z, Li Y, Sun P, Li D, Li P, et al. Poly(I: C) combined with multi-epitope protein vaccine completely protects against virulent foot-and-mouth disease virus challenge in pigs. *Antiviral Res* (2013) 97:145–53. doi:10.1016/j.antiviral.2012.11.009
67. Barrette RW, Szczepanek SM, Rood D, Challa S, Avery N, Vajdy M, et al. Use of inactivated *Escherichia coli* enterotoxins to enhance respiratory mucosal adjuvanticity during vaccination in swine. *Clin Vaccine Immunol* (2011) 18:1996–8. doi:10.1128/CVI.05273-11
68. Guo X, Jia H, Zhang Q, Yuan W, Zhu G, Xin T, et al. CpG-enriched plasmid enhances the efficacy of the traditional foot-and-mouth disease killed vaccine. *Microbiol Immunol* (2012) 56:332–7. doi:10.1111/j.1348-0421.2012.00438.x
69. Park ME, Lee SY, Kim RH, Ko MK, Lee KN, Kim SM, et al. Enhanced immune responses of foot-and-mouth disease vaccine using new oil/gel adjuvant mixtures in pigs and goats. *Vaccine* (2014) 32:5221–7. doi:10.1016/j.vaccine.2014.07.040
70. Lee J-H, Kang I-J, Kim A-R, Noh Y-S, Chung H-C, Park B-K. Increased humoral antibody response of foot-and-mouth disease virus vaccine in growing pigs pre-treated with poly-γ-glutamic acid. *J Vet Sci* (2016) 17:253–6. doi:10.4142/jvs.2016.17.2.253
71. Li Y, Xie F, Chen J, Fan Q, Zhai L, Hu S. Increased humoral immune responses of pigs to foot-and-mouth disease vaccine supplemented with ginseng stem and leaf saponins. *Chem Biodivers* (2012) 9:2225–35. doi:10.1002/cbdv.201100377
72. Xiao C, Rajput ZI, Liu D, Hu S. Enhancement of serological immune responses to foot-and-mouth disease vaccine by a supplement made of extract of cochinchina momordica seeds. *Clin Vaccine Immunol* (2007) 14:1634–9. doi:10.1128/CVI.00339-07
73. Chinsangaram J, Mason PW, Grubman MJ. Protection of swine by live and inactivated vaccines prepared from a leader proteinase-deficient serotype A12 foot-and-mouth disease virus. *Vaccine* (1998) 16:1516–22. doi:10.1016/S0264-410X(98)00029-2
74. Almeida MR, Rieder E, Chinsangaram J, Ward G, Beard C, Grubman MJ, et al. Construction and evaluation of an attenuated vaccine for foot-and-mouth disease: difficulty adapting the leader proteinase-deleted strategy to the serotype O1 virus. *Virus Res* (1998) 55:49–60. doi:10.1016/S0168-1702(98)00031-8
75. Diaz-San Segundo F, Medina GN, Ramirez-Medina E, Velazquez-Salinas L, Koster M, Grubman MJ, et al. Synonymous deoptimization of foot-and-mouth disease virus causes attenuation in vivo while inducing a strong neutralizing antibody response. *J Virol* (2016) 90:1298–310. doi:10.1128/JVI.02167-15
76. Shao JJ, Wong CK, Lin T, Lee SK, Cong GZ, Sin FWY, et al. Promising multiple-epitope recombinant vaccine against foot-and-mouth disease virus type o in swine. *Clin Vaccine Immunol* (2011) 18:143–9. doi:10.1128/CVI.00236-10
77. Chan EW, Wong HT, Cheng SC, Yan WY, Zheng ZX, Sheng ZT, et al. An immunoglobulin G based chimeric protein induced foot-and-mouth disease specific immune response in swine. *Vaccine* (2000) 19:538–46. doi:10.1016/S0264-410X(00)00186-9
78. Dong YM, Zhang GG, Huang XJ, Chen L, Chen HT. Promising MS2 mediated virus-like particle vaccine against foot-and-mouth disease. *Antiviral Res* (2015) 117:39–43. doi:10.1016/j.antiviral.2015.01.005

79. Cubillos C, de la Torre BG, Bárcena J, Andreu D, Sobrino F, Blanco E. Inclusion of a specific T cell epitope increases the protection conferred against foot-and-mouth disease virus in pigs by a linear peptide containing an immunodominant B cell site. *Virol J* (2012) 9:66. doi:10.1186/1743-422X-9-66
80. Cubillos C, de la Torre BG, Jakab A, Clementi G, Borrás E, Bárcena J, et al. Enhanced mucosal immunoglobulin A response and solid protection against foot-and-mouth disease virus challenge induced by a novel dendrimeric peptide. *J Virol* (2008) 82:7223–30. doi:10.1128/JVI.00401-08
81. Huang H, Yang Z, Xu Q, Sheng Z, Xie Y, Yan W, et al. Recombinant fusion protein and DNA vaccines against foot and mouth disease virus infection in guinea pig and swine. *Viral Immunol* (1999) 12:1–8. doi:10.1089/vim.1999.12.1
82. Li Y, Stirling CMA, Denyer MS, Hamblin P, Hutchings G, Takamatsu HH, et al. Dramatic improvement in FMD DNA vaccine efficacy and cross-serotype antibody induction in pigs following a protein boost. *Vaccine* (2008) 26:2647–56. doi:10.1016/j.vaccine.2008.01.037
83. Wong HT, Cheng SCS, Chan EWC, Sheng ZT, Yan WY, Zheng ZX, et al. Plasmids encoding foot-and-mouth disease virus VP1 epitopes elicited immune responses in mice and swine and protected swine against viral infection. *Virology* (2000) 278:27–35. doi:10.1006/viro.2000.0607
84. Borrego B, Argilaguet JM, Pérez-Martín E, Dominguez J, Perez-Filgueira M, Escribano JM, et al. A DNA vaccine encoding foot-and-mouth disease virus B and T-cell epitopes targeted to class II swine leukocyte antigens protects pigs against viral challenge. *Antiviral Res* (2011) 92:359–63. doi:10.1016/j.antiviral.2011.07.017
85. Song H, Wang Z, Zheng D, Fang W, Li Y, Liu Y, et al. A novel mucosal vaccine against foot-and-mouth disease virus induces protection in mice and swine. *Biotechnol Lett* (2005) 27:1669–74. doi:10.1007/s10529-005-2727-4
86. Wang G, Pan L, Zhang Y, Wang Y, Zhang Z, Lü J, et al. Intranasal delivery of cationic PLGA nano/microparticles-loaded fmdv DNA vaccine encoding IL-6 elicited protective immunity against FMDV challenge. *PLoS One* (2011) 6:e27605. doi:10.1371/journal.pone.0027605
87. Blignaut B, Visser N, Theron J, Rieder E, Maree FF. Custom-engineered chimeric foot-and-mouth disease vaccine elicits protective immune responses in pigs. *J Gen Virol* (2011) 92:849–59. doi:10.1099/vir.0.027151-0
88. Zheng H, Lian K, Yang F, Jin Y, Zhu Z, Guo J, et al. Cross-protective efficacy of engineering serotype A foot-and-mouth disease virus vaccine against the two pandemic strains in swine. *Vaccine* (2015) 33:5772–8. doi:10.1016/j.vaccine.2015.09.055

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 01 June 2016
doi: 10.3389/fvets.2016.00044

Parameter Values for Epidemiological Models of Foot-and-Mouth Disease in Swine

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In the event of a foot-and-mouth disease (FMD) incursion, response strategies are required to control, contain, and eradicate the pathogen as efficiently as possible. Infectious disease simulation models are widely used tools that mimic disease dispersion in a population and that can be useful in the design and support of prevention and mitigation activities. However, there are often gaps in evidence-based research to supply models with quantities that are necessary to accurately reflect the system of interest. The objective of this study was to quantify values associated with the duration of the stages of FMD infection (latent period, subclinical period, incubation period, and duration of infection), probability of transmission (within-herd and between-herd *via* spatial spread), and diagnosis of a vesicular disease within a herd using a meta-analysis of the peer-reviewed literature and expert opinion. The latent period ranged from 1 to 7 days and incubation period ranged from 1 to 9 days; both were influenced by strain. In contrast, the subclinical period ranged from 0 to 6 days and was influenced by sampling method only. The duration of infection ranged from 1 to 10 days. The probability of spatial spread between an infected and fully susceptible swine farm was estimated as greatest within 5 km of the infected farm, highlighting the importance of possible long-range transmission through the movement of infected animals. Finally, while most swine practitioners are confident in their ability to detect a vesicular disease in an average sized swine herd, a small proportion expect that up to half of the herd would need to show clinical signs before detection *via* passive surveillance would occur. The results of this study will be useful in within- and between-herd simulation models to develop efficient response strategies in the event an FMD in swine populations of disease-free countries or regions.

Keywords: FMD, transmission, meta-analysis, simulation model, Delphi technique

OPEN ACCESS

Edited by:
Marta Hernandez-Jover, Charles Sturt University, Australia

Reviewed by:
Evan Sergeant, AusVet Animal Health Services, Australia
Hans-Hermann Thulke, Helmholtz Centre for Environmental Research – UFZ, Germany
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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *16 March 2016*
Accepted: *17 May 2016*
Published: *01 June 2016*

Citation:
Kinsley AC, Patterson G, VanderWaal KL, Craft ME and Perez AM (2016) Parameter Values for Epidemiological Models of Foot-and-Mouth Disease in Swine. Front. Vet. Sci. 3:44. doi: 10.3389/fvets.2016.00044

INTRODUCTION

As the world's largest beef producer and second largest pork producer, the United States (US) is a major player in the world livestock market (1). The US's ability to export livestock and livestock products is highly dependent on maintaining a foot-and-mouth disease (FMD)-free status. Although an epidemic has not occurred since the eradication of FMD from the US in 1929, the threat of reintroduction remains due to international travel and trade as seen in recent outbreaks in, for example, the UK, Taiwan, the Netherlands, and France (2, 3). In an effort to contain and control FMD as proficiently as possible, it is common for an affected country to adopt a policy to cease

animal movements and depopulate infected animals. However, a strong understanding of FMD spread under regional conditions is essential for efficient preparedness, response, and utilization of resources. Therefore, it is important to carry out analytical studies for strategic and response planning before an outbreak occurs, which may be helped by the formulation, parameterization of, and experimentation with, disease models.

Infectious disease simulation models use mathematics to mimic the dispersion of disease in a population and can be useful in elucidating the mechanisms by which pathogens spread, as well as the underlying processes that influence animal movements, in the geographical region where infection occurs. Stochastic simulation models account for uncertainty and biological fluctuation by using probability distributions to encode for one or more of the variables in the model. However, there are often gaps in evidence-based research to supply models with quantities that are necessary to accurately reflect the system of interest. Researchers and veterinarians with extensive experience may help to fill those gaps and build confidence around the quantity of interest when feasibility restricts the amount of data that can be collected.

The efficacy and speed of FMD virus transmission is dependent on the strain of the virus, the contact structure between hosts, and susceptibility of species involved (4). Therefore, it is critical to develop species-specific transmission values that describe the time course of infection for the host and the probability of transmission. Pigs have played a role in recent outbreaks of FMD. For instance, in the 2011 outbreak in South Korea, the index case occurred on a pig farm where misdiagnosis led to rapid nationwide dissemination, resulting in the ultimate infection of approximately 3,700 farms and the culling of 3.48 million susceptible animals (5).

Here, we quantified parameters associated with FMD transmission in swine using a meta-analysis of the peer-reviewed literature and expert opinion. A modified Delphi technique was applied during a meeting with individuals possessing an average of over 12 years of experience with FMD. In addition, swine practitioners were asked to estimate the proportion of the herd that would need to show clinical signs for the diagnosis of a vesicular disease to occur. Our results will be of use for the parameterization of within- and between-herd FMD transmission models in the US and other FMD-free countries and regions.

MATERIALS AND METHODS

Meta-Analysis

A meta-analysis (6) was conducted to quantify values associated with the time course of FMD infection in swine and was composed of four main components, namely, (1) literature search, (2) inclusion criteria (3) definition of parameter values obtained through the meta-analysis, and (4) statistical analysis including the effects of experimental bias.

Literature Search

Literature searches were conducted using two electronic databases, PubMed and Agricola. The searches were conducted using multiple keywords and expressions ("foot-and-mouth disease"[MeSH Terms]) OR ("foot-and-mouth"[All Fields] AND "disease"[All Fields]) OR "foot-and-mouth disease"[All Fields] OR ("foot"[All Fields] AND "mouth"[All Fields] AND "disease"[All Fields]) OR ("foot and mouth disease"[All Fields]) AND ("swine"[MeSH Terms] OR "swine"[All Fields]) AND ("transmission"[Subheading] OR "transmission"[All Fields]) and swine AND foot and mouth disease AND transmission, respectively. Titles and abstracts were imported into RefWorks citation manager for review.

Inclusion Criteria

Inclusion criteria for this study include experimental studies that investigate direct and indirect transmission of FMD between unvaccinated domesticated swine with individual-level infection data.

Definition of Parameters Estimated through the Meta-Analysis

Parameter values associated with the time course of FMD infection were defined as the latent period, subclinical period, incubation period, and duration of infection (**Figure 1**). The duration of the stages of FMD infection were described as the following: the latent period (t_0–t_1) was considered the time from exposure to the time sample collection resulted in the first positive sample (oral swabs, nasal swabs, or blood); the subclinical period (t_1–t_2) was described as the time from sample collection resulted in the first positive test to the development of clinical signs (increased body temperature, lameness, dullness, reluctance to stand, and presence of vesicular lesions), and the duration of infection (t_1–t_3) was described as the time from sample collection of the first positive test until sample collection of the last positive test result (virus isolation or RT-PCR). The latent period, subclinical period, clinical period, and incubation period were determined from transmission studies using the first positive test and clinical signs in contact pigs. Studies that reported these time periods in hours were converted into days and were rounded to the nearest day. For studies that reported the incubation period and latent period, the subclinical period was calculated by subtracting the duration of the latent period from the incubation period.

Statistical Analysis

One parametric survival regression model was fit for each of the stages of infection (latent period, subclinical period,

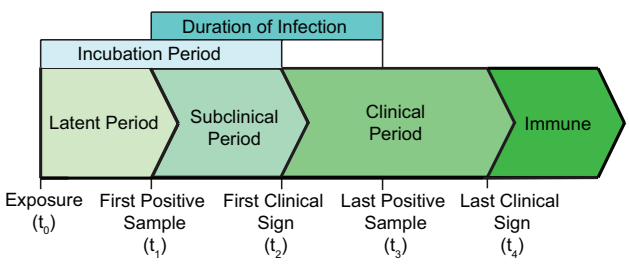

FIGURE 1 | Time course of FMD infection in pigs infected through contact with an inoculated pig.

incubation period, and duration of infection) to identify factors that influence the stages of FMD infection extracted from experimental studies. This method is an adaptation of the time-to-event modeling method used by Mardones et al. (7) to estimate the time ratio of an event in an accelerated failure time (AFT) regression model. The AFT model was fitted using the survreg function in the Survival package in R (8). The survival regression model assumed that the baseline hazard function followed a Weibull distribution, which is appropriate for data exhibiting a monotonic hazard rate. The time ratio of the AFT model describes the relative increase in time to the event compared to the baseline. The following factors were fit in the regression: diagnostic test, duration of contact with inoculated pig, reference laboratory, ratio of inoculated seeder pigs to susceptible contact pigs, sampling method, and strain (**Table 1**). Survival data were fitted and compared through a stepwise approach using the Akaike information criteria (AIC) (9). Factors, covariates, and interactions terms that produce the lowest AIC were calculated using the stepAIC function in the MASS package in R (10, 11) to select the most informative variables. Individual factors that resulted in a statistically significant model ($p < 0.05$) were included in the final model. A frailty term, comparable to a random effect in regression models, was included in the models to adjust for the variability between individual experiments. The frailty term was retained in the final model only if it improved the AIC.

Probability distribution functions were fit by investigating distributions commonly used and those used in FMD simulation models (7, 12, 13) and included: binomial, exponential, Inverse Gaussian, Poisson, Pearson 5, Weibull, Log-logistic, and normal distributions. Continuous and discrete theoretical distributions of the duration of the stages of FMD infection were selected using the Anderson–Darling goodness of fit test for continuous data and the Chi-square test for discrete data using @Risk (14) (**Figure 2**). Bin size was selected using the Freedman–Diaconis Rule. We then considered the conceptual aspects of the distributions and choose the simplest, most accurate distribution.

TABLE 1 | Variables considered in the accelerated failure time model.

Variable	Explanation	Description
Diagnostic test	Test chosen for the detection of FMDV	RT-PCR Virus isolation
Duration of contact	Time that infected inoculated pigs and susceptible pigs were housed together	Quantified in days
Reference laboratory	Laboratory at which the experiment was conducted	Lelystad Pirbright Plum Island
Ratio of inoculated to contact pigs	Number of inoculated pigs/ number of susceptible pigs in contact	Quantified as the number of inoculated/the number of susceptible
Sample	Tissue or excreta collected for FMDV identification	Serum Nasal swabs Oropharyngeal fluid
Strain	Strain of FMDV used to infect inoculated pig	O/TAW/97 O/NET/2001 O/HKN/21/70 O/UKG/01 O/SKR/2000 O/TAW/0/2/99 A24 Cruzeiro O1 Manisa Asia 1 Shamir

Expert Selection

Five individuals external to the University of Minnesota were selected based on their training and experience with FMD. Expert experience ranged from 12 to 35 years working with FMD, including experts with specialized area of knowledge in academia ($n = 2$), field experience ($n = 3$), government work ($n = 4$), and laboratory experiments ($n = 3$).

Data Collection

Data were collected utilizing a modification of the Delphi technique, an accepted method of obtaining data on a real world issue (15). Here, we used a two-round approach to reach consensus on transmission data relating to FMD.

Round 1

Through an open-ended questionnaire, experts were asked questions about the incubation period, mortality rates (adult pigs and piglets), probability of transmission, and spatial spread (at 1, 5, 10, and 50 km from an infected farm). The questionnaire was created based on extensive literature review, and the questions were the same for all experts.

These data were recorded by the respondents on paper, reviewed, and transferred to electronic format. The questionnaire was used as a survey instrument to collect data in Round 2.

Round 2

In the second round, each participant was asked to review the items from the initial questionnaire to discuss the reasoning supporting the response. In the case of incompatible answers, responses were discussed until unanimous understanding and consensus was reached.

Swine Practitioner Survey

Twenty surveys were administered to swine practitioners attending the 2015 Leman Swine Conference in St. Paul, MN, USA. The survey asked practitioners to estimate the proportion of a swine herd (typical size) that would need to show clinical signs before a vesicular disease was suspected.

Data Analysis

Survey responses were recorded and distributions were fit for FMD incubation period, disease-associated mortality rate, transmission probability, spatial spread, and proportion of the herd clinical for diagnosis to occur. Questionnaire results were described using the BetaPERT probability distribution function for the minimum, most likely, and maximum values for the mortality rates, probability of transmission, and spatial spread (**Table 2**). The estimation of mortality is the percentage of the herd that died due to disease. It was estimated separately for adult pigs and piglets.

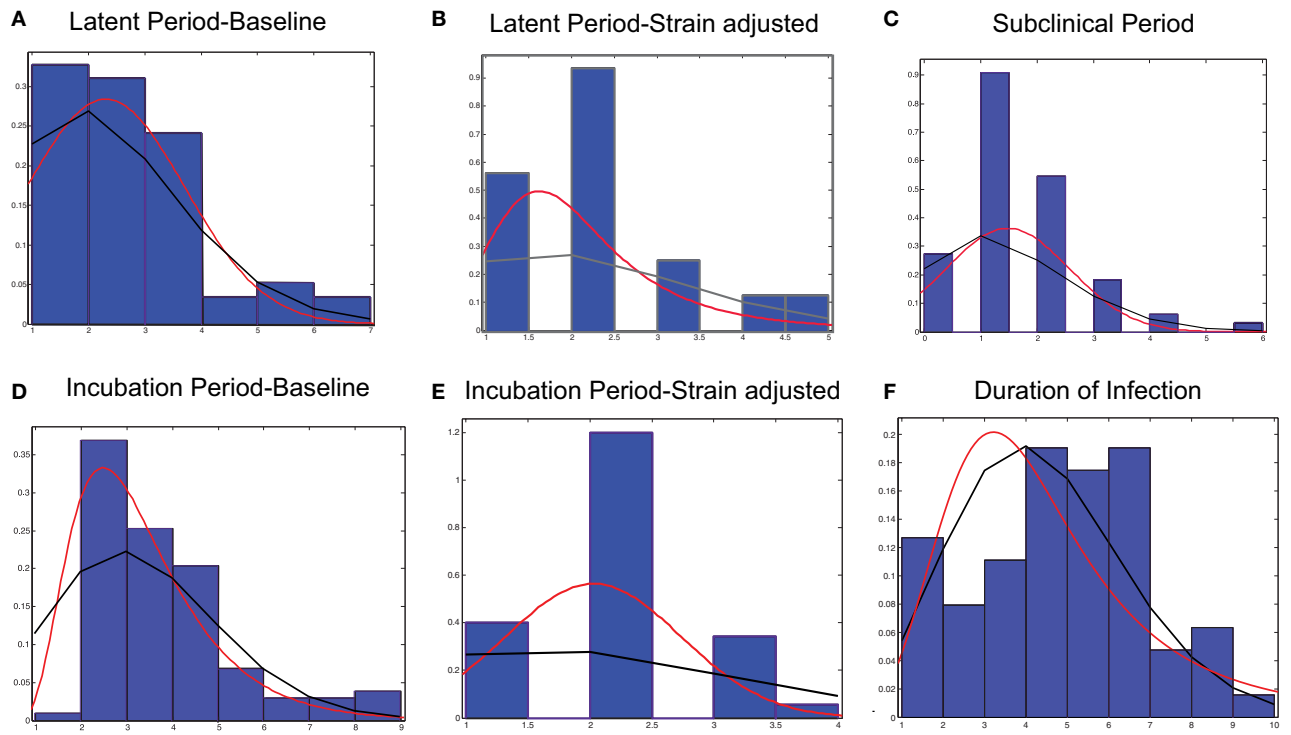

FIGURE 2 | Frequency distributions and probability distributions for the stages of FMD infection in pigs using data obtained through experimental studies. **(A)** Latent period-baseline, **(B)** latent period-strain adjusted, **(C)** subclinical period, **(D)** incubation period-baseline, **(E)** incubation period-strain adjusted, and **(F)** duration of infection. The lines represent continuous (red) and discrete (black) probability distributions.

TABLE 2 | Estimations of disease induced mortality rates and the probability of transmission given direct contact.

Parameter description	Distribution
Adult mortality (%)	BetaPERT (12.5, 20.8, 40.0)
Piglet mortality rate (%)	BetaPERT (18.3, 58.3, 23.0)
Transmission probability (direct contact %)	BetaPERT (46, 84, 97.5)

Values were obtained through expert opinion.

The probability of spatial transmission was defined as the probability that farm *j* becomes infected by farm *i* through a route described in any manner other than through the direct movement of animals. The probability of spatial transmission was estimated at a distance of 1, 5, 10, and 50 km from the infected premises. The expert-solicited most likely probability of spread was plotted at each distance and a non-linear function was fitted to the data in MATLAB using the Curve Fitting App (16).

RESULTS

Meta-Analysis

Literature Search

The PubMed and Agricola search resulted in 216 and 54 articles, respectively. Literature search results were screened for duplicate articles. Individual titles and abstracts were read to determine if the article met the inclusion criteria. Articles that did not specifically address FMD virus transmission between swine were excluded. After removing duplicate articles and excluding studies that did not meet the inclusion criteria, seven articles remained. The articles were published between 2003 and 2012 and include three serotypes (O, A, and Asia1) and nine strains (**Table 3**). Experiments were conducted at three reference laboratories, including the Central Institute for Animal Disease Control

TABLE 3 | Experimental studies used to fit distributions for the latent, subclinical, and infectious period.

Reference	Reference laboratory	Strain	Number of pigs (contact only)
Alexandersen et al. (17)	Pirbright	O/TAW/97	12 (6)
Eblé et al. (18)	Lelystad	O/TAW/97	50 (25)
Howey et al. (19)	Pirbright	O/UKG/01	12 (0)
Orsel et al. (20)	Lelystad	O/NET/2001	34 (25)
Pacheco and Mason (4)	Plum Island	O/HKN/21/70 O/TAW/97 O/UKG/01 O/SKR/2000 O/TAW/0/2/99	42 (18)
Pacheco et al. (21)	Plum Island	A24 Cruzeiro O1 Manisa Asia 1 Shamir	30 (18)
Quan et al. (22)	Pirbright	O/UKG/01	70 (38)
Van Roermund et al. (23)	Lelystad	O/NET/2001	36 (24)

TABLE 4 | Accelerated failure time model fitted for the latent period, and incubation period (Weibull distribution, shape parameter latent period = 2.34, shape parameter incubation period = 3.41).

Time period	Variable		Time ratio	β	95% CI	*p*-Value
Latent period	Strain	A24 Cruzeiro	0.28	−1.27	(−2.21, 0.32)	<0.01
		O/HKN/70	0.23	−1.47	(−2.45, −0.49)	<0.005
		O/NET/2001	0.31	−1.17	(−2.24, −0.11)	<0.05
		O/TAW/97	0.30	−1.22	(−2.12, −0.32)	<0.01
Incubation period	Strain	O/HKN/70	0.43	−0.85	(−1.31, −0.38)	<0.001
		O/TAW/97	0.59	−0.53	(−0.92, −0.14)	<0.001
		O/SKR/00	0.53	−0.64	(−1.14, −0.15)	0.01

Baseline latent period = 3.63 days, baseline incubation period = 4.66 days.

(CIDC, Lelystad, The Netherlands), the Institute for Animal Health (IAH, Pirbright, UK), and the Plum Island Animal Disease Center (PIADC, New York, NY, USA).

Time Course of FMD Infection

Latent Period

The experimental studies obtained through the literature review revealed that the latent period ranged from 1 to 7 days. This was in agreement with the experts' response in which the latent period ranged from 1 to 5 days with the majority of pigs testing positive on day 1 (data not shown). The first stepwise AIC calculation indicated that the latent period was significantly influenced by strain, reference laboratory, and time of introduction and whether the pig was infected through inoculation or contact. While inoculation *via* heel bulb or intravenous injection is a common technique, it may not be a realistic approach to estimate the duration of infectious stages of FMD in a population infected through direct contact. Because inoculated animals are not biologically representative of natural conditions and have a decreased latent period, inoculated pigs were excluded from the analysis. The final model included strain and sampling method, and a frailty term for the individual experiments, suggesting a baseline latent period of 3.63 days ($p < 0.001$) (**Table 4**). Samples collected through oropharyngeal swabs resulted in shorter latent periods. The latent period was adjusted for strain by separating those with a significantly shorter time ratio (**Table 4**) and fit to Binomial, Normal, and Log-logistic distributions (**Figures 2A,B**; **Table 5**).

Subclinical Period

A wide range of values, 0–6 days, was estimated for the subclinical period obtained through the experimental studies. The stepwise AIC calculations indicated that inclusion of the route of infection (inoculation vs. contact) in the model produced the best prediction for the subclinical period. Since inoculated animals are not biologically representative of natural conditions and have a decreased subclinical period, inoculated pigs were excluded from the analysis. After excluding animals infected through inoculation, sample method was the only covariate that remained in the best prediction model according to the AIC. However, inclusion of the sampling method produced a non-significant result. The subclinical period was fit to Binomial and Normal distributions (**Figure 2C**; **Table 5**).

TABLE 5 | Descriptive statistics of (a) discrete and (b) continuous distributions fit to the stages of FMD infection in pigs.

Stage of infection	Distribution	Parameters
Latent period-baseline (t_0–t_1)	(a) Binomial	(a) $N = 58$, $p = 0.04$
	(b) Normal	(b) $\mu = 2.31$, $\sigma = 1.40$
Latent period-adjusted (t_0–t_1)	(a) Binomial	(a) $N = 97$, $p = 0.02$
	(b) Log-logistic	(b) $\mu = 0.65$, $\sigma = 0.28$
Subclinical period (t_1–t_2)	(a) Binomial	(a) $N = 66$, $p = 0.02$
	(b) Normal	(b) $\mu = 1.48$, $\sigma = 1.10$
Incubation period-baseline (t_0–t_2)	(a) Binomial	(a) $N = 103$, $p = 0.03$
	(b) Inverse Gaussian	(b) $\mu = 3.36$, $\lambda = 16.97$
Incubation period-adjusted (t_0–t_2)	(a) Binomial	(a) $N = 35$, $p = 0.06$
	(b) Normal	(b) $\mu = 2.03$, $\sigma = 0.71$
Duration of infection (t_1–t_3)	(a) Poisson	(a) $\lambda = 5.19$
	(b) Log-logistic	(b) $\mu = 1.50$, $\sigma = 0.40$

Baseline and adjusted values correspond to results of the accelerated failure time model. Definition of parameter values by distribution: binomial – N = number of Bernoulli trials, p = probability of success; normal – μ = mean, σ = standard deviation; Log-logistic – μ = scale, σ = shape; Inverse Gaussian – μ = mean, λ = shape; and Poisson – λ = mean number of events per interval.

Incubation Period

The incubation period was characterized by values obtained through the literature review, which ranged from 1 to 9 days (**Figures 2D,E**; **Table 5**) and was concurrent with the values obtained through expert opinion (min = 2, max = 9) (data not shown). The final model included strain and sampling method and a frailty term for the individual experiments, suggesting a baseline latent period of 4.66 days ($p < 0.001$) (**Table 4**). The incubation period of the experimental data was adjusted for strain and fit to Binomial and Inverse Gaussian distributions (**Figures 2D,E**; **Table 5**).

Duration of Infection

The duration of infection ranged from 1 to 10 days and was fit to a Poisson and Log-logistic distribution (**Figure 2F**; **Table 5**). The stepwise AIC calculation indicated that inclusion of the reference laboratory produced the model with the lowest AIC, with the baseline duration of infection estimated to be 6.23 days. Inclusion of the reference laboratory and frailty term for the individual experiment in the final model resulted in a statistically significant model ($p < 0.001$).

Expert Opinion

Spatial Transmission

The probability of transmission from infected farm *i* to susceptible farm *j* was estimated by expert opinion at a distance of 1, 5, 10, and 50 km from the infected premise and was described by the expression $P(x) = a \times e^{(-bx)} + c$ where the coefficients and the corresponding 95% CI were $a = 0.3693$ (−1.079, 1.818), $b = 0.1182$ (−0.9134, 1.15), and $c = 0.3307$ (−0.6243, 1.286) (adjusted $R^2 = 0.745$) (**Figure 3**).

Swine Practitioner Survey

Swine practitioners estimated the proportion of the herd that would need to show clinical signs for the diagnosis of a vesicular disease to occur. This estimation ranged from 1 to 50% with a mean of 11.2% and a median of 5.75 (**Figure 4**). An Inverse Gaussian distribution was the best fit according to the Anderson–Darling goodness of fit test.

DISCUSSION

To the best of our knowledge, this is the first study aimed to quantify parameters associated with FMD transmission in swine for use in transmission models, using both expert opinion and meta-analyses of published studies. We employed a modified Delphi technique to individuals with at least 12 years of experience with FMD. In addition, we asked swine practitioners to estimate the proportion of the herd that would be affected for the diagnosis of a vesicular disease to occur which can be used to estimate the proportion of the herd that would be subclinical at the time of diagnosis. Results reported here will be valuable for developing simulation models of FMD transmission in swine farms.

Existing models of FMD vary in approach. As a result, the parameter values required for the models also differ. A common approach to quantify parameter values is to use existing disease data. For instance, a recent review of data-driven models of FMD revealed that data from 12 different epidemics have been used in models and that more than half used data from the 2001 UK epidemic (24), where pigs were not largely involved. However, transmission characteristics of FMD infection are influenced by biological processes specific to the strain of FMD virus, host, and environmental factors, such as the rate of contact (17, 25) and variations of parameter values, associated with these factors should be considered.

In a previous study (7), the duration of infection stages of FMD was reported for serotype O. They found that experimental conditions, such as host species involved in the transmission study and specific virus strain, significantly influenced the time course of disease. By utilizing a stepwise regression analysis similar to that described by Mardones et al. (7), we were able to update the parameters distributions with current studies of FMD transmission in swine. Moreover, we were able to provide a range of values that play a key role in between-farm disease transmission models including time to detection and the probability of spatial spread.

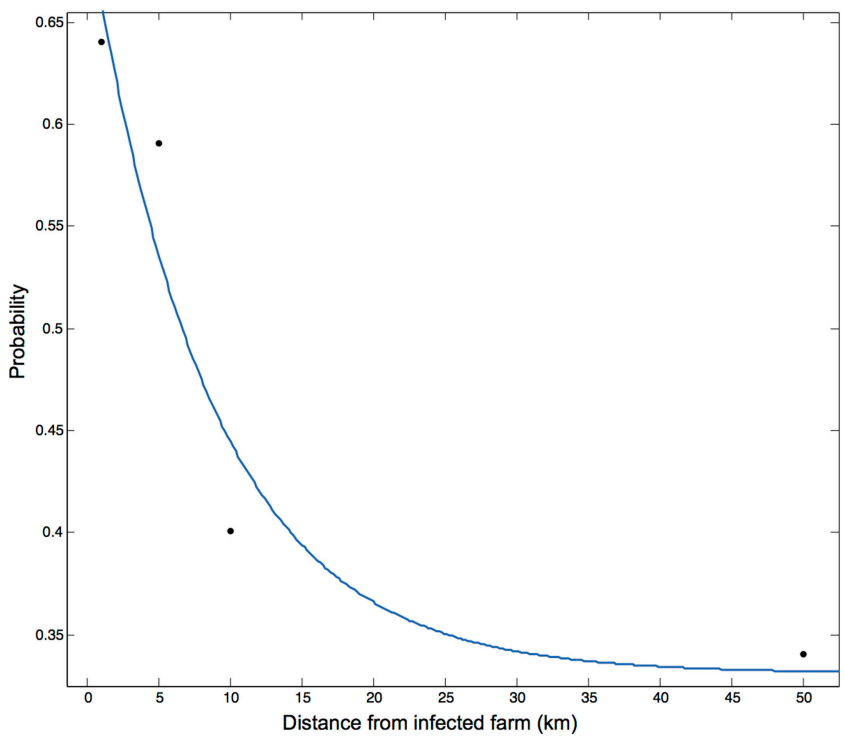

FIGURE 3 | Transmission kernel calculated from the probability of transmission through indirect contact at 1, 5, 10, and 50 km prior to the implementation of control measures. The probability of transmission is described by the equation $P(x) = 3.693 \times e^{(-0.118x)} + 0.3307$ where $P(x)$ is the probability of spatial transmission between infected farm *i* and susceptible farm *j* located *x* distance apart in kilometers. Values were obtained through expert opinion.

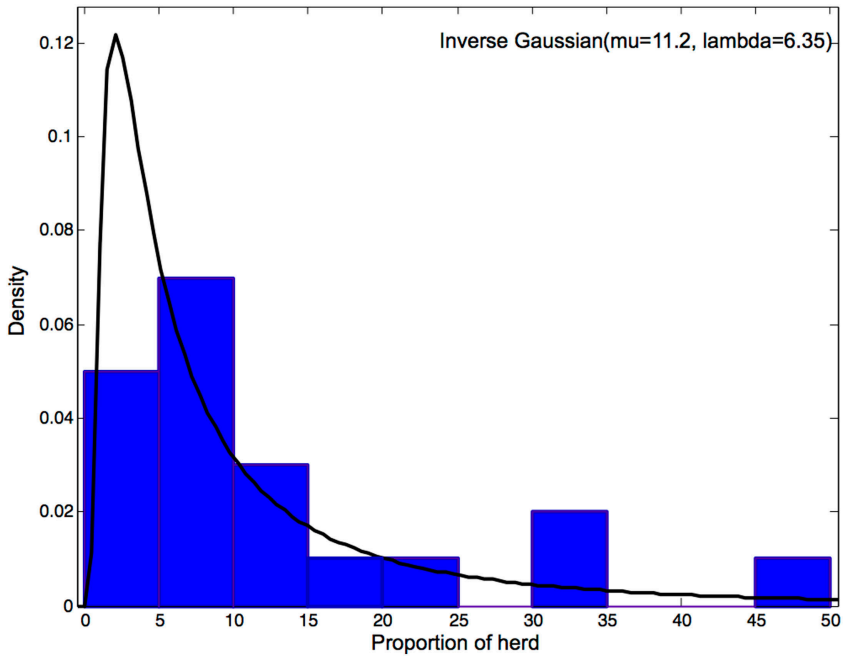

FIGURE 4 | Distribution of the estimated proportion of the herd showing clinical signs for diagnosis of a vesicular disease to occur.

The studies included in our analyses aim to understand the determinants of transmission through controlled experiments by varying factors within each experiment and measuring the impact of that factor on the kinetics of viral shedding and the manifestation of clinical disease. The factors measured in the experiments were extracted from the studies and included strain, duration of contact, route of infection, ratio of inoculated to contact pigs, and method of sampling. Of these factors, we found that the latent period and incubation period was shorter in inoculated animals than animals infected through direct contact. While the inoculation of donor animals is essential to reliably produce infectious animals with clinical disease, using the time course of disease in these animals to estimate the latent period is not appropriate as direct inoculation evades the host first line of defenses against infection (21). Current studies of FMD in swine suggest that initial virus entry occurs at the lymphoid tissues of the pharyngeal region followed by low-level viremia, then replication and development of vesicles in epithelial tissues (17, 24). Much greater amplification of the virus occurs in the epithelial cells leading to a substantially greater, detectable level of viremia in the pig (26, 27). It is likely that pigs infected through intradermal heel bulb or intravenous inoculation bypass the initial phase of infection leading to shorter latent periods than pigs infected through contact.

The frequency distribution for the latent period, subclinical period, incubation period, and duration of infection are consistent with those estimated in the Mardones et al. (7) paper. The frequency distributions are right skewed with relatively short tails. But the range of the values obtained in this study was consistently shorter for each of the stages of infection, and the duration of infection was shorter for a greater proportion of individuals represented in this study. This is likely due to the differences in the experimental design of the studies captured in our literature search such as the duration of the experiment and strain of virus. Also, in agreement with the study by Mardones et al. (7), we found that strain and method of sampling significantly influence the latent period and incubation period of FMD infection. These findings suggest that models will benefit from the inclusion of strain-specific factors and that sampling oropharyngeal fluid may be helpful in identifying infected individuals in the early stages of an outbreak or during active surveillance.

For the definitive diagnosis of FMD to occur, clinical disease must be recognized, and the identification of live virus must occur. In an FMD-free country, a producer or veterinarian identifies the lesions in the index case through passive surveillance. Once the index case has been confirmed, and the outbreak is underway, diagnosis may occur solely through clinical signs. While it seems implausible that up to 50% of a herd would be showing clinical signs before clinical disease is recognized, individuals who work with animals on a daily basis may fail to recognize the clinical signs due to inexperience (28–30). For instance, during the 2001 UK State Veterinary Service FMD investigations, veterinary officers found that up to 90% of 527 pigs on the index farm had lesions consistent with FMD (31). Delay in the time to diagnosis in the index case can greatly increase the probability of between-herd transmission likely leading to a larger outbreak. However, these results represent the belief of the limited number of practitioners surveyed in the study here and may not be representative of every swine farm in the country.

An additional caveat is that we used the opinion of experts to quantify parameter values associated with FMD infection. Although the experts in our study had a wide range of experience and extensive amount of time working with FMD, there is such a high degree of uncertainty quantifying values associated with transmission at the population level that error is possible. Between-farm values estimated from this study are useful for parameterizing or model fitting and should be interpreted in light of current research and continually updated for use in disease simulation models. However, for estimating distributions for stages of infection, expert opinion was used as a confirmatory cross-validation of the results of the meta-analysis.

In conclusion, we found that the stages of FMD infection were influenced by route of infection, strain, and sampling method. While modeling efforts may not need to be conducted for every strain of interest, strain variation should be accounted for in the model. Additionally, the probability of spatial spread between an infected and fully susceptible swine farm is greatest within 5 km of the infected farm, highlighting the importance of possible transmission beyond this through the movement of infected animals. Finally, while most swine practitioners are confident in their ability to detect a vesicular disease with few animals showing clinical signs; yet, a small proportion expect that up to half of the herd would need to show clinical signs before detection occurred.

AUTHOR CONTRIBUTIONS

Each of the authors were substantial contributors to the conception or design of the work (AK, KV, GP, and AP); or the acquisition (AK, KV, GP, and AP), analysis (AK, GP, KV, and AP), or interpretation of data for the work (AP, KV, GP, AP, and MC), and drafting the work or revising it critically for important intellectual content (AP, KV, GP, AP, and MC). AP, KV, GP, AP, and MC have approved the final version for publication and are in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This work was partially supported by Minnesota's Discovery, Research and InnoVation Economy (MNDRIVE), the National Pork Board (NPB), the USDA Agricultural Research Service (USDA/ARS), and the Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fvets.2016.00044

REFERENCES

1. United States Department of Agriculture. *Animal Production (03/06/2015) 02/19/2016*. Available from: www.usda.gov/wps/portal/usda/usdahome?navid=ANIMAL_PRODUCTION
2. Yang PC, Chu RM, Chung WB, Sung HT. Epidemiological characteristics and financial costs of the 1997 foot-and-mouth disease epidemic in Taiwan. *Vet Rec* (1999) 145:731–4.
3. Garner MG, Beckett S. Modelling the spread of foot-and-mouth disease in Australia. *Aust Vet J* (2005) 83:758–66. doi:10.1111/j.1751-0813.2005.tb11589.x
4. Pacheco JM, Mason P. Evaluation of infectivity and transmission of different Asian foot-and-mouth disease viruses in swine. *J Vet Sci* (2010) 11:133–42. doi:10.4142/jvs.2010.11.2.133
5. Park J, Lee K, Ko Y, Kim S, Lee H, Shin Y, et al. Control of foot-and-mouth disease during 2010-2011 epidemic, South Korea. *Emerg Infect Dis* (2013) 19:655–60. doi:10.3201/eid1904.121320
6. Field AP, Gillett R. How to do a meta-analysis. *Br J Math Stat Psychol* (2010) 63:665–94. doi:10.1348/000711010X502733
7. Mardones F, Perez A, Sanchez J, Alkhamis M, Carpenter T. Parameterization of the duration of infection stages of serotype O foot-and-mouth disease virus: an analytical review and meta-analysis with application to simulation models. *Vet Res* (2010) 41:45. doi:10.1051/vetres/2010017
8. Therneau T. *A Package for Survival Analysis in S. R Package Version 2.37-4*. Box, 980032:23298–20032 (2013). Available from: http://CRAN.R-project.org/package=survival
9. Burnham KP, Anderson DR. Multimodel inference understanding AIC and BIC in model selection. *Sociol Methods Res* (2004) 33:261–304. doi:10.1177/0049124104268644
10. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing (2012).
11. Yamashita T, Yamashita K, Kamimura R. A stepwise AIC method for variable selection in linear regression. *Commun Stat Theory Methods* (2007) 36:2395–403. doi:10.1080/03610920701215639
12. Bates TW, Thurmond MC, Carpenter TE. Description of an epidemic simulation model for use in evaluating strategies to control an outbreak of foot-and-mouth disease. *Am J Vet Res* (2003) 64:195–204. doi:10.2460/ajvr.2003.64.195
13. Carpenter TE, Thurmond MC, Bates TW. A simulation model of intraherd transmission of foot and mouth disease with reference to disease spread before and after clinical diagnosis. *J Vet Diagn Invest* (2004) 16:11–6. doi:10.1177/104063870401600103
14. Palisade Corporation. *@RISK. Version 7.0*. Ithaca, NY: Palisade Corporation (2016).
15. Hsu C, Sandford BA. The Delphi technique: making sense of consensus. *Pract Assess Res Eval* (2007) 12:1–8. Available online: pareonline.net/getvn.asp?v=12&n=10
16. The MathWorks, Inc. *MATLAB and Statistics Toolbox Release 2012b*. Natick, MA: The MathWorks, Inc (2012).
17. Alexandersen S, Quan M, Murphy C, Knight J, Zhang Z. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J Comp Pathol* (2003) 129:268–82. doi:10.1016/S0021-9975(03)00045-8
18. Eblé P, De Koeijer A, Bouma A, Stegeman A, Dekker A. Quantification of within-and between-pen transmission of foot-and-mouth disease virus in pigs. *Vet Res* (2006) 37:647–54. doi:10.1051/vetres:2006026
19. Howey R, Quan M, Savill NJ, Matthews L, Alexandersen S, Woolhouse M. Effect of the initial dose of foot-and-mouth disease virus on the early viral dynamics within pigs. *J R Soc Interface* (2009) 6:835–47. doi:10.1098/rsif.2008.0434
20. Orsel K, De Jong M, Bouma A, Stegeman J, Dekker A. Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* (2007) 25:6381–91. doi:10.1016/j.vaccine.2007.06.010
21. Pacheco JM, Tucker M, Hartwig E, Bishop E, Arzt J, Rodriguez LL. Direct contact transmission of three different foot-and-mouth disease virus strains in swine demonstrates important strain-specific differences. *Vet J* (2012) 193:456–63. doi:10.1016/j.tvjl.2012.01.012
22. Quan M, Murphy C, Zhang Z, Durand S, Esteves I, Doel C, et al. Influence of exposure intensity on the efficiency and speed of transmission of foot-and-mouth disease. *J Comp Pathol* (2009) 140:225–37. doi:10.1016/j.jcpa.2008.12.002

23. Van Roermund H, Eblé P, de Jong M, Dekker A. No between-pen transmission of foot-and-mouth disease virus in vaccinated pigs. *Vaccine* (2010) 28:4452–61. doi:10.1016/j.vaccine.2010.04.019
24. Pomeroy L, Bansal S, Tildesley M, Moreno-Torres K, Moritz M, Xiao N, et al. Data-driven models of foot-and-mouth disease dynamics: a review. *Transbound Emerg Dis* (2015). doi:10.1111/tbed.12437
25. Quan M, Murphy C, Zhang Z, Alexandersen S. Determinants of early foot-and-mouth disease virus dynamics in pigs. *J Comp Pathol* (2004) 131:294–307. doi:10.1016/j.jcpa.2004.05.002
26. Alexandersen S, Oleksiewicz MB, Donaldson AI. The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time-course study using TaqMan RT-PCR. *J Gen Virol* (2001) 82:747–55. doi:10.1099/0022-1317-82-4-747
27. Stenfeldt C, Pacheco JM, Rodriguez LL, Arzt J. Early events in the pathogenesis of foot-and-mouth disease in pigs; identification of oropharyngeal tonsils as sites of primary and sustained viral replication. *PLoS One* (2014) 9(9):e106859. doi:10.1371/journal.pone.0106859
28. Bates TW, Thurmond MC, Hietala SK, Venkateswaran KS, Wilson TM, Colston BW Jr, et al. Surveillance for detection of foot-and-mouth disease. *J Am Vet Med Assoc* (2003) 223:609–14. doi:10.2460/javma.2003.223.609
29. Wee SH, Park JY, Joo YS, Lee JH, An SH. Control measures implemented during the 2002 foot-and-mouth disease outbreak in the Republic of Korea. *Vet Rec* (2004) 154:598–600. doi:10.1136/vr.154.19.598
30. McLaws M, Ribble C. Description of recent foot and mouth disease outbreaks in nonendemic areas: exploring the relationship between early detection and epidemic size. *Can Vet J* (2007) 48:1051–62.
31. Gibbens JC, Sharpe CE, Wilesmith JW, Mansley LM, Michalopoulou E, Ryan JB, et al. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. *Vet Rec* (2001) 149:729–43. doi:10.1136/vr.149.24.729

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer [BB] declared a past supervisory role with one of the authors [AP] to the handling Editor, who ensured that the process met the standards of a fair and objective review.

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ORIGINAL RESEARCH
published: 22 September 2016
doi: 10.3389/fvets.2016.00085

A Comparative Assessment of the Risks of Introduction and Spread of Foot-and-Mouth Disease among Different Pig Sectors in Australia

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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *04 July 2016*
Accepted: *09 September 2016*
Published: *22 September 2016*

Citation:
Hernández-Jover M, Schembri N, Holyoake PK, Toribio J-ALML and Martin PAJ (2016) A Comparative Assessment of the Risks of Introduction and Spread of Foot-and-Mouth Disease among Different Pig Sectors in Australia. Front. Vet. Sci. 3:85. doi: 10.3389/fvets.2016.00085

Small-scale pig producers are believed to pose higher biosecurity risks for the introduction and spread of exotic diseases than commercial pig producers. However, the magnitude of these risks is poorly understood. This study is a comparative assessment of the risk of introduction and spread of foot-and-mouth disease (FMD) through different sectors of the pig industry: (1) large-scale pig producers; (2) small-scale producers (<100 sows) selling at saleyards and abattoirs; and (3) small-scale producers selling through informal means. An exposure and consequence assessments were conducted using the World Organization for Animal Health methodology for risk analysis, assuming FMD virus was introduced into Australia through illegal importation of infected meat. A quantitative assessment, using scenario trees and Monte Carlo stochastic simulation, was used to calculate the probabilities of exposure and spread. Input data for these assessments were obtained from a series of data gathering exercises among pig producers, industry statistics, and literature. Findings of this study suggest there is an *Extremely low* probability of exposure (8.69×10^{-6} to 3.81×10^{-5}) for the three sectors of the pig industry, with exposure through direct swill feeding being 10–100 times more likely to occur than through contact with infected feral pigs. Spread of FMD from the index farm is most likely to occur through movement of contaminated fomites, pigs, and ruminants. The virus is more likely to spread from small-scale piggeries selling at saleyards and abattoirs than from other piggeries. The most influential factors on the spread of FMD from the index farm is the ability of the farmer to detect FMD, the probability of FMD spread through contaminated fomites and the presence of ruminants on the farm. Although small-scale producers selling informally move animals less frequently and do not use external staff, movement of pigs to non-commercial pathways could jeopardize animal traceability in the event of a disease outbreak. This study suggests that producers' awareness on and engagement with legislative and industry requirements in relation to biosecurity and emergency animal disease management needs to be improved. Results from this study could be used by decision-makers to prioritize resource allocation for improving animal biosecurity in the pig industry.

Keywords: biosecurity, surveillance, emergency animal disease management, risk assessment, foot-and-mouth disease

INTRODUCTION

Small landholders are commonly thought to pose biosecurity risks to mainstream livestock production, although the magnitude and significance of these risks have not been previously evaluated. Practices of small-scale pig producers are believed to be associated with a higher risk of introduction and spread of exotic diseases than those of larger producers (1–5). Previous research suggests that small-scale pig producers selling at livestock markets (saleyards) had poor on-farm biosecurity practices, poor disease knowledge and understanding of swill feeding, and limited veterinary contact (6–11). Similar concerns in relation to biosecurity and animal disease management were reported in a qualitative study among small-scale pig producers selling through informal means in Australia conducted in 2009, which provided an insight into the implementation of and attitudes toward biosecurity among this sector of the industry (12).

Characteristics of Australia, such as geographical isolation and quarantine procedures, provide the country with a privileged disease free status for major livestock diseases, like foot-and-mouth disease (FMD). However, the potential for the introduction of exotic diseases still exists. Illegal introduction of meat products by incoming passengers from infected countries is the highest risk source of entry of FMD into Australia (13–15). Between 1997 and 2000, there was a 29% increase in declared and detected animal products brought by passengers entering Australia from FMD-infected countries and for the highest risk group of countries the increase was 43% (14). Pigs are highly susceptible to FMD and once infected excrete high concentrations of the virus in aerosol form, being considered a major amplifying host for this disease (16). Feeding of infected meat scraps has been identified as one of the major pathways of introducing FMD into a free country (13, 15, 17). The source of a FMD outbreak in South Africa in 2000 was meat scraps from a ship's garbage being fed to pigs (18) and during the 2001 FMD outbreak in the United Kingdom, a small-scale pig farm where unprocessed pig swill was fed to pigs, was considered the source case of the virus introduction (19). Similarly than for FMD, illegal introduction of meat products and subsequent swill feeding has also been suggested as the cause of outbreaks of classical swine fever (CSF) in the European Union in the 2000s and the introduction of African swine fever (ASF) in Eastern Europe in 2007 (20, 21). Previous studies have investigated the risk of introduction of and subsequent exposure to emergency animal diseases, such as FMD, CSF, and ASF, from the illegal importation of meat products (20, 22, 23). Hartnett et al. (22) quantified the risk of FMD introduction and exposure in Great Britain and Costard et al. (20) assessed the risk of ASF introduction and exposure in Europe. In both studies, poor biosecurity practices, especially among backyard producers and the presence of feral pigs were identified as highly influential on the probability of exposure of domestic pigs to these viruses.

The Productivity Commission (14) and Buetre et al. (24) assessed the impact of an FMD outbreak in Australia, considering a number of outbreaks of varying intensity. The most significant consequence of an FMD outbreak in Australia, independently of the location within the country, would be the immediate closure of the export market of livestock products to FMD-free countries, such as Japan and United States of America, which would remain for at least 3 months after eradication. The direct economic impacts of a FMD outbreak in Australia would be mainly due to the cost of control and eradication and a loss of revenue to affected livestock commodities from a decrease in export and domestic sales. The most recent assessment estimated a direct economic impact of $5.6 to $51.8 billion over a 10-year period, depending on the size of the outbreak. In addition, these financial effects would also have significant social impacts at an individual, household, and community levels, such as mental health issues and reduced welfare and well-being (24).

Spread of disease from the index farm will depend on on-farm biosecurity practices and animal movement patterns of pig enterprises. Understanding these practices among the different sectors of the pig industry is crucial to assess the risk of exotic disease introduction and spread posed by each of these sectors. This study conducts a comparative exposure and partial consequence assessment among different sectors of the pig industry in Australia. The aim of this study is to investigate how the FMD virus, which is assumed to be introduced into the country through illegal importation of contaminated meat, could exposure pigs at the index piggery and subsequently spread from this piggery. The sectors of the pig industry considered are: (1) large-scale or mainstream pig producers; (2) small-scale producers (<100 sows) selling through saleyards and abattoirs; and (3) small-scale producers selling through informal means. These assessments quantify the nature and magnitude of the biosecurity risks posed by each sector of the pig industry. This information could support decision-makers for the prioritization of resources allocation for improving biosecurity in the pig industry.

MATERIALS AND METHODS

Exposure and Consequence Assessment Models

This comparative risk assessment follows the World Organization for Animal Health (OIE) methodology for risk analysis (25) and uses scenario tree models to represent the potential pathways of exposure and spread and subsequently calculate the corresponding probabilities of these occurring. Scenario trees provide an effective way of identifying pathways and information requirements and a framework for a quantitative analysis (26, 27).

An entry assessment as outlined by the OIE risk analysis methodology was not performed in this study as this assessment assumed that FMD had already been introduced into Australia. The assumption was that the virus was introduced through illegal importation of FMD-infected salted or cured meat and an estimated amount of introduced infected meat per year of 5 kg was used. The exposure assessment describes the potential pathways for pigs from the three different types of piggeries getting exposed to the FMD virus and estimates the probability of these pathways to occur. The partial consequence assessment describes the potential pathways of spread of FMD virus from the index farm and estimates the probability of this spread occurring. The

assessment of the impacts of the resulting FMD outbreaks after virus introduction is not reported in this manuscript. The scenario trees were implemented in Microsoft Excel (PC/Windows XP, 2006) and probabilities were determined using Monte Carlo stochastic simulation modeling with the @RISK software (@Risk 6.0, Palisade Corporation, USA). The outcome probabilities for each pathway of exposure and spread were calculated as a product of all conditional probabilities describing the nodes of each specific pathway. The overall probability of exposure and spread for each type of piggery were obtained by adding the probabilities for each of the exposure and spread pathways, respectively, given these pathways are independent (27). Each simulation consisted of 50,000 iterations sampled using the Latin hypercube method with a fixed random seed of one.

Population Framework

Different definitions on small-scale pig producers can be found. The Australian pig body representative, Australian Pork Limited (APL), defines small landholder as those pig producers with less or equal than 50 sows and/or trading less or equal to 1000 pigs per year (28). Biosecurity Australia in their Import Risk Analysis for Pig Meat (13) classified backyard pig producers as those with less than 10 sows, small pig-producing enterprises those with between 10 and 99 sows, and commercial enterprises those with more than 99 sows. This classification was based on the assumption that management practices, such as feeding, husbandry, and motivation to keep pigs, where significantly different between these groups. Research into small-scale pig producers trading via saleyards in eastern Australia has shown no differences on on-farm practices among producers with 1–100 sows (8).

For the purpose of this assessment, small-scale pig producers are those with less than 100 sows with those piggeries with more than 100 sows being defined as commercial enterprises. Moreover, this assessment considers the differences on livestock trade patterns among small-scale pig producers as some livestock movements could pose higher risk for disease transmission. As a consequence, small-scale producers were subsequently classified into two groups; those selling through saleyards and abattoirs, and those selling mainly through informal means. Informal sales included internet, word-of-mouth, family and friends, and local businesses.

Data Sources

Data used to populate the exposure and partial consequence assessments were obtained from different data gathering exercises, published literature, and industry statistics. Below is a description of the data gathering exercises to collect information to populate the models used in these assessments.

Postal Questionnaire, Interviews, and Focus Groups with Pig Producers Selling through Saleyards in Eastern Australia

A three-part study involving pig producers at six saleyards situated in eastern Australia was conducted in a 12-month period starting at the end of 2006 (6). The first part of the study was the distribution of a postal questionnaire, which gathered basic data on the demographics and husbandry practices, among all producers who traded pigs at saleyards during the 2005 calendar year ($n = 815$). The second part of the study involved face-to-face interviews with producers ($n = 106$) who indicated their willingness to participate during the postal questionnaire, along with volunteers opportunistically recruited from the study saleyards. The interview collected detailed information on demographics, husbandry practices, nutrition, herd health, biosecurity practices, movement practices, animals identification systems, and communication networks (8, 9, 35, 42, 43). The final part of the study consisted in nine one-off focus group discussions, with 5–12 producers in each discussion, to investigate in depth attitudes and behaviors of producers toward diseases, disease reporting, traceability, and communication networks (6, 42–44). Focus group participants ($n = 34$) were recruited on a voluntary basis from face-to-face interviews and from advertisements placed at the saleyards and in stock agent newsletters. This study included mainly small-scale producers selling through saleyards although there were a small proportion of large-scale producers and small-scale producers selling through informal means.

Case Study Interviews and Questionnaires with Small-Scale Pig Producers Selling through Informal Means

To improve our understanding of practices of small-scale pig producers selling by informal means (internet, word-of-mouth, family and friends, and local businesses), a total of 13 small-scale ($\leq$100 sows) pig producers using this marketing strategy were interviewed in New South Wales (12). This questionnaire, which was distributed using face-to-face interviews, gathered in-depth information on demographics and practices on husbandry, feeding, herd and health management, biosecurity, and pig movements. Producers were recruited at agricultural shows and through stage government databases. In addition, to collect supporting data in relation to practices of this sector of the pig industry, a shorter questionnaire covering similar topics was developed to be distributed by post among members of the Australian Pig Breeders Association ($n = 29$) and face-to-face among participants ($n = 24$) of pig industry field days.

Exposure Assessment

This assessment evaluates the probability of exposure of a pig from a piggery to FMD-infected meat that has been illegally introduced into the country. The assessment considers that the FMD-infected meat could end up in any household in the country, with or without pigs. Four different pathways have been identified as potential pathways of exposure of a pig at an index piggery for each of the three piggery types (small-scale piggery selling by informal means; small-scale piggery selling at saleyards and abattoirs; or large-scale piggery), depending on: (1) the type of household where the meat is destined to; (2) the proportion of waste discarded from this meat; (3) the involvement of feral pigs in the pathway; and (4) the probability of pig producers feeding swill to their pigs. These pathways of exposure are:

- *Exposure 1:* The FMD-infected meat ends up in a household without pigs and some of this meat is discarded as waste. This waste is then accessible to feral pigs and these pigs become infected with FMD. As the final step of the pathway, the

infected feral pigs travel to the index piggery getting in contact with domestic pigs from this piggery.

- *Exposure 2:* The FMD-infected meat ends up in the index piggery and some of this meat is discarded as waste. This waste is then fed directly to the domestic pigs in the piggery.
- *Exposure 3:* The FMD-infected meat ends up in the index piggery and some of this meat is discarded as waste. This waste is then accessible to feral pigs around the piggery and these pigs become infected with FMD. The feral pigs get in contact with domestic pigs from the same piggery.
- *Exposure 4:* The FMD-infected meat ends up in a non-index piggery, and some of this meat is discarded as waste. This waste is then accessible to feral pigs and these pigs become infected with FMD. The infected feral pigs travel to the index piggery getting in contact with domestic pigs from this piggery.

A scenario tree was developed to represent these four pathways of exposure and the same structure of the scenario tree was used for modeling the risk of FMD exposure among the three groups of pig producers considered in this study. **Figure 1** represents the scenario tree considering a small-scale piggery selling through saleyards and abattoirs is the index piggery. Some of the parameter estimates and input values differed between the three groups of pig producers. **Table 1** summarizes the nodes used for the exposure scenario tree and a detailed description of the nodes and input parameters used are provided in the online supplemental material.

Consequence Assessment

Once the first pig from a piggery (small- or large scale) is exposed to the FMD virus, different potential outbreak scenarios could occur depending on different factors. This partial consequence assessment evaluates the potential outbreak scenarios and their corresponding probabilities occurring. The main factors considered in the consequence assessment are the ability of the farmer to detect the disease, the presence of ruminants on the farm and the movement of animals, fomites, and people from the index farm. In the event that the infection in the index farm is not detected, the virus could spread beyond this property. Six main outbreak scenarios have been identified:

- *Scenario 1*: This scenario represents no spread beyond the index farm, which could occur in different situations: (1) in a piggery

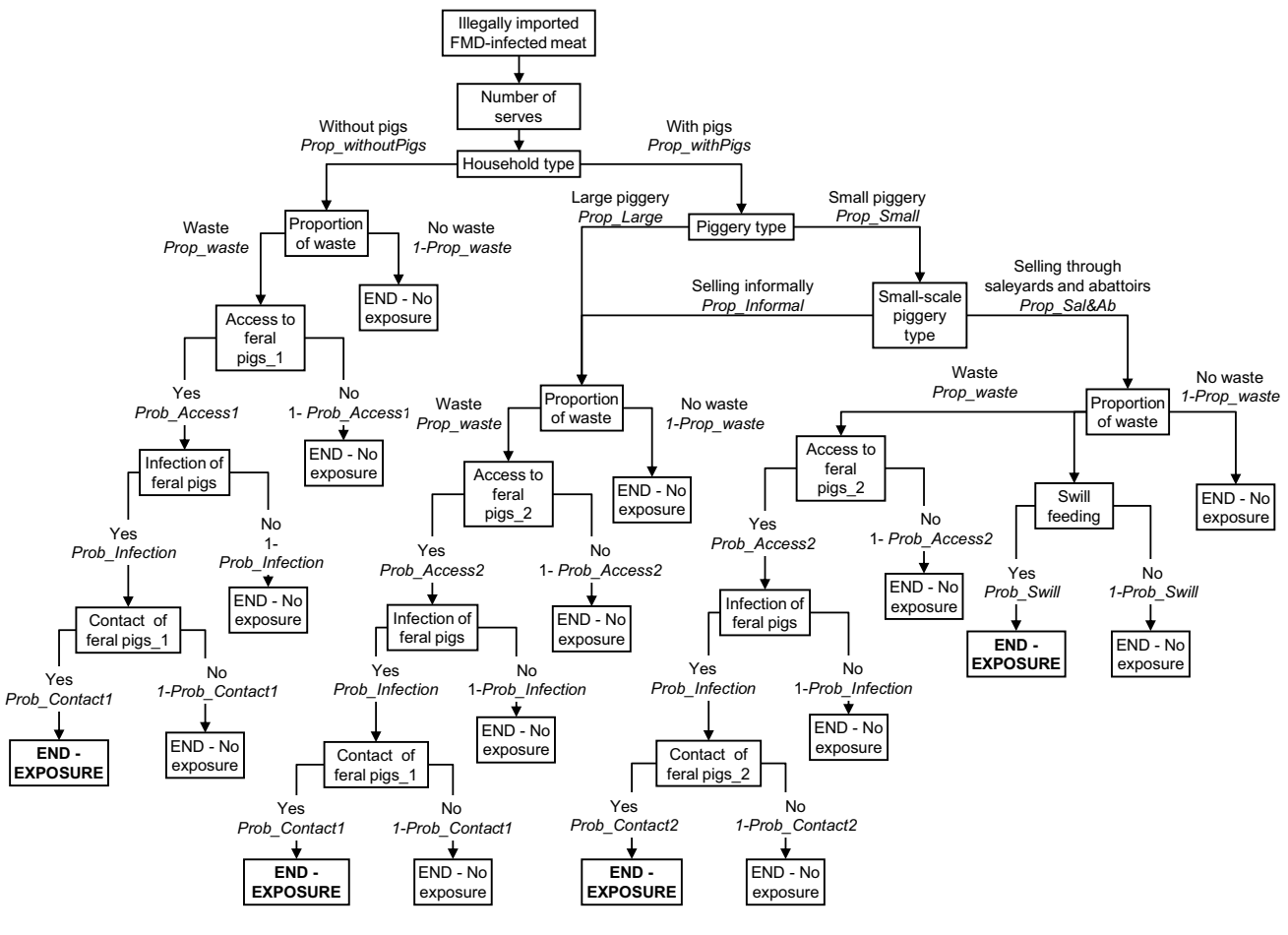

FIGURE 1 | Scenario tree representing the exposure pathways of a domestic pig from a small-scale piggery (<100 sows) selling through saleyards and abattoirs to foot-and-mouth disease (FMD) virus from FMD-infected meat illegally introduced into Australia.

TABLE 1 | Nodes, parameter estimates, and input values used for the exposure assessment estimating the probability of a piggery being exposed to FMD-infected meat illegally introduced into Australia through incoming passengers from overseas.

	Name	Outcome	Parameter estimates	Input value	Data sources
1	Household type	Without pigs With pigs	Proportion of households with and without pigs in Australia (*Prop_withPigs; Prop_withoutPigs*)	Total households: Pert (8.7, 9.2, 9.6 M) Households with pigs: large piggery (>100 sows) + small piggery + growers • Large piggery: 315 • Growers: 524 • Small piggery: Pert (1409, 1550, 620)	(8, 29, 30)
2	Proportion of waste	Waste No waste	Number of serves in 5 kg of meat Proportion of meat discarded as waste (*Prop_waste*)	Single serve size of meat: average, 50 g (25–100 g), Pert (0.025, 0.05, 0.1) Number of serves in 5 kg of meat: 5 kg/single serve size *Prop_Waste*: Pert (0.01, 0.02, 0.05)	(13, 31)
3	Piggery type	Large piggery (> 100 sows) Small piggery (<100 sows)	Proportion of large and small-scale piggeries in Australia (*Prop_Small*; *Prop_Large*)	Large-scale piggeries: 839 (315 breeding, 524 contract growers) Small-scale piggeries: Pert (1071, 1178, 1232)	(8)
4	Small-scale piggery type	Selling informally Selling through saleyards and abattoirs	Proportion of these two types of piggeries among small-scale piggeries (*Prop_Informal*; *Prop_Sal&Ab*)	Number small-scale piggeries: 589 Number of small-scale piggeries selling informally: Pert [Beta (38,553), +50%, +70%]	Number of small-scale piggeries selling informally: Pert [Beta (38,553), +50%, +70%] (8, 12); Questionnaire with specific groups of pigs producers
5	Access of feral pigs to waste	Yes No	Probability of waste from households without pigs getting in contact with feral pigs (*Prob_Access1*); probability of waste from piggeries getting in contact with feral pigs (*Prob_Access2*)	*Prop_Access1* = Cumul (Probability access and located, Proportion of households in different areas) • Probability of access and located = Probability of waste being accessible [*High* (Uniform (0.7, 1)) in remote areas, *Moderate* (Uniform (0.3, 0.7)) in rural areas and *Very low* (Uniform (0.001, 0.05)) at large towns] × Probability of waste being located by the feral pigs *Very low* [Uniform (0.001, 0.05)] in remote areas, *Extremely low* [Uniform (0.000001, 0.001)] in rural areas and *Negligible* [Uniform (0, 0.000001)] at large towns • Proportion of households: 3% remote, 11% rural, and 86% large towns *Prob_Access2* = Pert (*Prob_Access1*, +15%, +20%)	(13)
6	Infection of feral pigs	Yes No	Probability of the feral pigs being infected once they are in contact with the FMD-contaminated waste (*Prob_Infection*)	*Prob_Infection* = Probability of the infected meat contains sufficient dose to cause infection of feral pigs *High* [Uniform (0.7, 1)] × Viability of the virus in the infected waste until the feral pig contacts with this waste *High* [Uniform (0.7, 1)]	(13, 15)
7	Contact of feral pigs with domestic pigs	Yes No	Probability feral pigs infected via waste from other households contact pigs from the index piggery (*Prob_Contact1*); probability feral pigs infected via waste originated in the index piggery contact pigs from the same piggery (*Prob_Contact2*)	*Prob_Contact2* = Proportion of producers reporting feral pigs around their property [Small-scale saleyard and abattoir, Beta (46, 127); small-scale selling informally, Beta (6, 17)]; large-scale, Pert (max–50%, max-20%, 23/149) *Prob_Contact1* = Pert (–50%, –20%, *Prob_Contact2*)	(8, 12); Questionnaire with specific groups of pigs producers
8	Swill feeding	Yes No	Probability of swill feeding (*Prob_Swill*) among producers	Small-scale selling through saleyards and abattoirs: Pert (Most likely – 50%, 19/109, Most likely +20%) Small-scale selling informally: Pert (Most likely – 50%, 5/22, Most likely +20%) Large-scale: Pert (Most likely – 50%, 6/41, Most likely +20%)	(8, 9, 12); Questionnaire with specific groups of pigs producers

with pigs only, when infection is detected and reported; (2) in a piggery with pigs and ruminants, when infection is detected in both species and reported; (3) in a piggery with pigs only, when infection is not detected, but there are no movement of pigs off the farm during the infective period either movements of contaminated fomites. In this last situation, the infection would die out before spread occurs. If ruminants are kept on the farm, these are very likely to become infected before FMD is detected in pigs and moved off the farm (see *Scenario 5*).

- *Scenario 2*: Infection is not detected (in pigs and ruminants) at the first exposed piggery and FMD virus is spread through movement of pigs off farm. This spread could be more or less significant and at local, regional, or national level, depending on the destination of the animals. Within *Scenario 2*, further scenarios were identified depending on the destination of the animals moving from the index farm when the infection is initially not detected by the farmer. These scenarios slightly differed between small and large-scale piggeries, and are described in **Table 2**.
- *Scenario 3*: Infection in the first exposed piggery is spread through movement of contaminated fomites. Spread through fomites can happen independently of farmer detection. If the farmer does not detect, spread of the virus to other properties through contaminated fomites is more likely than when detection occurs. However, if detection is delayed, spread through fomites can still happen. For this assessment, fomites were defined as mechanical vectors and included vehicles, equipment, and clothing.
- *Scenario 4*: Infection in the first exposed piggery is spread through movement of people carrying infective virus particles in the respiratory tract. As the previous scenario, spread through contaminated people is more likely to happen when the farmer does not detect the infection. When detection occurs but it is in late stages of the infection, spread through people could also be possible. Spread through people carrying the virus was considered separate to the spread through fomites as a person could be contaminated despite biosecurity measures being applied to avoid spread through fomites (e.g., disinfection of equipment).
- *Scenario 5:* Infection is not detected by the farmer (in pigs and ruminants) in the first exposed piggery and FMD virus is spread through movement of ruminants off farm. Even when infection is detected in pigs, movement of infected ruminants off the farm could occur before infection is detected in pigs. This spread could be more or less significant at a local, regional, or national level, depending on the destination of the animals. Information on the movement of ruminants off the farm, such as potential destinations and frequency of movements, was not collected during this assessment. However, information on the presence and number of ruminants kept on the farm was used to evaluate the likelihood of the spread through ruminant movement.
- *Scenario 6*: Infection in the first exposed piggery is spread through airborne transmission. Airborne transmission of FMD has been extensively investigated in the past (45, 46). It was not the objective of this assessment to assess the potential spread of FMD through airborne transmission as this could occur independently of the biosecurity practices of the piggery, evaluation of which is the main objective of this assessment.

Two different consequence scenario trees were developed to represent the previously described potential outbreak scenarios for small and large-scale piggeries. The only difference in the structure of these scenario trees was the potential destinations where pigs from the index farm could go once moved off the farm. **Figure 2** represents the consequence scenario tree used for modeling the probability of FMD spread from small-scale pig producers. **Table 3** describes the nodes, input values and data sources used for the small-scale piggery consequence scenario tree. A description of the scenario tree used for the consequence assessment for large-scale piggeries is shown in **Table 4**. A detailed description of the nodes and input parameters used for both consequence assessments are provided in the online supplemental material.

Sensitivity Analysis

The influence of some input parameters on the model outputs was investigated using the @Risk Advanced Sensitivity Analysis (@RISK 6.0, Palisade Corporation, USA). For the probability of exposure for each piggery type, the input parameters evaluated were the probability of pig producers swill feeding (*Prob_Swill)* and the amount of meat illegally introduced into Australia. For investigating which practices had the most influence on the probability of spread of the virus, the following spread scenarios were included in the sensitivity analysis: *Scenario 1* (no spread beyond the index farm), *Scenario 2* (spread through movement of pigs), *Scenario 3* (spread through contaminated fomites), and *Scenario 5* (spread through movement of ruminants). The input parameters investigated were the probability of the farmer detecting FMD in pigs (*Prob_1stDet)* and ruminants (*Prob_1stDet_Rum)*, the presence of ruminants on the farm (*Prop_Rum*), the probability of FMD transmission through contaminated fomites moving off the index farm (*Prob_Fomites*), and the probability of movement of pigs (*Prob_MovPigs*) and ruminants (*Prob_MovRum*) off the farm.

Probability input values were allowed to vary from 0 to 1 in tenths (0.1, 0.2, 0.3…) and the values used for the amount of meat illegally introduced into Australia were 10, 50, 100, and 200 kg. Each of the values for each input parameter was evaluated separately in a simulation of 5,000 iterations, while values for all other input variables were fixed to the base value.

RESULTS

Exposure Assessment Results

The exposure assessment evaluated potential pathways of exposure of a pig from a piggery to FMD-infected meat illegally introduced into Australia and estimated the probability of these pathways to occur. Four potential exposure pathways were identified in this assessment, which were mainly dependent on the type of household in Australia where the contaminated meat was destined to and the characteristics of these households. Description of the exposure pathways, the likelihood of each of these pathways to occur and the overall likelihood of exposure for

TABLE 2 | A description of the potential scenarios of spread of FMD virus from an infected small-scale (<100 sows) or large-scale piggery in Australia, due to movement of pigs off farm, according to different destinations.

Scenario	Piggery type	Description
a	Large/small scale	*Infected animals moving to another piggery (small-scale piggery for the small-scale scenario; large-scale piggery for the large-scale scenario) where the infection is detected and reported:* this scenario represents limited spread to a local pig community, depending on movement of other animals from the index farm until infection is detected at the second piggery, time of the detection at the second piggery, the presence of ruminants at this piggery and the movement of animals from this piggery before detection
b	Large/small scale	*Infected animals moving to another piggery (small-scale piggery for the small-scale scenario; large-scale piggery for the large-scale scenario) where the infection is not detected:* this scenario represents spread of the infection. The magnitude of this spread will depend on other animal movements from the index farm, the presence of ruminants at the second piggery, and the movement of animals from this piggery
c	Large scale	*Infected animals moving to a small-scale piggery where the infection is detected and reported:* this scenario is similar than *Scenario a*; however, the spread would be more limited as there would be less animals that could get infected at the second piggery
d	Large scale	*Infected animals moving to a small-scale piggery where the infection is not detected and infection is spread:* this scenario is similar than *Scenario b* although the spread would be more limited due to the lower number of animals that would be affected and could move from the second piggery
e	Small scale	*Infected animals are transferred to a person who keeps pigs as pets (private individual) and infection is detected and reported at this second location:* this scenario represents limited spread to a local pig community. Similar than *Scenario a*, the extent of the spread will depend on other animal movements from the first exposed piggery until infection is reported by the private individual, time of the detection and reporting at this second location, the presence of ruminants at this location and the movement of other animals from this location before detection
f	Small scale	*Infected animals are transferred to a person who keeps pigs as pets (private individual) and infection is not detected at this second location:* this scenario represents spread of the infection. The magnitude of this spread will depend on other animal movements from the first exposed piggery, the presence of ruminants at the second location and the movement of animals from this location. It is assumed that the extent of the spread of this scenario would be less significant than in *Scenario b* as a private individual keeping pigs as pets is less likely to move these or other animals off the farm
g	Small scale	*Infected animals moving to an agricultural show, where the infection is detected:* in this scenario, once the infection is detected, all movements to and from the show would be stopped. Spread to animals attending the agricultural show and also outside the show can occur depending on time of detection and movement of animals, fomites, and people off the show before detection
h	Small scale	*Infected animals moving to an agricultural show, where the infection is not detected:* spread of the infection would be more significant than any of the previous scenarios. Animals attending agricultural shows can travel from the same region, the same state, and also from other states in Australia. If infection is not detected at the show, all susceptible livestock could be infected, and infection could spread to any of the locations where animals are moved from the show. The spread could affect all susceptible livestock species at a local, regional, and national level, depending on destination of animals from the show and time of detection of the infection once infected animals move from this agricultural show
i	Small scale	*Infected animals moving to another property for home-kill:* independently of detection, animals will be killed so spread will be very limited, and its extent will depend on other animal movements from the first exposed piggery, the period of time until the infected animal is killed at the second property and the presence of ruminants in this property
j	Large/small scale	*Infected animals moving to a saleyard where infection is detected:* in this scenario, once infection is detected, all movements to and from the saleyard would stop. Spread would be limited to the first exposed piggery and animals attending the saleyard; however, spread could go beyond the animals attending at the saleyards if movement of animals and fomites from the saleyard occur before the detection of the infection. The extent of the spread of this scenario will also depend on other animal movements from the first exposed piggery until the infection is detected at the saleyard
k	Large/small scale	*Infected animals moving to a saleyard where infection is not detected:* spread of the infection could be significant at local and regional levels, although national spread could also occur as animals travel interstate to be sold at saleyards. Thus, the extent of the spread will depend on other animal movements from the first exposed piggery, movements of animals, and contaminated fomites from the saleyard and presence of ruminants at the saleyard
l	Large/Small-scale	*Infected animals moving to an abattoir where infection is detected:* once infection is detected at the domestic abattoir, all movements to and from the abattoir would be disrupted. This scenario represents a locally limited spread to the first exposed piggery and animals attending at the abattoir. Animal movement from the abattoir is uncommon; however, contaminated vehicles could spread the infection if they move from the abattoir before the infection is detected. The extent of the spread of this scenario will also depend on other animal movements from the first exposed piggery until the infection is detected at the abattoir
m	Large/small scale	*Infected animals moving to an abattoir where infection is not detected:* in this scenario, non-detected infected animals would be slaughtered and spread would most likely be limited to the local community, depending on other animal movements from the first exposed piggery and movement of infected animals and fomites from the abattoir

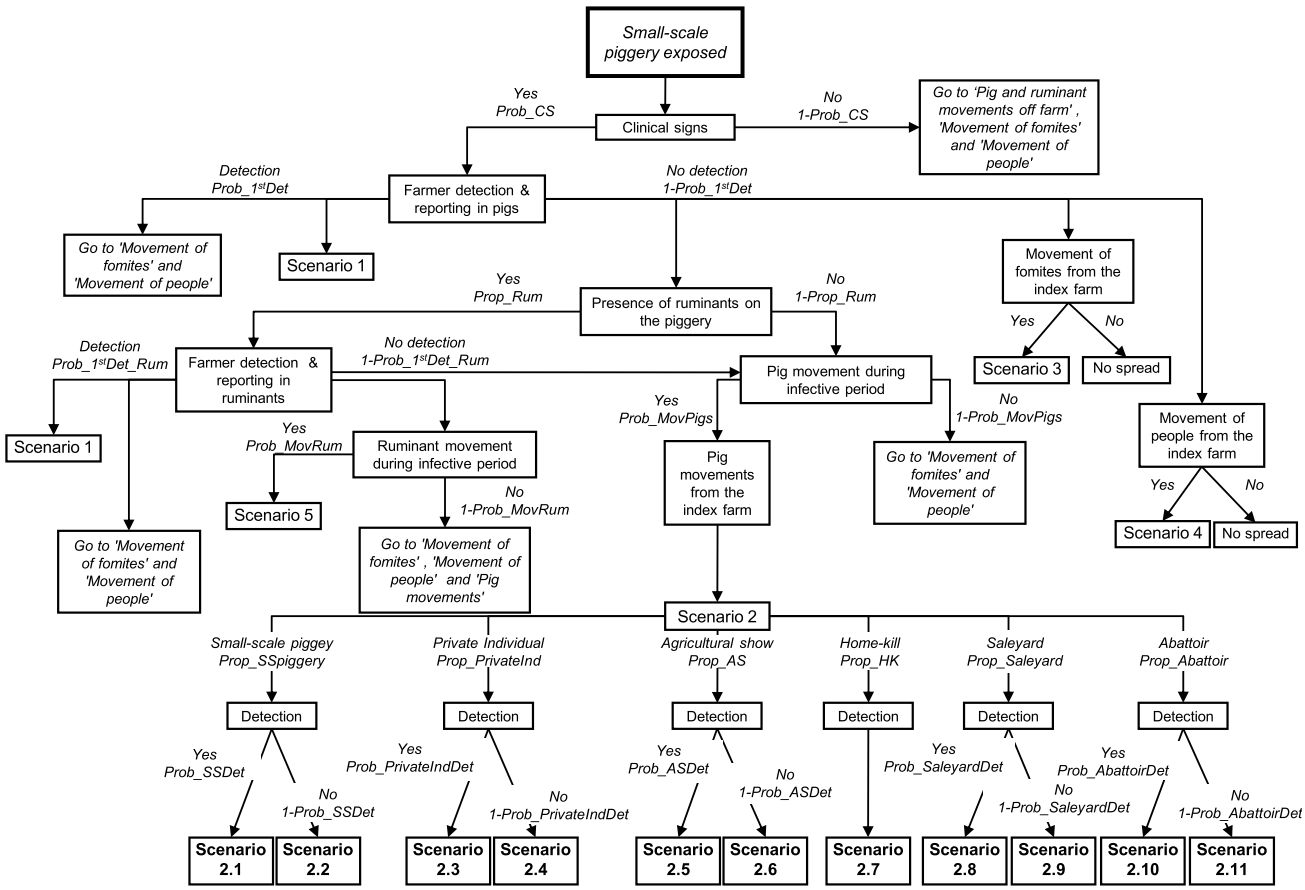

FIGURE 2 | Scenario tree used for a spread assessment investigating the potential outbreak scenarios of foot-and-mouth disease (FMD) from a small-scale piggery (<100 sows) in Australia.

the three assessments (small-scale pig producers selling through informal means, small-scale pig producers selling at saleyards and abattoirs and large-scale pig producers) are presented in **Table 5**.

Results indicate that the most likely pathway of exposure to FMD-infected meat illegally introduced into Australia among the three types of piggeries is through the direct feeding of the infected meat to the pigs (*Exposure 2*). The probability of this exposure pathway to occur is *extremely low* (qualitative descriptors based on Guidelines for Import Risk Analysis; DAFF, 2004), with one exposure estimated to occur for every 10,000–100,000 times FMD-infected meat is illegally introduced into the country, depending on the type of piggery. The lowest value was estimated for small-scale piggeries selling informally as shown in **Table 5**. Probabilities of other pathways were considered to be *negligible*. Exposure 1, which represents the exposure through feral pigs which have been infected from contaminated waste from a household without pigs, has a higher probability to occur than the other two pathways, given most households in Australia do not have pigs (99.9%) and the illegally introduced meat is more likely to be destined to these households than households with pigs. The overall probability of exposure was estimated 8.69×10^{-6}, 7.26×10^{-5}, and 3.81×10^{-5} (*extremely low*), for small-scale piggeries selling informally, those selling through saleyards and abattoirs and large-scale piggeries, respectively. The probabilities of exposure among small-scale piggeries selling through saleyards and abattoirs and large-scale piggeries are slightly higher than that for small-scale piggeries selling informally, due to the higher number of producers within the former categories, and the higher potential contact between feral and domestic pigs in these piggeries.

This assessment considered that households without and with pigs had even probability of illegally introducing meat into the country. When evaluating the probability of exposure of the pigs to the infected meat, once this meat is introduced to the piggery of concern, a *very low* probability (0.003–0.005) was obtained for all piggery types, given most of this meat will be consumed and only a small proportion will be waste that could be fed to the pigs.

Consequence Assessment Results

Following the exposure of a pig from a piggery, six main scenarios have been identified depending on the ability of the farmer to detect FMD and the movement of animals, fomites, and people from the index farm. These scenarios are the same for the three groups of pig producers.

TABLE 3 | Nodes, parameter estimates, and input values used for the partial consequence assessment estimating the probability of potential outbreak scenarios after a small-scale (<100 sows) piggery has been exposed to FMD-infected meat illegally introduced into Australia through incoming passengers from overseas.

Node	Name	Outcome	Parameter estimates	Input value	Data sources
1	Clinical signs	Yes No	Probability that an FMD-infected animal would display clinical signs (*Prob_CS; Prob_CS_2*)	Incubation period: lognormal [5, 2.7, Truncate (1, 12)]; infective period: uniform (14, 30); Time to the onset of clinical signs = Incubation period – 2 days *Prob_CS* = (Infective period – Incubation period)/Infective period *Prob_CS_2* = (Infective period – Time to the onset of clinical signs)/Infective period	(13, 15, 16)
2	Farmer detection and reporting in pigs	Detection No detection	Probability of the farmer from the index piggery detecting and reporting FMD in pigs for each type of piggery (*Prob_1stDet*)	*Prob_1stDet* = Probability of the farmer detection × Probability of the farmer reporting Probability of the farmer detection: • Small-scale selling at saleyards and abattoirs: Pert (0.32, 0.4, 0.48) • Small-scale selling informally: Pert (0.4, 0.5, 0.6) Probability of farmer reporting: • Small-scale selling at saleyards and abattoirs: Pert (0.48, 0.6, 0.72) • Small-scale selling informally: Pert (0.6, 0.75, 0.9)	(7, 8, 12); Questionnaire with specific groups of pigs producers
3	Presence of ruminants on the piggery	Yes No	Proportion of pig farms keeping also ruminants (*Prop_Rum*)	Small-scale selling at saleyards and abattoirs: Beta (461,93) Small-scale selling informally: Beta (33, 6)	As Node 2
4	Farmer detection & reporting in ruminants	Yes No	Probability of the farmer from the first exposed piggery detecting and reporting the FMD infection in ruminants (*Prob_1stDet_Rum*)	*Prob_1stDet_Rum* = Probability of the farmer detection × Probability of the farmer reporting Probability of the farmer detection: • Small-scale selling at saleyards and abattoirs: Pert (*Prob_1stDet*, +10%, +20%) • Small-scale selling informally: Pert (*Prob_1stDet*, +10%, +20%) Probability of farmer reporting: • Small-scale selling at saleyards and abattoirs: Pert (0.48, 0.6, 0.72) • Small-scale selling informally: Pert (0.6, 0.75, 0.9)	(15)
5	Pig movement during infective period	Yes No	Probability of pig movements during the infective period (*Prob_MovPigs*)	Small-scale selling at saleyards and abattoirs: Pert (–20%, 0.6, +20%) Small-scale selling informally: Pert (–20%, 0.3, +20%)	As Node 2
6	Ruminant movement during infective period	Yes No	Probability of ruminant movement during the infective period (*Prob_MovRum*)	Small-scale selling at saleyards and abattoirs: *Moderate* [Uniform (0.3, 0.7)] Small-scale selling informally: *Low* [Uniform (0.05, 0.3)]	As Node 2

(Continued)

TABLE 3 | **Continued**

Node	Name	Outcome	Parameter estimates	Input value	Data sources
7	Pig movement from the index farm	Small scale Piggery Private individual Agricultural show Home-kill Saleyard Abattoir	Proportion of movement of pigs to each of these destinations (*Prop_SSpiggery; Prop_PrivateInd; Prop_AS; Prop_HK; Prop_Saleyard; Prop_Abattoir*)	Beta (n + 1, s – n + 1) for each proportion Movements of small-scale selling at saleyards and abattoirs (*n* = 883): • Small-scale piggery: 113 • Private Individual: 26 • Agricultural Show: 5 • Home-kill: 192 • Saleyard: 455 • Abattoir: 82 Movements of small-scale selling informally (n = 57): • Small-scale piggery: 32 • Private Individual:10 • Agricultural Show: 4 • Home-kill: 0 • Saleyard: 0 • Abattoir: 1	As Node 2
8	Ruminant movement from the index farm	Saleyard Abattoir Contractor Independent property Export	Proportion of movement of ruminants to each of these destinations (*Prop_SaleyardRum; Prop_AbattoirRum; Prop_Contractor; Prop_IndProp; Prop_Export)*	*Not estimated*	(32–34)
9	Detection at small-scale piggery	Detection No detection	Probability that the farmer at the large-scale piggery receiving the infected pigs would detect infection (*Prob_SSDet*)	*Prob_SSDet* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (0.32, 0.4, 0.48) • Probability of farmer reporting: Pert (0.48, 0.6, 0.72)	As Node 2
10	Detection at private individual	Detection No detection	Probability that the farmer at the small-scale piggery receiving the infected pigs would detect infection (*Prob_PrivateIndDet*)	*Prob_PrivateIndDet* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (0.32, 0.4, 0.48) • Probability of farmer reporting: Pert (0.48, 0.6, 0.72)	As Node 2
11	Detection at agricultural show	Detection No detection	Probability of detection of FMD at agricultural shows (*Prob_ASDet*)	*Prob_ASDet* = SumProduct (Probability of detection at show type, Proportion of each show type) • Proportion of show types depending on animal health responsible [Beta (n + 1, s – n + 1)]: Exhibitors only (17/59), Staff (25/59), Vet (17/59) • Probability of detection at show type: Exhibitors only (*Prob_1st Det*), Staff [Pert (0.1, 0.3, 0.5)], Vet (1)	(35, 36)
12	Detection at home-kill	Detection No detection	Probability of detection of a FMD in properties for home-kill (*Prob_HKDet*)	*Prob_HKDet* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (0.32, 0.4, 0.48) • Probability of farmer reporting: Pert (0.48, 0.6, 0.72)	As Node 2

(Continued)

TABLE 3 | Continued

Node	Name	Outcome	Parameter estimates	Input value	Data sources
13	Detection at saleyards	Detection No detection	Probability of detection of FMD at saleyards (*Prob_SaleyardDet*)	*Prob_SaleyardDet* = SumProduct (Probability of detection at saleyard type, Proportion of each saleyard type) • Proportion of saleyard type: Domestic (10/13), Export (3/13) • Probability of detection at saleyard type: Domestic (median, 0.475; 5–95%, 0.343–0.599), Export (0.474; 0.334–0.603)	(35, 37)
14	Detection at abattoirs	Detection No detection	Probability of detection at pig domestic and export abattoirs (*Prob_AbattoirDet*)	*Prob_AbattoirDet* = SumProduct (Probability of detection at abattoir type, Proportion of each abattoir type) • Proportion of saleyard type: Domestic (19/26), Export (7/26) • Probability of detection at saleyard type: Domestic (0.430; 0.329–0.534), Export (0.861; 0.799–0.916)	(35, 37)
15	Movement of contaminated fomites from the index farm	Yes No	Probability of FMD transmission through contaminated fomites (*Prob_Fomites*)	Small-scale selling at saleyards and abattoirs: *Moderate* [Uniform (0.3, 0.7)] Small-scale selling informally: *Low* [Uniform (0.05, 0.3)]	As Node 2; (15, 38, 39)
16	Movement of contaminated people from the index farm[a]	Yes No	Probability of transmission through movement of people carrying the virus in their respiratory tract (*Prob_People*)	Small-scale selling at saleyards and abattoirs: *Low* [Uniform (0.05, 0.3)] Small-scale selling informally: *Very low* [Uniform (0.001, 0.05)]	As Node 2; (15, 40, 41)

[a]*Movement of people carrying FMD infective particles in the upper respiratory tract.*

TABLE 4 | Nodes, parameter estimates and input values used for the partial consequence assessment estimating the probability of potential outbreak scenarios after a large-scale (>100 sows) piggery has been exposed to FMD-infected meat illegally introduced into Australia through incoming passengers from overseas.

Node	Name	Outcome	Parameter estimates	Input values	Data sources
1	Clinical signs	Yes No	Probability that an FMD-infected animal would display clinical signs (*Prob_CS; Prob_CS_2*)	Incubation period: lognormal [5, 2.7, Truncate (1, 12)]; infective period: uniform (14, 30); time to the onset of clinical signs = Incubation period – 2 days *Prob_CS* = (Infective period – Incubation period)/Infective period *Prob_CS_2* = (Infective period – Time to the onset of clinical signs)/Infective period	(13, 15, 16)
2	Farmer detection & reporting in pigs	Detection No detection	Probability of the farmer from the index piggery detecting and reporting FMD in pigs (*Prob_1stDet*)	*Prob_1stDet* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (0.56, 0.7, 0.84) • Probability of farmer reporting: Pert (0.64, 0.80, 0.96)	(7, 8, 12); Questionnaire with specific groups of pigs producers
3	Presence of ruminant on the piggery	Yes No	Proportion of pig farms keeping also ruminants (*Prop_Rum*)	Beta (65, 24)	As Node 2
4	Farmer detection and reporting in ruminants	Yes No	Probability of the farmer from the first exposed piggery detecting and reporting the FMD infection in ruminants (*Prob_1stDet_Rum*)	*Prob_1stDet_Rum* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (*Prob_1stDet*, +10%, +20%) • Probability of farmer reporting: Pert (0.64, 0.80, 0.96)	(15)

(Continued)

TABLE 4 | **Continued**

Node	Name	Outcome	Parameter estimates	Input values	Data sources
5	Pig movement during infective period	Yes No	Probability of pig movements during the infective period (*Prob_MovPigs*)	Pert (−10%, −5%, 1)	As Node 2
6	Ruminant movement during infective period	Yes No	Probability of ruminant movement during the infective period (*Prob_MovRum*)	*High* [Uniform (0.7, 1)]	As Node 2
7	Pig movement from the index farm	Large-scale piggery Small-scale piggery Saleyard Abattoir	Proportion of movement of pigs to each of these destinations (*Prop_LCpiggery; Prop_SCpiggery; Prop_Saleyard; Prop_Abattoir*)	Large-scale piggery: Pert (−20%, 0.10, +20%) Small-scale piggery: Pert (−20%, 0.10, +20%) Saleyard: Pert (−20%, 0.15, +20%) Abattoir: Pert (−20%, 0.65, +20%)	As Node 2
8	Ruminant movement from the index farm	Saleyard Abattoir Contractor Independent property Export	Proportion of movement of ruminants to each of these destinations (*Prop_SaleyardRum; Prop_AbattoirRum; Prop_Contractor; Prop_IndProp; Prop_Export)*	*Not estimated*	(32–34)
9	Detection and reporting at large-scale piggery	Detection No detection	Probability that the farmer at the large-scale piggery receiving the infected pigs would detect infection (*Prob_LSDet*)	*Prob_LSDet* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (0.56, 0.7, 0.84) • Probability of farmer reporting: Pert (0.64, 0.80, 0.96)	As Node 2
10	Detection and reporting at small-scale piggery	Detection No detection	Probability that the farmer at the small-scale piggery receiving the infected pigs would detect infection (*Prob_SSDet*)	*Prob_SSDet* = Probability of the farmer detection × Probability of the farmer reporting • Probability of the farmer detection: Pert (0.32, 0.4, 0.48) • Probability of farmer reporting: Pert (0.48, 0.6, 0.72)	As Node 2
11	Detection at saleyards	Detection No detection	Probability of detection of FMD at saleyards (*Prob_SaleyardDet*)	*Prob_SaleyardDet* = SumProduct (Probability of detection at saleyard type, Proportion of each saleyard type) • Proportion of saleyard type: Domestic (10/13), Export (3/13) • Probability of detection at saleyard type: Domestic (median, 0.475; 5–95%, 0.343–0.599), Export (0.474; 0.334–0.603)	(35, 37)
12	Detection at abattoirs	Detection No detection	Probability of detection at pig domestic and export abattoirs (*Prob_AbattoirDet*)	*Prob_AbattoirDet* = SumProduct (Probability of detection at abattoir type, Proportion of each abattoir type) • Proportion of saleyard type: Domestic (19/26), Export (7/26) • Probability of detection at saleyard type: Domestic (0.430; 0.329–0.534), Export (0.861; 0.799–0.916)	(35, 37)
13	Movement of contaminated fomites from the index farm	Yes No	Probability of FMD transmission through contaminated fomites (*Prob_Fomites*)	*Moderate* [Uniform (0.3, 0.7)]	As Node 2; (15, 38, 39)
14	Movement of contaminated people from the index farm[a]	Yes No	Probability of transmission through movement of people carrying the virus in their respiratory tract (*Prob_People*)	*Low* [Uniform (0.05, 0.3)]	As Node 2; (15, 40, 41)

[a]*Movement of people carrying FMD infective particles in the upper respiratory tract.*

TABLE 5 | Quantitative (median, 5 and 95 percentiles) and qualitative estimates of the likelihood of exposure of a pig from a piggery to FMD-infected meat previously illegally introduced into Australia, according to potential pathways of exposure and piggery type.

Exposure pathway	Description[a]	Quantitative and qualitative estimates[b]		
		Small-scale piggeries selling through informal means	**Small-scale piggeries selling at saleyards and abattoirs**	**Large-scale piggeries**
1	The FMD-infected meat gets to a household without pigs and some is discarded as waste – waste accessible to feral pigs, which become infected – Infected feral pigs get in contact with a pig from the exposure piggery	1.51×10^{-7} (1.32×10^{-8} to 2.74×10^{-4}) Negligible	1.59×10^{-7} (1.45×10^{-8} to 2.99×10^{-4}) Negligible	7.62×10^{-8} (7.30×10^{-9} to 1.00×10^{-4}) Negligible
2	The FMD-infected meat gets to the exposure piggery and some is discarded as waste – waste is directly fed to a pig from the same the piggery	7.80×10^{-6} (3.82×10^{-6} to 1.66×10^{-5}) Extremely low	6.65×10^{-5} (3.29×10^{-5} to 1.33×10^{-4}) Extremely low	3.46×10^{-5} (1.65×10^{-5} to 7.14×10^{-5}) Extremely low
3	The FMD-infected meat gets to the exposure piggery and some is discarded as waste – waste accessible to feral pigs, which become infected – infected feral pigs get in contact with a pig from the same piggery	4.00×10^{-12} (3.72×10^{-13} to 6.91×10^{-9}) Negligible	4.23×10^{-11} (3.94×10^{-12} to 7.63×10^{-8}) Negligible	1.19×10^{-11} (1.10×10^{-12} to 1.59×10^{-8}) Negligible
4	The FMD-infected meat gets to a non-exposure piggery, and some is discarded as waste – waste accessible to feral pigs, which become infected – infected feral pigs get in contact with a pig from the exposure piggery	4.98×10^{-11} (4.16×10^{-12} to 8.80×10^{-8}) Negligible	2.43×10^{-11} (2.23×10^{-12} to 4.54×10^{-8}) Negligible	1.64×10^{-11} (1.54×10^{-12} to 2.22×10^{-8}) Negligible
Overall	The FMD-infected meat gets in contact with pigs from the exposure piggery	8.69×10^{-6} (4.03×10^{-6} to 2.83×10^{-4}) Extremely low	7.26×10^{-5} (3.36×10^{-5} to 3.98×10^{-4}) Extremely low	3.81×10^{-5} (1.77×10^{-5} to 1.40×10^{-4}) Extremely low

[a]*Exposure piggery = small-scale pig producers selling through informal means, small-scale pig producers selling through saleyards and abattoirs or large-scale pig producers according to the assessment.*

[b]*Quantitative estimates are the output distribution of a simulation stochastic model with 50,000 iterations; qualitative estimates are based on the median and the likelihood ranges described at the Guidelines for Import Risk Analysis (DAFF, 2004).*

Assessment of the Risks of FMD Spread from Small-Scale Pig Producers

Table 6 shows a description of the potential outbreak scenarios and the likelihood of each of these scenarios to occur once FMD virus has been introduced into a small-scale piggery. The likelihood of five of the six main potential outbreak scenarios has been evaluated in this assessment. Among these scenarios and for small-scale producers selling through informal means, the most likely potential outbreak scenarios, with a similar likelihood of occurring, are *Scenario 1*, *Scenario 2*, *Scenario 3*, and *Scenario 5*. *Scenario 1*, representing no spread of FMD beyond the index farm, has a 0.193 (*Low*) probability to occur, as the ability of the farmer to detect FMD in pigs is estimated low. The probability of *Scenarios 2* and *5* to occur is estimated 0.137 and 0.100, respectively (*Low*). In these scenarios, the FMD-infected animals at the index farm are not detected and the virus spread from this farm through movement of pigs or ruminants. The extent of the spread and the impact of the consequences will depend on the destination of the animals moving off the farm. The likelihood of *Scenario 3* to occur is estimated similar, with a probability of 0.175 (*Low*). This scenario represents the spread of the FMD virus through the movement of contaminated fomites independently of the farmer detection. The virus could spread to another farm, saleyards, or abattoirs. The low frequency of movement of pigs and ruminants from this type of piggeries is the main reason why there is a low probability of spread through movement of animals (*Scenarios 2* and *5*) and fomites (*Scenario 3*).

Within Scenario 2, **Table 2** describes the potential outbreak scenarios through movement of pigs from a small-scale piggery selling informally according to the destination of the FMD-infected pigs. The most likely scenario is the spread of FMD virus to another small-scale piggery where the FMD-infected pigs are not detected (*Scenario b*), with a likelihood of occurring of 0.102 (*Low*). This scenario is more likely to occur than the rest of scenarios involving movement of pigs off the index farm as the main destination of pigs from a small-scale piggery selling through informal means is another small-scale piggery. The less likely scenarios are those involving spread to saleyards and abattoirs.

Among the five main potential outbreak scenarios from a small-scale piggery selling at saleyards and abattoirs, the most likely scenarios are *Scenario 2*, *Scenario 3*, and *Scenario 5*, all with a *Moderate* likelihood of occurring (**Table 6**). The likelihood of *Scenarios 2* and *3* for this group of producers was estimated higher than those obtained for small-scale piggeries selling through informal means, given movements of pigs from small-scale piggeries selling at saleyards and abattoirs were reported to be more frequent than those from small-scale piggeries selling informally and a lower proportion of these producers had boots and/or overalls for on-farm use only. Spread of FMD virus through movement of people carrying the virus in their respiratory tract (*Scenario 4)* was estimated to be 0.175 (*Low*) as the virus only survives for up to 28 h in the human respiratory tract, these farms do not usually employ external staff to work in the piggery, and over half of these farms have controlled entry of visitors and wash their hands after

TABLE 6 | Quantitative (median, 5 and 95 percentiles) and qualitative estimates of the likelihood of the potential outbreak scenarios for the introduction and spread of foot-and-mouth disease in small-scale piggeries in Australia.

Outbreak Scenarios	Quantitative and qualitative estimate[a]		Description[b]
	Small-scale piggery selling through informal means	Small-scale piggery selling at saleyards and abattoirs	
1	0.193 (0.122–0.272), Low	0.092 (0.060–0.122), Low	Infection detected at the exposure farm: no FMD spread beyond the exposure farm
2	0.137 (0.107–0.171), Low	0.382 (0.325–0.445), Moderate	Infection not detected at the exposure farm: spread of FMD through movement of pigs off farm (any destination)
a	0.006 (0.003–0.012), Very low	0.014 (0.010–0.018), Very low	FMD spread to another small-scale piggery where infected pigs are detected
b	0.102 (0.074–0.132), Low	0.054 (0.042–0.067), Low	FMD spread to another small-scale piggery where infected pigs are not detected
e	0.011 (0.006–0.017), Very low	0.004 (0.003–0.006), Very low	FMD spread to a private individual (pigs kept as pets) where infected pigs are detected
f	0.031 (0.018–0.050), Very low	0.012 (0.008–0.017), Very low	FMD spread to a private individual (pigs kept as pets) where infected pigs are not detected
g	0.009 (0.004–0.018), Very low	0.002 (0.001–0.003), Very low	FMD spread to an agricultural show where infected pigs are detected
h	0.009 (0.004–0.019), Very low	0.002 (0.001–0.004), Very low	FMD spread to an agricultural show where infected pigs are not detected
i	0.035 (0.021–0.054), Very low	0.097 (0.080–0.117), Low	FMD spread to a property where pigs are killed for home consumption (home-kill)
j	0.001 (0.000–0.005), Very low	0.119 (0.090–0.153), Low	FMD spread to a saleyard (livestock market) where infected pigs are detected
k	0.001 (0.000–0.006), Very low	0.133 (0.099–0.168), Low	FMD spread to a saleyard (livestock market) where infected pigs are not detected
l	0.003 (0.000–0.008), Very low	0.020 (0.013–0.027), Very low	FMD spread to a domestic abattoir where infected pigs are detected
m	0.003 (0.001–0.010), Very low	0.026 (0.019–0.033), Very low	FMD spread to a domestic abattoir where infected pigs are not detected
3	0.175 (0.062–0.287), Low	0.499 (0.320–0.680), Moderate	Spread of FMD virus from the index farm through movement of contaminated fomites
4	0.026 (0.003–0.048), Very low	0.175 (0.062–0.287), Low	Spread of FMD virus from the index farm through movement of people carrying infective particles of virus in the respiratory tract
5	0.100 (0.037–0.183), Low	0.421 (0.270–0.600), Moderate	Infection not detected at the exposure farm: spread of FMD through movement of ruminants off farm (any destination)

[a]Quantitative estimates are the output distribution of a simulation stochastic model with 50,000 iterations; qualitative estimates are based on the median and the likelihood ranges described at the Guidelines for Import Risk Analysis (DAFF, 2004).

[b]Exposure piggery = small-scale pig producers selling through informal means or small-scale pig producers selling through saleyards and abattoirs according to the assessment.

handling the pigs. However, this estimate is higher than the estimate for small-scale piggeries selling through informal means, as the latter group of producers have better on-farm practices, which could avoid the spread of the virus through contaminated people. *Scenario 1* (0.092, *Low*), representing detection of FMD at the index farm and no spread beyond this farm, has a similar estimate than for the previous group of producers. This scenario has the lowest probability of occurring as the probability of the farmer detecting and reporting at these piggeries was estimated lower than for the other groups of producers.

Regarding those scenarios involving movement of pigs off the index farm to different destinations, the most likely scenarios to occur in this group of pig producers are *Scenario j* (0.119, *Low*) and *k* (0.133, *Low*), representing movement of pigs to saleyards, where infection is or is not detected, respectively.

Assessment of the Risks of FMD Spread from Large-Scale Pig Producers

The six main outbreak scenarios are the same than those described for small-scale pig producers; however, scenarios involving movement of pigs off the index farm differed depending on the destination of these animals. **Table 7** shows the potential outbreak scenarios and the likelihood of each of these scenarios to occur once FMD virus has been introduced into a large-scale piggery. If FMD is introduced into a large-scale piggery, the most likely scenarios to occur are *Scenario 1* and *Scenario 3*. *Scenario 1*, with a probability of 0.317 (*Moderate*), represents detection of FMD at the index farm and no spread beyond this farm. The likelihood of this scenario in large-scale piggeries is higher than that in both groups of small-scale piggeries, as the ability to detect disease among large-scale producers is estimated higher than that for small-scale producers and there is a lower proportion of large-scale producer with ruminants on the farm, limiting the potential spread off the farm through this species. The likelihood of *Scenario 3* to occur was estimated 0.499 (*Moderate*). This estimate is the same than the estimate for this scenario for small-scale piggeries selling at saleyards and abattoirs. Spread of FMD through movement of pigs (*Scenario 2*) when the infection has not been detected has a probability of occurring of 0.263 (*Low*). This probability is higher than that for the same scenario in small-scale piggeries selling through informal means, due to the more frequent movements of pigs, but lower than that estimated for small-scale piggeries selling through saleyards and abattoirs, as the producer in a large-scale piggeries is more likely

TABLE 7 | Quantitative (median, 5 and 95 percentiles) and qualitative estimates of the likelihood of the potential outbreak scenarios for the introduction and spread of foot-and-mouth disease in large-scale piggeries (>100 sows) in Australia.

Outbreak Scenarios	Quantitative and qualitative estimate[a]	Description
1	0.317 (0.199–0.444), Moderate	Infection detected at the exposure farm: no FMD spread beyond the exposure farm
2	0.263 (0.176–0.372), Low	Infection not detected at the exposure farm: spread of FMD through movement of animals off farm (any destination)
a	0.024 (0.018–0.032), Very low	FMD spread to another large-scale piggery where infected pigs are detected
b	0.023 (0.016–0.035), Very Low	FMD spread to another large-scale piggery where infected pigs are not detected
c	0.011 (0.008–0.014), Very low	FMD spread to a small-scale piggery where infected pigs are detected
d	0.044 (0.032–0.055), Very Low	FMD spread to a small-scale piggery where infected pigs are not detected
j	0.031 (0.021–0.042), Very low	FMD spread to a saleyard (livestock market) where infected pigs are detected
k	0.039 (0.028–0.059), Very low	FMD spread to a saleyard (livestock market) where infected pigs are not detected
l	0.157 (0.118–0.197), Low	FMD spread to an abattoir where infected pigs are detected
m	0.150 (0.105–0.235), Low	FMD spread to an abattoir where infected pigs are not detected
3	0.499 (0.320–0.680), Moderate	Spread of FMD virus from the index farm through movement of contaminated fomites
4	0.175 (0.062–0.287), Low	Spread of FMD virus from the index farm through movement of people carrying infective particles of virus in the respiratory tract
5	0.218 (0.0.116–0.334), Low	Infection not detected at the exposure farm: spread of FMD through movement of ruminants off farm (any destination)

[a]*Quantitative estimates are the output distribution of a simulation stochastic model with 50,000 iterations; Qualitative estimates are based on the median and the likelihood ranges described at the Guidelines for Import Risk Analysis (DAFF, 2004).*

to detect FMD than that in the small-scale piggery. Similarly, the likelihood of spread through movement of ruminants (*Scenario 5*; 0.218, *Low*) is lower than that in small-scale piggeries selling at saleyards and abattoirs but higher than that in small-scale piggeries selling informally.

Among the scenarios of spread due to movement of pigs off the index farm (*Scenario 2*), the most likely scenarios were those where pigs were sent to the abattoir (*Scenario l* and *m,* approximately 0.150, *Low*), as this was the most common destination of pigs from large-scale piggeries.

Sensitivity Analysis

The sensitivity of the probability of exposure and the spread scenarios to some of the input variables was evaluated in this assessment. The input variable with most influence on the probability of exposure for all exposure groups is the amount of FMD-infected meat illegally introduced into Australia. However, even when the model assumes that 200 kg of FMD-infected meat has been introduced into Australia, the probability that pigs would be exposed to FMD is *Very low* in large-scale and small-scale producers selling at saleyards and abattoirs and *Extremely low* in small-scale producers selling informally. When the probability of producers feeding swill to the pigs was increased to 0.9, there was a two- to sevenfold increase in the probability of exposure for all exposure groups; however, the probability was still considered *Extremely low*.

Results of the sensitivity analysis on *Scenario 1* and *Scenario 2* for each group of pig producers are presented in **Figure 3**. For *Scenario 1* (no spread beyond the index farm) among small-scale pig producers, the input parameter most influential on the output of the model is the presence of ruminants on the farm. A significant proportion of these producers stated having ruminants on the farm. If the proportion of producers with ruminants in the piggery is decreased to only 10% (base value 82–85%), the likelihood of *Scenario 1*, increases 35 and 18% in the small-scale producers selling informally and in those selling at saleyards and abattoirs, respectively. For large-scale producers, the probability of the farmer detecting FMD in pigs is the input value with the most influence on the output probability of *Scenario 1*, with 18% increase in the probability of FMD not spreading form the index farm when detection in pigs is 0.9 (base value 0.56). A similar influence of the probability of FMD detection is seen among small-scale producers selling informally. The probability of spread through movement of infective pigs off the farm (*Scenario 2*) decreases when increasing the probability of the farmer detecting in pigs and ruminants for the three groups of pig producers. The influence of these input variables is more significant among large-scale and small-scale pig producers selling at saleyards and abattoirs (decrease of up to 25%). As expected, movement of pigs off farm influences the probability of *Scenario 2* for all piggery types. The probability of spread through movement of contaminated fomites has a significant influence on the occurrence *Scenario 3*, given this model assume that the virus would spread through fomites independently of the farmer detecting the disease. When *Prob_Fomites* is reduced to 0.1 (from a base value of 0.5 for large-scale piggeries and small-scale piggeries selling through saleyards and abattoirs), there is a fivefold decrease in the probability of spread through this pathway. These results stress the importance of maintaining appropriate biosecurity practices to minimize the spread of FMD through fomites, such as pig transport and visitor vehicles, clothes and equipment. For *Scenario 5* (spread through movement of ruminants), the input value with the most influence on the model output is the probability of movement of ruminants during the infective period. However, the influence is different among the

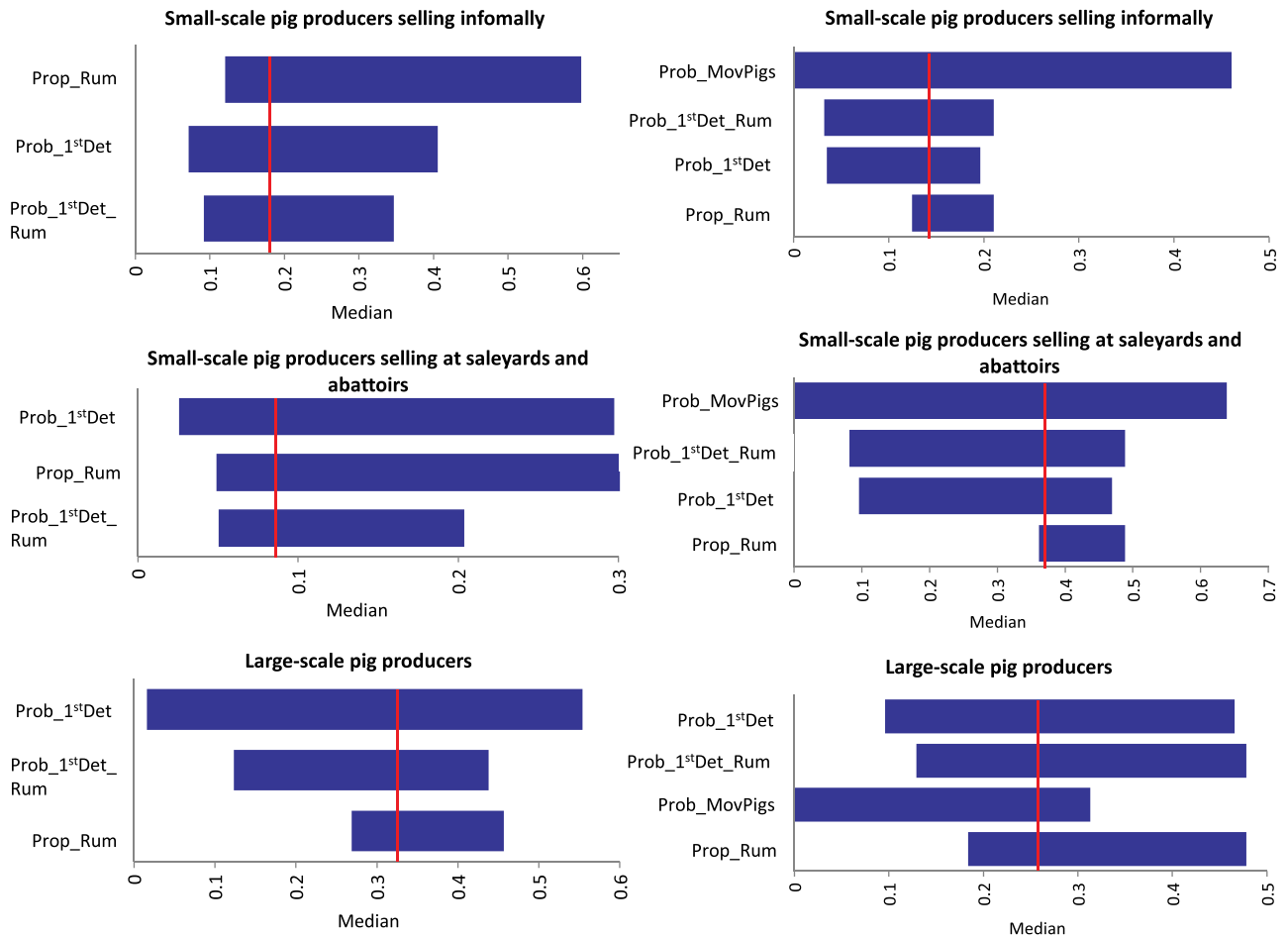

FIGURE 3 | Outputs of a sensitivity analysis investigating the influence of different input variables to the median (vertical line) probability of two spread scenarios [left: no spread beyond the index farm (*Scenario 1*); right: spread through movement of infective pigs (*Scenario 2*)] of foot-and-mouth disease that has previously been introduced in a piggery in Australia (Sensitivity analysis with 5,000 iterations using @Risk's Advanced Sensitivity Analysis) (*Prob_1stDet_Rum*, Probability of farmer detecting FMD in ruminants; *Prob_1stDet*, Probability of farmer detecting FMD in pigs; *Prob_MovPigs*, Probability of movement of pigs during infective period; *Prop_Rum*, Presence of ruminants).

three groups of pig producers as seen in **Figure 4**. The probability of detecting FMD in ruminants has also an important influence on the probability of *Scenario 5* occurring, especially among small-scale piggeries selling through saleyards and abattoirs, which are those with an estimated lower probability of detection (base value 0.24). When detection in these piggeries is improved to 0.89, the probability of *Scenario 5* occurring decreases by 13.5-folds.

DISCUSSION

Several studies have suggested that small-scale pig producers are more likely to introduce and spread emergency animal diseases (EAD) compared to large-scale pig producers (1, 8, 10, 11, 47, 48), with the lack of appropriate isolation for incoming animals, the use of saleyards for trading pigs, their poor knowledge on EADs and their low compliance with legislative requirements for keeping pigs being the main reasons for this suggestion. The current study is the first comparative assessment on the FMD introduction and spread risks posed by small and large-scale piggeries in Australia, which has used extensive data on producers' practices. Data used to populate the models in this study is based on several quantitative and qualitative studies among small-scale pig producers in Eastern Australia conducted between 2005 and 2009. These studies, which aimed to investigate biosecurity and surveillance among this sector of the pig industry, have been previously described as providing baseline information on practices and attitudes toward biosecurity among small-scale pig producers in Australia (8). Subsequent studies suggest that biosecurity practices among pig producers have not significantly changed since (48–50). However, although estimates used for the current assessments are considered an accurate representation of practices among pig producers in the country, some practices of producers located in Western areas of the country might not be appropriately represented. The model assumed that FMD was introduced through the illegal importation of 5 kg of cured or

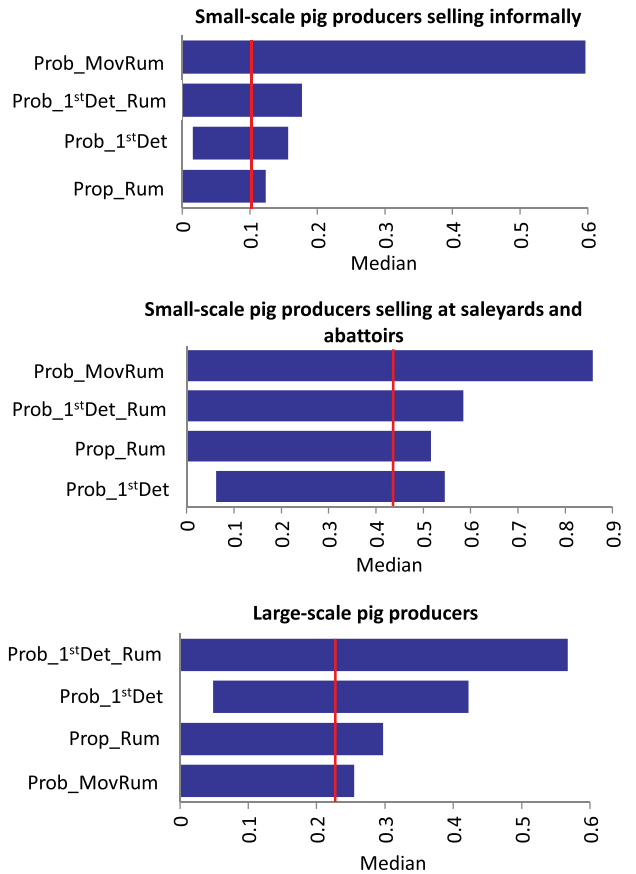

FIGURE 4 | Influence of different input variables to the median (vertical line) of the scenario of spread through movement of infective ruminants of foot-and-mouth disease that has previously been introduced in a piggery in Australia (Sensitivity analysis with 1,000 iterations using @Risk's Advanced Sensitivity Analysis) (*Prob_1stDet_Rum*, probability of farmer detecting FMD in ruminants; *Prob_1stDet*, probability of farmer detecting FMD in pigs; *Prop_Rum*, presence of ruminants; *Prob_MovRum*, probability of movement of ruminants during infective period).

salted meat and that the virus survived until exposure occurred. We acknowledge that to accurately estimate the probability of exposure of domestic pigs due to illegal introduction of infected meat, virus survival should be considered; however, the aim of this study was to identify biosecurity practices posing a risk for disease introduction and spread in piggeries and compare this risk between different sectors of the pig industry. These sectors were, small-scale piggeries selling through informal means, small-scale piggeries selling at saleyards and abattoirs and large-scale piggeries. Results of the probabilities of exposure and spread posed by each sector of the industry should be considered relative among the three sectors of the industry.

The probability of exposure of a piggery to the FMD virus, which has been introduced into Australia through the illegal importation of 5 kg of FMD-infected cured or salted meat products was estimated *Extremely low* for the three groups of piggeries. One of the factors driving this low probability is the fact that this assessment considered households without and with pigs having an even probability of illegally introducing meat into the country and most households in Australia do not keep pigs. As such, the probability of the illegally introduced meat being destined to a household with pigs is extremely low. There is no available data providing scientific evidence about households with pigs being more likely to illegally introduce FMD-infected meat into Australia than other households. However, data on illegal movements of meat products are needed to confirm this assumption. The most likely pathway of exposure according to this assessment is through the direct feeding of the infected meat to the pigs. In addition, the sensitivity analysis indicates that exposure is highly influenced the probability of producers to swill feed pigs. Swill feeding was estimated to be more likely among small-scale producers; however, estimates of swill feeding were incorporated in the model with significant uncertainty as they were based on information on feeding practices of producers collected during questionnaires and interviews (8, 9, 12). More accurate information on swill feeding incidence among large and small-scale pig producers in Australia would improve validity of the results. Mathews (51), in a report assessing Australia's current level of preparedness and capacity to prevent and respond to an outbreak of FMD, identified the effectiveness of swill feeding prohibitions, especially among periurban and small-scale pig producers, as one of the areas that required attention. As previously described, swill feeding has been identified as posing the major risk for FMD introduction and establishment in Australia, through illegally introduced FMD-contaminated meat or dairy products (15, 51). Similarly, the illegal importation of meat, which is subsequently fed to pigs as swill, has been identified as the potential cause of the introduction of emergency diseases, such as CSF and ASF (20, 22, 23). As Schembri et al. (8) indicates, a program involving appropriate swill feeding investigations and effective education and enforcement strategies, supported by a consistent national definition of swill feeding is required to improve current data on swill feeding incidence and producers' awareness and compliance.

According to results from these assessments, once FMD has been introduced into a piggery, the most likely pathway of spread is through contaminated fomites (*Scenario 3*). Spread of FMD through contaminated vehicles, equipment or clothing with poor or absent appropriate disinfection has been reported as an important pathway of FMD spread from infected properties (15, 52, 53). Spread through this pathway is estimated less likely to occur among small-scale piggeries selling informally due to the low frequency of animal and vehicle movements and the non-use of external staff in these properties compared to other piggeries.

In the current assessment, a similar probability of spread through this pathway was estimated for large- and small-scale piggeries selling at saleyards and abattoirs. Large-scale pig producers have been reported to have better on-farm biosecurity practices in Australia (8, 48) and other countries, such as Finland (47) and United States (54), with these studies suggesting that large-scale producers might perceive the impact of disease as more significant for their enterprise. However, pig movements off farm among large-scale enterprises are frequent and all employ

external staff, which could contribute to the spread of the virus through contaminated fomites. By contrast, among small-scale pig producers selling at saleyards and abattoirs pig movements off farm are not as frequent as those among large-scale pig producers. This scenario was considered independent of the farmer detecting the infection, as movement of contaminated fomites could occur before detection of clinical signs occurs, given shedding of the virus could start during the pre-clinical phase of the disease (15, 53). It could be argued that the earlier FMD is detected the less likely the virus would spread through fomites. According to available data, large-scale producers are considered more likely to early detect FMD-infected animals than small-scale producers, and as a consequence, spread through fomites in large-scale piggeries would be less likely to occur. Given this has not been considered in this assessment, the probability of *Scenario 3* to occur in large-scale piggeries could be somewhat overestimated.

The next spread scenario with highest probability among all producers was the spread of FMD through movement of pigs from the index farm (*Scenario 2*). Similarly than for *Scenario 3*, spread through movement of pigs was less likely to occur among small-scale piggeries selling informally due to the lower number of pigs kept on the farm and frequency of pig movements off the farm. However, although the probability of this scenario of spread was lower than for other piggeries, movement of pigs to non-commercial pathways could jeopardize animal traceability in the event of a disease outbreak. Some of these pig properties are not registered within government and/or industry databases, challenging the ability to trace back animal movements in the event of an EAD outbreak and increasing the potential magnitude of the outbreak. A recent study among 198 small-scale pig producers in Australia reported over 85% of participant producers moving pigs off their property in the last 12 months, ~10% of producers not recording animal movements and 3% not having a legally required property identification code for their property (49). Mathews (51) identified the poor understanding on the number and location of small holder producers in Australia and the need for a national register as critical for the management of EADs. For this scenario, the main differences between the three sectors of the pig industry were the different destinations of pigs being moved off the property. While pigs from small-scale piggeries selling informally are mainly moved to other properties keeping pigs, pigs from small-scale piggeries selling at saleyards and abattoirs are mainly sent to saleyards and pigs from large-scale piggeries are sent to abattoirs. This will affect the potential magnitude of the spread of FMD before the outbreak is detected and the potential strategies to reduce the risks of spread. The ability of detecting FMD-infected animals at saleyards and abattoirs, where animals from different origins are commingled, is crucial for limiting the spread of the disease. The estimates of FMD detection used in the current assessments are based on a study by Hernández-Jover et al. (35), who conducted a quantitative evaluation of the likelihood of exotic disease detection with passive disease surveillance activities for pigs at saleyards and abattoirs in eastern Australia. This study indicates that although the probability of detecting FMD at these locations was high when assuming a herd and unit design prevalence of 1 and 30%, respectively, the probability of early detecting FMD at these venues could be improved. This study identified the improvement of disease awareness of saleyard and abattoir stockmen, increased presence of inspectors at these venues and identification of high-risk herds as approaches for enhancing the capacity of the country for early detection of emerging animal diseases. As suggested by several studies, the use of a risk-based surveillance approach, with surveillance being focused at locations with high-risk animals could result in more efficient allocation of resources (55–57).

The sensitivity analysis indicates that for all piggery types, spread of the virus is highly influenced by the probability of the farmer detecting FMD and the presence of ruminants on the farm. No spread beyond the index farm (*Scenario 1*) was more likely to occur in large-scale piggeries as these are considered more likely to detect disease and a lower proportion of these producers keep ruminants (8, 12). Early detection and reporting of FMD is crucial for limiting the spread of the virus and minimizing the potential impact of an outbreak. According to data available, small-scale pig producers have low awareness of EADs and the concept of shared responsibility in relation to the management of EADs. In addition, their contact with veterinarians is low and a lack of trust with government agencies has been identified (8, 49). As a consequence, these data suggest small-scale pig producers would be unlikely to detect FMD before the virus was spread to other livestock in other properties. Similarly, several previous studies identified lack of EAD awareness and negative attitudes toward disease reporting among livestock producers (58–61). Mathews (51) suggested that an incursion of FMD into Australia would not be readily detected, and identified these factors as contributing to the delayed detection of an FMD outbreak. Supporting this suggestion, modeling studies estimated an expected time to FMD detection after being introduced into the country of 22–47 days (53, 56).

CONCLUSION

These assessments provide information regarding the relative order of magnitude of the risks of FMD introduction and spread among the three sectors of the pig industry, as well as the biosecurity practices posing higher risks among piggeries for each of the sectors considered. This information can support decision-making when prioritizing resource allocation for improving the capability of the pig industry to prevent and respond against emergency animal disease outbreaks. According to the results of this assessment, direct feeding of the infected meat to the pigs (swill feeding) is the most likely pathway of exposure, and the probability of this to occur is slightly higher among small-scale producers. If FMD is to be introduced into a piggery in Australia, spread is more likely to occur if this piggery is a small-scale piggery selling at saleyards and abattoirs with movement of contaminated fomites and movement of pigs and ruminants off the farm the most likely pathways of spread. Presence of ruminants on the farm and the probability of the farmer detecting FMD are the most influential factors for the spread of the virus. Although large-scale piggeries have higher probability of FMD spread than small-scale piggeries selling informally, they are easy to locate, are members of the pig industry body APL and do not use

non-commercial venues to market their pigs. These would limit the potential extent of an outbreak. Small-scale piggeries selling informally do not pose a higher likelihood of spread of disease than the other sectors of the industry; however, if spread from these piggeries occurs, non-traceable movement of pigs would increase the magnitude of an outbreak.

This study suggests that there is a need for improving engagement with biosecurity and animal health management of pig producers and agricultural shows, saleyards and abattoirs stakeholders understanding producers' current attitudes and behaviors toward biosecurity and animal health management, especially among small-scale producers, and collaboration among government and industry stakeholders are crucial for the development of effective extension strategies that could lead to practice change in relation to biosecurity and EAD management.

AUTHOR CONTRIBUTIONS

MH-J led the study, building and implementing the risk assessment models, and writing the manuscript. NS led the data gathering exercises of this study, participated in the estimation of parameter estimates, and providing input in the writing of the manuscript. PH and J-AT participated in the design of the data gathering exercises, provided input in the scenario tree model building and the estimation of the input parameters, and provided input on the writing of the manuscript. PM provided guidance and ongoing input on the building of the risk assessment models, estimation of input parameters and implementation of the models, as well as the writing of the manuscript.

ACKNOWLEDGMENTS

This research was funded and supported by the Australian Biosecurity Cooperative Research Centre for Emerging Infectious Diseases. The authors thank the participating saleyards, abattoirs, government and industry stakeholders, and pig producers for their collaboration in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fvets.2016.00085

REFERENCES

1. Ribbens S, Dewulf J, Koenen F, Mintiens K, De Sadeleer L, de Kruif A, et al. A survey on biosecurity and management practices in Belgian pig herds. *Prev Vet Med* (2008) 83(3–4):228–41. doi:10.1016/j.prevetmed.2007.07.009
2. Hollier C, Reid M, Fenton K. *Engaging Small Lifestyle Farmers in Natural Resource Management (Contract No.: RIRDC Publication No 07/097 RIRDC Project No DAV-201A)*. Rutherglen: Rural Industries Research and Development Corporation (2007).
3. Hollier C, Reid M. *Small Lifestyle Farms: Improving Delivery Mechanisms for Sustainable Land Management: A Report for the Cooperative Venture for Capacity Building. Rural Industries Research and Development (Contract No.: RIRDC Publication No 07/098 RIRDC Project No DAV-201A)*. Victoria: Rural Industries Research and Development Corporation (2007).
4. Aslin H, Kelson S, Smith J, Lesslie R. *Peri-Urban Landholders and Bio-Security Issues – A Scoping Study*. Canberra: Bureau of Rural Sciences (2004).
5. Aslin H, Mazur N. *Biosecurity Awareness and Peri-Urban Landholders: A Case Study Approach*. Canberra: Sciences BoR (2005).
6. Schembri N. *Biosecurity Practices of Producers Trading Pigs at Saleyards*. Camden: University of Sydney (2009).
7. Schembri N, Hart K, Petersen R, Whittington R. Assessment of the management practices facilitating the establishment and spread of exotic diseases of pigs in the Sydney region. *Aust Vet J* (2006) 84(10):341–8. doi:10.1111/j.1751-0813.2006.00035.x
8. Schembri N, Hernandez-Jover M, Toribio JA, Holyoake PK. On-farm characteristics and biosecurity protocols for small-scale swine producers in eastern Australia. *Prev Vet Med* (2015) 118(1):104–16. doi:10.1016/j.prevetmed.2014.11.008
9. Schembri N, Hernández-Jover M, Toribio JA, Holyoake PK. Feeding of prohibited substances (swill) to pigs in Australia. *Aust Vet J* (2010) 88(8):294–300. doi:10.1111/j.1751-0813.2010.00604.x
10. Van Metre DC, Barkey DQ, Salman MD, Morley PS. Development of a syndromic surveillance system for detection of disease among livestock entering an auction market. *J Am Vet Med Assoc* (2009) 234(5):658–64. doi:10.2460/javma.234.5.658
11. East I, Davis J, Sergeant E, Garner M. Structure, dynamics and movement patterns of the Australian pig industry. *Aust Vet J* (2014) 92(3):52–7. doi:10.1111/avj.12141
12. Schembri N, Holyoake PK, Hernández-Jover M, Toribio JLML. A qualitative study of the management and biosecurity practices of 13 interviewed pig owners selling via informal means in New South Wales, Australia. *Anim Prod Sci* (2010) 50(9):852–62. doi:10.1071/AN09226
13. DAFF. *Generic Import Risk Analysis (IRA) for Pig Meat: Final Import Risk Analysis Report*. Canberra: Biosecurity Australia (2004).
14. Productivity Commission. *Impact of a Foot and Mouth Disease Outbreak on Australia, Research Report*. Canberra: Productivity Commission (2002).
15. Animal Health Australia. *Disease Strategy: Foot-and-Mouth Disease (Version 3.4). Australian Veterinary Emergency Plan (AUSVETPLAN)*. Canberra: ACT (2014).
16. Alexandersen S, Donaldson AI. Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. *Epidemiol Infect* (2002) 128(2):313–23. doi:10.1017/S0950268801006501
17. Geering WA. Foot and mouth disease. In: Department of Primary Industries and Energy, editor. *A Qualitative Assessment of Current Exotic Disease Risks for Australia: Bureau of Rural Resources*. Canberra: Energy DoPIa (1990). p. 39–41.
18. Nunn MJ. *Foot-and-Mouth Disease in the United Kingdom. FMD Relief and Recovery Workshop*. Canberra: Commonwealth of Australia (2001).
19. Bourn J. *The 2001 Outbreak of Foot and Mouth Disease*. London: National Audit Office (2002).
20. Costard S, Jones BA, Martínez-López B, Mur L, de la Torre A, Martínez M, et al. Introduction of African swine fever into the European Union through Illegal importation of pork and pork products. *PLoS One* (2013) 8(4):e61104. doi:10.1371/journal.pone.0061104
21. Sánchez-Vizcaíno JM, Mur L, Martínez-López B. African swine fever (ASF): five years around Europe. *Vet Microbiol* (2013) 165(1–2):45–50. doi:10.1016/j.vetmic.2012.11.030
22. Hartnett E, Adkin A, Seaman M, Cooper J, Watson E, Coburn H, et al. A quantitative assessment of the risks from illegally imported meat contaminated with foot and mouth disease virus to Great Britain. *Risk Anal* (2007) 27(1):187–202. doi:10.1111/j.1539-6924.2006.00869.x
23. Corso B. Likelihood of introducing selected exotic diseases to domestic swine in the continental United States of America through uncooked swill. *Rev Sci Tech* (1997) 16(1):199–206.
24. Buetre B, Wicks S, Kruger H, Millist N, Yainshet A, Garner G, et al. *Potential Socio-Economic Impacts of an Outbreak of Foot-and-Mouth Disease in Australia*. Canberra: Commonwealth of Australia (2013).
25. World organization for Animal Health (OIE), editor. Chapter 2.1. Import risk analysis. *Terrestrial Animal Health Code*. World organization for Animal Health (OIE) (2009).

26. MacDiarmid SC, Pharo HJ. Risk analysis: assessment, management and communication. *Rev Sci Tech* (2003) 22(2):397–408.
27. Martin RAJ, Cameron AR, Greiner M. Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. *Prev Vet Med* (2007) 79(2–4):71–97. doi:10.1016/j.prevetmed.2006.09.008
28. APIQ. *Small Holder Manual*. Canberra: Australian Pork Limited (2015).
29. Australian Bureau of Statistics. *3236.0 – Household and Family Projections, Australia, 2011 to 2036 2015*. Available from: http://www.abs.gov.au/ausstats/abs@.nsf/mf/3236.0
30. APL. *Australian Pig Annual 2012–2013*. Canberra: Australian Pork Limited (2014).
31. Gale P. *Risk Assessment: Use of Composting and Biogas Treatment to Dispose of Catering Waste Containing Meat*. Marlow: Department for Environment, Food and Rural Affairs (2002).
32. AusVet Animal Health Services. *A Review of the Structure and Dynamics of the Australian Beef Cattle Industry*. Canberra: AusVet Animal Health Services (2006).
33. AusVet Animal Health Services. *A Review of the Structure and Dynamics of the Australian Dairy Cattle Industry*. Canberra: AusVet Animal Health Services (2005).
34. Hassall & Associates Pty Ltd. *The Structure and Dynamics of Australia's Sheep Population*. Dubbo: Hassall & Associates Pty Ltd (2006).
35. Hernández-Jover M, Cogger N, Martin PAJ, Schembri N, Holyoake PK, Toribio JALML. Evaluation of post-farm-gate passive surveillance in swine for the detection of foot and mouth disease in Australia. *Prev Vet Med* (2011) 100(3–4):171–86. doi:10.1016/j.prevetmed.2011.03.011
36. Cha E, Toribio JALML, Thomson PC, Holyoake PK. Biosecurity practices and the potential for exhibited pigs to consume swill at agricultural shows in Australia. *Prev Vet Med* (2009) 91(2–4):122–9. doi:10.1016/j.prevetmed.2009.05.010
37. Cutler RS, Holyoake PK. *The Structure and Dynamics of the Pig Meat Industry*. Ocean Grove (2007).
38. Alexandersen S, Quan M, Murphy C, Knight J, Zhang Z. Studies of quantitative parameters of virus excretion and transmission in pigs and cattle experimentally infected with foot-and-mouth disease virus. *J Comp Pathol* (2003) 129(4):268–82. doi:10.1016/S0021-9975(03)00045-8
39. Orsel K, Bouma A, Dekker A, Stegeman JA, Jong dMCM. Foot and mouth disease virus transmission during the incubation period of the disease in piglets, lambs, calves, and dairy cows. *Prev Vet Med* (2009) 88(1):158–63. doi:10.1016/j.prevetmed.2008.09.001
40. Kitching RP, Hutber AM, Thrusfield MV. A review of foot-and-mouth disease with special consideration for the clinical and epidemiological factors relevant to predictive modelling of the disease. *Vet J* (2005) 169:197–209. doi:10.1016/j.tvjl.2004.06.001
41. Morgan ER, Lundervold M, Medley GF, Shaikenov BS, Torgerson PR, Milner-Gulland EJ. Assessing risks of disease transmission between wildlife and livestock: the Saiga antelope as a case study. *Biol Conserv* (2006) 131(2):244–54. doi:10.1016/j.biocon.2006.04.012
42. Hernández-Jover M, Schembri N, Toribio JALML, Holyoake PK. Biosecurity risks associated with current identification practices of producers trading live pigs at livestock sales. *Animal* (2008) 2(11):1692–9. doi:10.1017/S1751731108003066
43. Hernández-Jover M, Gilmour J, Schembri N, Sysak T, Holyoake PK, Beilin R, et al. Use of stakeholder analysis to inform risk communication and extension strategies for improved biosecurity amongst small-scale pig producers. *Prev Vet Med* (2012) 104(3–4):258–70. doi:10.1016/j.prevetmed.2011.12.006
44. Hernández-Jover M, Schembri N, Toribio JA, Holyoake P. Evaluation of the implementation of new traceability and food safety requirements in the pig industry in eastern Australia. *Aust Vet J* (2009) 87(10):387–96. doi:10.1111/j.1751-0813.2009.00483.x
45. Donaldson AI, Alexandersen S. Predicting the spread of foot and mouth disease by airborne virus. *Rev Sci Tech* (2002) 21(3):569–75. doi:10.20506/rst.21.3.1362
46. Gloster J, Champion HJ, Sorensen JH, Mikkelsen T, Ryall DB, Astrup P, et al. Airborne transmission of foot-and-mouth disease virus from Burnside Farm, Heddonon-the-Wall, Northumberland, during the 2001 epidemic in the United Kingdom. *Vet Rec* (2003) 152(17):525–33. doi:10.1136/vr.152.17.525
47. Sahlström L, Virtanen T, Kyyro J, Lyytikainen T. Biosecurity on Finnish cattle, pig and sheep farms – results from a questionnaire. *Prev Vet Med* (2014) 117(1):59–67. doi:10.1016/j.prevetmed.2014.07.004
48. Hernández-Jover M, Taylor M, Holyoake P, Dhand N. Pig producers' perceptions of the influenza pandemic H1N1/09 outbreak and its effect on their biosecurity practices in Australia. *Prev Vet Med* (2012) 106(3–4):284–94. doi:10.1016/j.prevetmed.2012.03.008
49. Hernández-Jover M, Hayes L, Toribio J-ALML. *Smallholder Production in Australia*. Wagga Wagga: Charles Sturt University (2014).
50. Brookes VJ, Hernandez-Jover M, Neslo R, Cowled B, Holyoake P, Ward MP. Identifying and measuring stakeholder pnces for disease prioritisation: a case study of the pig industry in Australia. *Prev Vet Med* (2014) 113(1):118–31. doi:10.1016/j.prevetmed.2013.10.016
51. Mathews K. *A Review of Australia's Preparedness for the Threat of Foot-and-Mouth Disease*. Canberra: Australian Government Department of Agriculture, Fisheries and Forestry (2011).
52. Morgan IR. *Spread of Foot-and-Mouth Disease: A Review Prepared for the Australian Meat Research Corporation*. Melbourne: Victorian Institute of Animal Science (2006).
53. East IJ, Martin PAJ, Langstaff I, Iglesias RM, Sergeant ESG, Garner MG. Assessing the delay to detection and the size of the outbreak at the time of detection of incursions of foot and mouth disease in Australia. *Prev Vet Med* (2016) 123:1–11. doi:10.1016/j.prevetmed.2015.12.005
54. Bottoms K, Poljak Z, Dewey C, Deardon R, Holtkamp D, Friendship R. Evaluation of external biosecurity practices on southern Ontario sow farms. *Prev Vet Med* (2013) 109(1–2):58–68. doi:10.1016/j.prevetmed.2012.08.013
55. Schemann AK, Hernández-Jover M, Hall W, Holyoake PK, Toribio JA. Assessment of current disease surveillance activities for pigs post-farmgate in New South Wales. *Aust Vet J* (2010) 88(3):75–83. doi:10.1111/j.1751-0813.2009.00543.x
56. Martin PAJ, Langstaff I, Iglesias RM, East IJ, Sergeant ESG, Garner MG. Assessing the efficacy of general surveillance for detection of incursions of livestock diseases in Australia. *Prev Vet Med* (2015) 121(3–4):215–30. doi:10.1016/j.prevetmed.2015.06.017
57. Stärk KD, Regula G, Hernandez J, Knopf L, Fuchs K, Morris RS, et al. Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. *BMC Health Serv Res* (2006) 6(1):20. doi:10.1186/1472-6963-6-20
58. Higgins V, Bryant M, Hernández-Jover M, McShane C, Rast L. Harmonising devolved responsibility for biosecurity governance: the challenge of competing institutional logics. *Environ Plann A* (2016) 48(6):1133–51. doi:10.1177/0308518X16633471
59. Barclay E. *Local Community Preparedness for an Emergency Animal Disease Outbreak*. Canberra: Rural Industries Research and Development Corporation (2005).
60. Palmer S, Fozdar F, Sully M. The effect of trust on West Australian farmers' responses to infectious livestock diseases. *Sociol Ruralis* (2009) 49(4):360–74. doi:10.1111/j.1467-9523.2009.00495.x
61. Elbers ARW, Gorgievski-Duijvesteijn MJ, Zarafshani K, Koch G. To report or not to report: a psychosocial investigation aimed at improving early detection of avian influenza outbreaks. *Rev Sci Tech* (2010) 29(3):435–49. doi:10.20506/rst.29.3.1988

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer FS and handling editor declared their shared affiliation, and the handling editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Veterinary Science

PERSPECTIVE
published: 03 January 2017
doi: 10.3389/fvets.2016.00117

Proactive Risk Assessments and the Continuity of Business Principles: Perspectives on This Novel, Combined Approach to Develop Guidance for the Permitted Movement of Agricultural Products during a Foot-and-Mouth Disease Outbreak in the United States

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Animal diseases such as foot-and-mouth disease (FMD) have the potential to severely impact food animal production systems. Paradoxically, the collateral damage associated with the outbreak response may create a larger threat to the food supply, social stability, and economic viability of rural communities than the disease itself. When FMD occurs in domestic animals, most developed countries will implement strict movement controls in the area surrounding the infected farm(s). Historically, stopping all animal movements has been considered one of the most effective ways to control FMD and stop disease spread. However, stopping all movements in an area comes at a cost, as there are often uninfected herds and flocks within the control area. The inability to harvest uninfected animals and move their products to processing interrupts the food supply chain and has the potential to result in an enormous waste of safe, nutritious animal products, and create animal welfare situations. In addition, these adverse effects may negatively impact agriculture businesses and the related economy. Effective disease control measures and the security of the food supply thus require a balanced approach based on science and practicality. Evaluating the risks associated with the movement of live animals and products before an outbreak happens provides valuable insights for risk management plans. These plans can optimize animal and product movements while preventing disease spread. Food security benefits from emergency response plans that both control the disease and keep our food system functional. Therefore, emergency response plans must aim to minimize the unintended negative consequence to farmers, food processors, rural communities, and ultimately consumers.

Keywords: continuity of business, public–private partnership, risk assessments, permitted movements, foot-and-mouth disease, animal disease, outbreak response, proactive risk assessment

OPEN ACCESS

Edited by:
Preben William Willeberg, Technical University of Denmark, Denmark

Reviewed by:
Anette Boklund, Technical University of Denmark, Denmark
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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *12 August 2016*
Accepted: *12 December 2016*
Published: *03 January 2017*

Citation:
Goldsmith TJ, Culhane MR, Sampedro F and Cardona CJ (2017) Proactive Risk Assessments and the Continuity of Business Principles: Perspectives on This Novel, Combined Approach to Develop Guidance for the Permitted Movement of Agricultural Products during a Foot-and-Mouth Disease Outbreak in the United States. Front. Vet. Sci. 3:117. doi: 10.3389/fvets.2016.00117

Outbreaks happen when the right host population meets the right infectious agent at the right time. When highly contagious animal diseases not currently found in a country or region are introduced into a naïve population, the result can be explosive spread. For this reason, the response to such a foreign animal disease (FAD) must be rapid and well-planned. Historically, response to highly a contagious FAD like foot-and-mouth disease (FMD) has focused on the eradication of the disease and a return to disease-free status as rapidly as possible (1). This approach, commonly referred to as "stamping out," requires the rapid identification of infected premises, quick depopulation and, for premises with susceptible species in the control area that are at risk for infection but not known to be infected, there are often quarantine and strict movement controls. The scenario in which there are uninfected but susceptible animals near infected premises is likely to occur and is a predicament for regulatory officials. Stopping all movements of animals, products, and potential fomites from infected premises utilizing a stamping out approach is an obvious thing to do to control disease, but what does one do with not known to be infected herds and all of the food they produce?

Everyday there are food products and animals that move from farms in a "just in time" production system. If an FAD outbreak occurs, for those premises in a control area, the product and animal movements can add up quickly. The inability to harvest uninfected animals and move their products to further processing interrupts the food supply chain and has the potential to result in an enormous waste, adversely impact agricultural businesses, and create animal welfare situations. This was more fully recognized following the 2001 outbreak of FMD in the United Kingdom. Of the 6 million animals culled during the 2001 outbreak of FMD in the UK, an estimated one-third died under various types of "welfare cull" (2, 3). These 2 million animals represent the potentially non-infected population that were killed due to the lack of a prepared response that includes continuity of business (COB) considerations for the permitted movement of non-infected animals and animal products. Furthermore, it is increasingly unacceptable globally to destroy large numbers of "healthy" or "non-infected" animals. Thus, current and future response plans should consider COB principles are part of the planning for an FAD response. COB planning is meant to prepare for animal health emergencies and to address what to do with premises and herds that are not known to be infected but may be adversely affected by disease response activities. COB planning tools and guidance can facilitate the managed movement of animals and their products.

Continuity of business principles have been adopted by the United States Department of Agriculture (USDA) Animal Health and Plant Inspection Service (APHIS) Veterinary Services (VS) and were used to guide the permitted movements of products in highly pathogenic avian influenza outbreaks in 2015 and 2016. The stated goals of USDA APHIS VS for a FAD response (4) include COB principles:

> The APHIS goals of an FAD response are to (1) detect, control, and contain the disease in animals as quickly as possible; (2) eradicate the disease using strategies that seek to stabilize animal agriculture, the food supply, and the economy and that protect public health and the environment; and (3) provide science- and risk-based approaches and systems to facilitate continuity of business for non-infected animals and non-contaminated animal products.
>
> Achieving these three goals will allow individual livestock facilities, States, Tribes, regions, and industries to resume normal production as quickly as possible. The objective is to allow the United States to regain disease-free status without the response effort causing more disruption and damage than the disease outbreak itself.

Once USDA APHIS VS adopted COB in principle, it became clear that tools were needed to guide the specific decisions that would balance product movement with outbreak control. To that end, a process was developed utilizing risk assessments performed proactively (i.e., before an outbreak happens) to develop and evaluate science-based guidelines and the associated risk of specific movements from premises located in control areas that are not known to be infected. These risk assessments, done before an epidemic occurs, also take into consideration the potential factors or strategies to mitigate risk that may be put in place during an outbreak. This proactive risk assessment process is a transparent and scientifically accepted method to evaluate commodity and disease specific pathways of transmission. Proactive risk assessments specifically identify pathways where risk exists and explore the necessary mitigations for reducing the risks. The results of the process can help determine the disease transmission risk of specific product movement and inform the responsible regulatory officials and industry stakeholders who are designing emergency preparedness and COB plans before an outbreak. Ultimately, the planning process will allow for informed decision-making regarding managed movement and the COB plan implementation during an outbreak.

The approach used to develop the proactive risk assessment utilizes a collaborative process involving state and federal regulatory officials, academia, and members of private industry (public–private partnerships) and follows the general risk analysis framework that is presented in the OIE Terrestrial Animal Health Code (5). While the intention of the OIE framework is to prevent the entry of animal pathogens and evaluating the risk of susceptible animal exposure through the importation of live animals, animal products, and commodities into a country, the framework fits well for the situation of addressing risk of spread through movements within a control area.

Throughout this process, the input from the food animal industry is crucial as it helps to supply data to support the work and decide what potential mitigation strategies can be realistically adopted by the industry in the event of an FAD outbreak. State and federal regulators' input is also important as they provide the procedures and policies for managed movement as well as the logistics to implement the emergency preparedness plans. The effect of proposed mitigations on existing risk pathways is evaluated and then used to inform the development of movement guidelines that can then become permitted movement guidance. Without development of proactive risk assessments, similar

decisions about movements would have to be made quickly during an outbreak and sometimes without a full understanding of risk. This is one of the main advantages of this approach. The collaborative, proactive approach makes it possible to compile the best available information, model scenarios, understand movement risks, and form mitigation strategies relevant to current production practices. The mitigation strategies can then be put in place and the movements can occur all while following the prescribed regulatory structure. This allows COB to be included in preparedness planning and increases the adoption and awareness of guidelines and tools pre-event.

The collaborative public–private partnership approach is thus a key component to the development of the proactive risk assessments. Just as they would need to work together in an outbreak, these sectors collaborate in developing the proactive risk assessments and guidelines for managed product movement for specific commodities. The process itself communicates findings to the collaborating groups and supports the development of networks of individuals that include public and private stakeholders. Regulators learn about food animal industry systems and practice while industry representatives learn about regulatory processes and requirements. This is a key part of the risk communication step of risk analysis.

Ultimately, the decision to allow managed movements in an outbreak from individual premises in an established control area will be the decision of the responsible regulatory official. The COB plans and guidance materials are practical and useable tools for decision makers. However, it is important to recognize that final decisions in an outbreak situation may have many other constraints like resource limitations, political restrictions, or biological considerations that have not been anticipated in the development of the risk assessment. Also, one of the main limitations is that no one can know exactly what the next outbreak will look like. Although guidance documents can incorporate what is known from past outbreaks, biological agents have a way of acquiring novel characteristics and presenting themselves in new ways. For that reason, guidelines developed through this process to support COB are just that—*guidelines*—and not requirements. The judgment needed to balance disease control, and COB must be made in the context of the ongoing outbreak; this is the intense burden of the responsible regulatory officials.

In the end, outbreaks are expensive, time consuming, and a serious threat to food security and business. No one wants them to happen but when they do, the negative impacts on farmers, food processors, rural communities, and consumers can be lessened with planned responses that include the development of proactive COB guidelines. Regulatory requirements that stop the movement of all animals and animal-derived products may likely result in disease eradication but may just as likely have serious deleterious effects on the entire food supply chain. The development of COB plans that simultaneously address the challenges of controlling an FAD outbreak, maintaining the supply of food to the consumer, and ensuring the viability of the food industry represent an important step in FAD response.

AUTHOR CONTRIBUTIONS

TG was the lead author of the manuscript and formed the perspectives. FS, CC, and MC contributed to the writing and editing of the manuscript.

FUNDING

The authors of this perspectives article would like to acknowledge the support of this work discussed above through USDA APHIS Cooperative Agreement 15-1900-1493-CA.

REFERENCES

1. OIE World Organization for Animal Health. *OIE – Guidelines for Animal Disease Control – May 2014*. Paris, France (2014). Available from: http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/A_Guidelines_for_Animal_Disease_Control_final.pdf
2. Davies G. The foot and mouth disease (FMD) epidemic in the United Kingdom 2001. *Comp Immunol Microbiol Infect Dis* (2002) 25:331–43. doi:10.1016/S0147-9571(02)00030-9
3. Department for Environment Food & Rural Affairs. *Animal Health and Welfare: FMD Data Archive*. Sand Hutton, York, United Kingdom (2004). Available from: http://footandmouth.fera.defra.gov.uk/
4. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. *Foreign Animal Disease Preparedness and Response Plan (FAD Prep) Manual, October 2015*. Bethesda, MD (2015). Available from: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/documents_manuals/fadprep_manual_2.pdf
5. OIE World Organization for Animal Health. *2016 OIE – Terrestrial Animal Health Code; Chapter 2.1 – Import Risk Analysis*. Paris, France (2016). Available from: http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_import_risk_analysis.htm

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer AB and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 31 October 2016
doi: 10.3389/fvets.2016.00097

Prioritization of Managed Pork Supply Movements during a FMD Outbreak in the US

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In the event of a foot-and-mouth disease (FMD) outbreak in the United States, local, state, and federal authorities will implement a foreign animal disease emergency response plan restricting the pork supply chain movements and likely disrupting the continuity of the swine industry business. To minimize disruptions of the food supply while providing an effective response in an outbreak, it is necessary to have proactive measures in place to ensure minimal disease spread and maximum continuation of business. Therefore, it is critical to identify candidate movements for proactive risk assessments: those that are both most likely to contribute to disease spread and most necessary for business continuity. To do this, experts from production, harvest, retail, and allied pork industries assessed 30 common pork supply movements for risk of disease spread and industry criticality. The highest priority movements for conducting a risk assessment included the movement of weaned pigs originating from multiple sow farm sources to an off-site nursery or wean to finish facility, the movement of employees or commercial crews, the movement of vaccination crews, the movement of dedicated livestock hauling trucks, and the movement of commercial crews such as manure haulers and feed trucks onto, off, or between sites. These critical movements, along with several others identified in this study, will provide an initial guide for prioritization of risk management efforts and resources to be better prepared in the event of a FMD outbreak in the United States. By specifically and proactively targeting movements that experts agree are likely to spread the disease and are critical to the continuity of business operations, potentially catastrophic consequences in the event of an outbreak can be limited.

Keywords: swine, risk prioritization, business continuity, movement restrictions, FMD

OPEN ACCESS

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Specialty section:
This article was submitted to Veterinary Epidemiology and Economics, a section of the journal Frontiers in Veterinary Science

Received: *30 June 2016*
Accepted: *17 October 2016*
Published: *31 October 2016*

Citation:
Patterson GR, Mohr AH, Snider TP, Lindsay TA, Davies PR, Goldsmith TJ and Sampedro F (2016) Prioritization of Managed Pork Supply Movements during a FMD Outbreak in the US. Front. Vet. Sci. 3:97. doi: 10.3389/fvets.2016.00097

INTRODUCTION

In the event of a foot-and-mouse disease (FMD) outbreak in the United States., local, state, and federal authorities will implement an emergency response plan as described in the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) Foreign Animal Disease Preparedness and Response Plan (1). This response includes a control and eradication strategy that will utilize depopulation, quarantine, vaccination, and managed movement control measures applied throughout the swine industry. The document recognizes the need to develop a strategic plan to address managed movement control and its implications for continuity of business in foreign animal disease preparedness planning (1).

Continuity of business, in the context of the food supply, means the ability of a farm or food processor to continue key operations of production and distribution of safe, high quality food, and agricultural commodities despite disruption of normal operational procedures (1, 2). These key operations are critical to business vitality and may cause severe economic losses for the industry if disrupted for prolonged periods of time by managed movement controls (3). In order for any managed movement to take place, incident commanders must issue official movement permits for animals or commodities that have an acceptable level of risk. These permits need to be guided by a risk assessment or science-based evaluation (2).

Completing a risk assessment in a timely manner during an outbreak is typically impractical and not conducive for the coordination of managed movement (4). Developing risk assessments requires significant time, thereby potentially delaying the movement of pigs or pork products that may represent negligible risk for disease spread. Throughout the swine industry, there is a heavy reliance on continuous movement of animals, and the timely delivery of animal feed, supplies, and products. Even brief disruptions in the supply of products or movement of animals can result in devastating economic losses as well as serious animal welfare concerns, as available inventory capacity is often limited (3, 5).

Risk assessments conducted proactively, before an outbreak, can identify mitigation strategies to reduce the potential for disease spread and facilitate business continuity. This is done by supporting the timely movement of animals and products that represent an acceptable low risk for disease spread, while providing additional resources and safeguards to restrict those movements that pose a high risk of spreading disease. It is this balance between "acceptable risk" of disease spread and importance for business continuity that incident commanders will be seeking when issuing managed movement orders. Invariably, there may exist movements that are both critical to business continuity, but also pose a high risk for disease spread. These movements are important candidates for conducting a proactive risk assessment due to the anticipated negative consequences for the overall industry if they are not completed in a timely manner. However, little information is available about which specific movements in the pork supply chain are critical to both the potential for disease spread and the economic viability of the industry. Identifying critical movements at the intersection of these two factors is essential for effectively guiding an emergency response in the face of a transboundary disease outbreak such as FMD in swine.

The objective of this study is to establish a framework for prioritizing critical movements within the pork supply chain according to experts' perception of the likelihood of spreading FMD and the importance of the movements for the continuity of business.

MATERIALS AND METHODS

Recruitment of Experts

To effectively evaluate the risk and impact of various movements in the pork supply chain, opinions were solicited from experts who were actively engaged within the swine industry, including pork producers, veterinarians, and academics. Experts were recruited from multiple parts of the production chain, so collectively they would be able to evaluate risk across all of the movements. An online survey (6) was distributed *via* email to the American Association of Swine Veterinarians (AASV) mailing list, and respondents were encouraged to forward the invitation to other industry professionals in an effort to capture a diversity of responses. AASV is a non-profit educational professional society for veterinarians that specialize in swine health and management for the purposes of pork production. To help recruit more experts, announcements about the survey were made at the 2015 Leman Swine Health Conference and at the 2015 World Pork Expo, which are two technical meetings attended by a large group of AASV members, as well as many other swine industry producers and professionals each year. The announcements were followed with an email and link to the survey in the weekly AASV e-newsletter.

Fifty-one experts completed the survey, and an additional 19 provided partial responses (a further 8 consented to participate but did not answer any question, so no information about them is known). Experts indicated their line of work in the survey, and respondents included swine producers ($n = 9$), harvest industry ($n = 4$), retail/distribution ($n = 10$), and allied industries ($n = 47$). Those in allied industries could specify one or more industries. Of those who specified ($n = 31$), responses included veterinarians ($n = 25$), non-veterinarian academic or government workers ($n = 4$), and media/industry ($n = 2$). Pork producers were asked additional demographic questions regarding the size of their production, the type of operation, and the frequency of pig movements. Producers and those in allied industries were also asked about the size and location of their facilities or location of their involvement, and whether they had biosecurity protocols in place.

Prioritization of Critical Movements

To prioritize the critical movements among the pork supply chain, questions were included on the survey to elicit expert opinion on FMD-related threats with the goal of identifying movements that have the highest perceived risk of disease spread and that are understood to be most critical to the business operation. Thirty common pork supply movements were identified based on the structure of the current pork production chain in the United States (7). Included were movements of all live pigs, genetic material, feed, equipment, personnel, and materials that are common to the multistage production systems that predominate in the United States. Movements of finished pork products post-harvest were also considered. These movements were divided into five main categories of the pork production chain: equipment, genetics, general (live animals), harvest and processing, and personnel (**Table 1**).

Experts were asked to assign each of the thirty movements to one of the four categories describing its risk of disease spread: no or slight risk, low risk, some risk, or high risk of FMD disease spread. Then, they were asked to estimate the time at which the restriction of each movement during an outbreak would have a significant negative consequence on business (e.g., high likelihood of bankruptcy and negative impact on animal welfare).

TABLE 1 | Consensus scores for perceived risk of FMD spread, and the mean time until a negative impact on business continuity would occur for each movement.

Category	Number	Consensus high risk of disease spread	Majority placement for risk of disease spread	Time to negative business impact	Movement description
Equipment and feed	1	59	Unclear consensus	2–7 days	Feed onto production sites
	2	54	Unclear consensus	7–14 days	Supplies onto production sites
	3	76	High	7–14 days	Shared equipment onto production sites
	4[a]	79	High	2–7 days	Contracted or shared livestock trucks onto production sites
	5	60	Unclear consensus	2–7 days	Dedicated livestock trucks among company production sites
Live animals	6	60	Unclear consensus	2–7 days	Weaned pigs to off-site nursery, wean to finish, or finishing (single source)
	7	78	High	7–14 days	Finishing pigs direct to slaughter
	8	69	Unclear consensus	14–21 days	Replacement gilts into a sow unit
	9[a]	90	High	2–7 days	Weaned pigs to off-site nursery, wean to finish, or finishing (multiple sources)
	10	65	Unclear consensus	7–14 days	Feeder pigs to finishing (e.g., from nursery to finishing)
	11	63	Unclear consensus	14–21 days	Cull sows and boars direct to slaughter
	12	84	High	14–21 days	Off size and cull pigs, sows, and boars to sale barn/buying station
	13[a]	80	High	7–14 days	Off size and cull pigs, sows, and boars from sale barn/ buying station to slaughter
	14	82	High	14–21 days	Feeder pigs from sale barn to production site
	15	71	Unclear consensus	2–7 days	Dead stock to off-site disposal (landfill, rendering, etc.)
	16	55	Unclear consensus	14–21 days	Manure to field application off-site
Genetic	17	52	Unclear consensus	14–21 days	Replacement gilts and boars into production system isolation
	18	70	Unclear consensus	14–21 days	Replacement gilts and boars onto production site
	19	50	Unclear consensus	2–7 days	Semen into a production system (breeding herd)
Harvesting and processing	20	44	Unclear consensus	2–7 days	Fresh carcasses to off-site processing
	21	43	Unclear consensus	2–7 days	Raw inedibles (byproducts) from harvest site to further processing
	22	30	Unclear consensus	7–14 days	Rendered inedibles from harvest site to further processing
	23	19	Low	2–7 days	Finished products to distributing
	24	23	Low	2–7 days	Fresh products to point of service
	25	19	Low	7–14 days	Ready to eat products to point of service
Personnel	26[a]	76	High	2–7 days	Employees onto, off, and/or between production site(s)
	27	72	Unclear consensus	7–14 days	Routine service providers (e.g., plumbers, electricians, etc.) onto, off, and/or between sites
	28	67	Unclear consensus	7–14 days	Veterinarians onto, off, and/or between sites
	29[a]	82	High	7–14 days	Vaccination crews into, off, and/or between sites
	30[a]	95	High	7–14 days	Commercial crews onto, off, and/or between sites (e.g., manure haulers, feed trucks, and livestock haulers)

[a]*Indicates a priority movement identified in this study.*

Time was expressed in a continuous scale using a slider with four labels from shortest (i.e., most critical, less than 48 h) to longest (i.e., least critical, more than 60 days). The slider was initially positioned at the longest time label. Respondents were instructed to assume all movements would take place according to their biosecurity protocols, if one existed, and to assume that movements were expected to occur the day after a restriction was implemented.

Data Analysis

Perceptions of the risk of disease spread were assessed using an ordinal categorical scale (numeric values were never shown), so contiguous categories were not necessarily uniformly distanced from each other. Further, experts' notion of the difference between any two contiguous choices could vary greatly. Therefore, it was not statistically appropriate to calculate means for this variable to assess overall perceptions of disease risk. Instead, these perceptions were analyzed by taking the upper two choices ("some risk" and "high risk") as to indicate a substantial risk in the movement and the lower choices ("no or slight risk" or "low risk") as to indicate no substantial risk. Therefore, scores were calculated based on the percent of experts who assigned a substantial risk for that movement (referred to as "high" for short from here on), with higher or lower percentages indicating a majority consensus. These "consensus" scores were used to identify the movements in which a substantial majority of experts (over 75%) agreed that the movement carries a high risk of disease spread. Conversely, a low percentage on these scores (below 25%) should be interpreted as a substantial consensus that a movement has low risk of disease spread, as it indicates the majority of experts determined the movement to have no or slight risk of disease spread.

The other per-movement measure, time until critical business impact, was reported on a continuous slider scale, so the mean position of the sliders was calculated for each movement. However, because the labels placed on the scale were chosen to provide easily relatable time frames for respondents rather than to provide equidistant points on the timescale, absolute means of the positions were not a meaningful measure of time to critical business impact (e.g., a slider positioned two-thirds of the way between the labels >48 h and 7 days does not map onto an exact time value). For this reason, the mean times for each movement are reported as categories in **Table 1**, but are plotted based on their mean position in **Figure 1** [for data and analysis scripts, see Ref. (6)].

Each movement was plotted based on the consensus scores of high risk of disease spread and the average time at which business would be critically impacted if the movement were stopped (**Figure 1**). To determine which movements would be best candidates for proactive risk assessments, individual movements were identified in which there was at least a 75% consensus of a high risk of disease spread, and a critical (time-sensitive) importance to business continuity, defined as negative business impact within 7 days (appearing in the top-right quadrant of the plot). Conversely, movements that were deemed by experts to have a low or negligible risk of disease spread, combined with a minimal impact on business continuity (lower left quadrant) were also identified.

RESULTS AND DISCUSSION

The recruited experts worked or owned facilities in many areas across the country. Of those who answered the demographic questions, 8 out of 10 producers and harvest industry respondents reported to own or manage farms or production facilities in multiple states, and most respondents in allied industries (34 out of 41; 82%) reported involvement in multiple states. Respondents also reported to work in each of the seven regions of

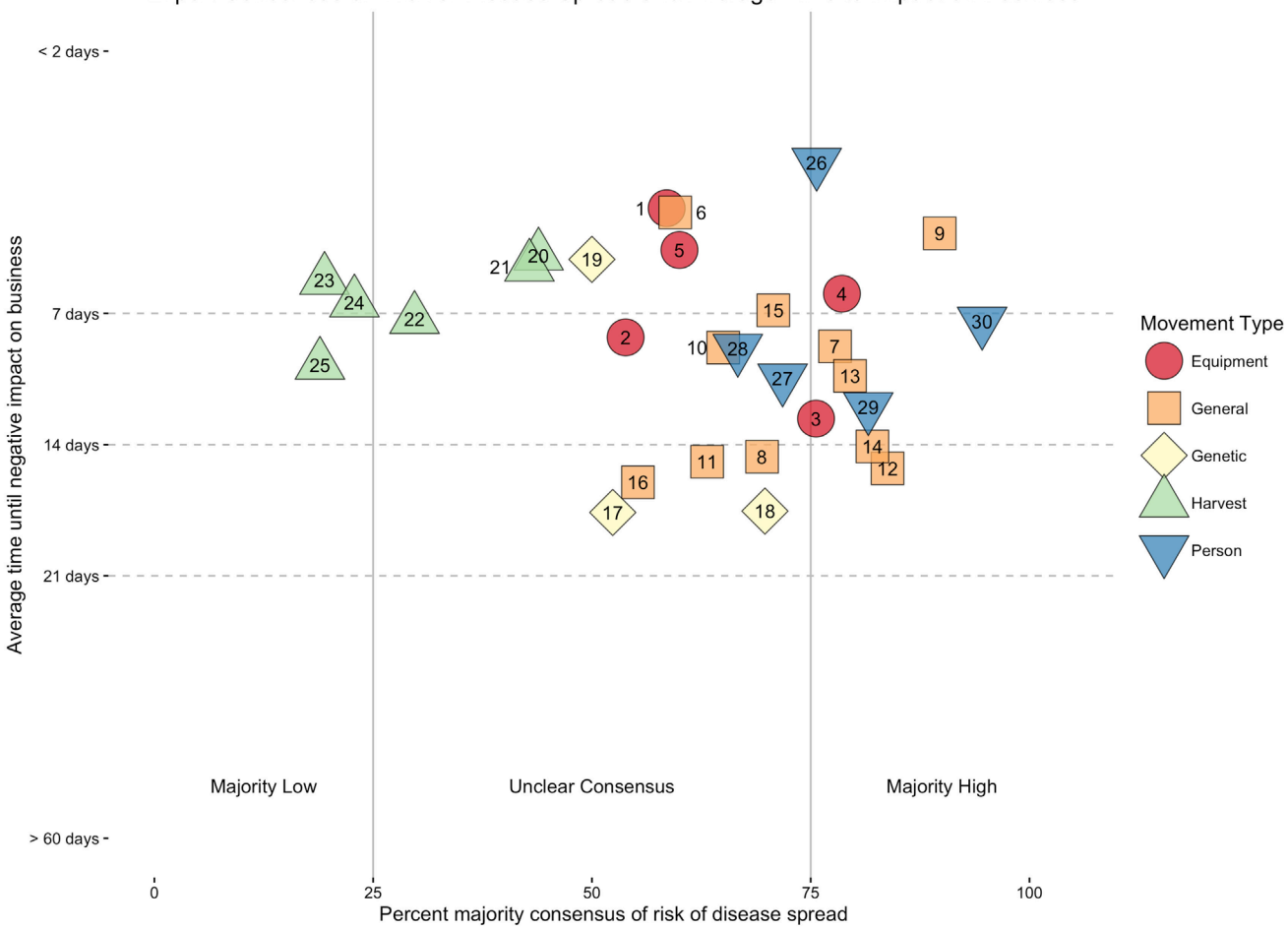

FIGURE 1 | Risk of disease spread consensus scores and mean time until a negative business impact (bars represent SEM) for each of the pork supply chain movements. A specific description of each movement ID number can be found in **Table 1**. Detailed analysis of each movement, along with the precise breakdown of expert responses for each movement, is best appreciated in the interactive web-based version of this publication (http://z.umn.edu/pattersonfmd2016).

the contiguous 48 United States. The vast majority of respondents reported having established biosecurity protocols for live animal sites or visits (42 out of 50; 84%).

When only considering the risk of disease spread, there were 10 movements in which most of the experts (greater than 75%) indicated that the movement had some or high risk of disease spread. The movements with the highest consensus of high risk of disease spread, in order of agreement, are (1) commercial crews onto, off, and/or between sites (95%); (2) weaned pigs to off-site nursery, wean to finish, or finishing (multiple sources) (90%); (3) off size and cull pigs, sows, and boars to sale barn/buying station (84%); (4) feeder pigs from sale barn to production site (82%); and (5) vaccination crews into, off, and/or between sites (82%; see **Table 1**). These results are consistent with current Food and Agriculture Organization of the United Nations (FAO) guidelines for animal disease risk management. According to FAO, animal diseases are spread most often by: the movements of live animals and animal products; by the transport of fomites, people, and equipment between farms; and animal comingling areas such as sale barns and slaughter plants (8).

When only considering impact on business, there were eight movements for which the mean of experts' scores indicated business would be severely affected within 1 week of restriction. The movements with the shortest mean reported time to critical business impact were (1) employees onto, off, and/or between production site(s), (2) feed onto production sites, and (3) weaned pigs to an off-site nursery, wean to finish, or finishing (single source) (see **Figure 1** and **Table 1**).

These results are consistent with the study conducted by Bargen and Whiting (5), which determined the time sensitivity of weaned pig movement off of sow farms. This was done by studying 15 sow farms in the Manitoba province of Canada and determined that if weaned pig movement off-site is restricted, the time to critical overcrowding was approximately 5 days (0.66 ± 0.88 weeks). Also, it makes logical sense that those movements pertaining to the basic husbandry of swine (feed availability, water, and environmental management), which are overseen by daily chore personnel, will have serious implications for animal health and welfare if disrupted (9).

Table 1 shows consensus scores for perceived risk of FMD disease spread, along with the mean time until a negative impact on business would occur across all 30 movement types. Movements with at least a 75% consensus of a high risk of disease spread, in combination with a time-sensitive window (less than 14 days) were defined as priority movements, in which proactive risk assessments would be most advantageous. There were three movements that met the criteria with the shortest time-sensitive window of 2–7 days. They were, in order of the highest percentage consensus for risk of disease spread: (1) weaned pigs to off-site nursery, wean to finish, or finishing (multiple sources), (2) contracted or shared livestock trucks onto, off, and/or between sites production sites, and (3) employees onto, off, and/or between sites. There were two additional priority movements that also had at least a 80% consensus for high risk of disease spread, however, had a longer time-sensitive window (7–14 days). These were, in order of the highest percentage consensus: (4) commercial crews onto, off, and/or between sites (e.g., manure haulers, feed trucks, and livestock haulers); (5) vaccination crews into, off, and/or between sites; and Off size and cull pigs, sows, and boars from sale barn/buying station to slaughter.

Returning to the movement of top priority, there are a number of features of moving weaned pigs to an off-site barn from multiple sources that may have prompted the experts to identify this particular movement as highest priority. There are a number of long-term health and logistical benefits that are captured when a barn is filled quickly with pigs of a similar age, which often requires inputs from multiple sow farm sources (9, 10). The nature of this movement, which may involve a trailer carrying weaned pigs to stop and pick additional pigs at multiple farms before arriving at its final destination, carries a risk of spreading disease to the farms visited. Additionally, when FMD virus naive pigs are presumably mixed with infected pigs at the destination site, virus spread is amplified in the new hosts, which will complicate further containment efforts (3).

Conversely, the movement of weaned pigs off a sow farm is a regular and essential function within the pork supply chain. In most cases, there is limited space available on sow farms to house weaned piglets for prolonged periods of time, and space must be made available frequently for the newest group of weaned pigs (5). For these reasons and according to the experts, this particular movement would have the strongest implications for the swine industry.

It is also noteworthy that movements related to the basic husbandry of swine, such as the movement of chore personnel and feed trucks, were widely considered to have a high risk for disease spread and of critical importance for business continuity. Movement restrictions that limit some of the basic needs of domestic swine (such as food and water) will unsurprisingly cause serious negative consequences if not tended to. This reality must be considered despite the high risk of spreading disease further. Previous studies on this topic have not examined the importance of personnel movement to provide basic animal husbandry, which highlights the need to consider these movements in national emergency preparedness plans.

Proactive risk assessments may also identify movements that should proceed in an FMD outbreak: those that that have a low risk of disease spread and would critically impact business in a short time if stopped. Two movements were perceived by experts to fall into this category. Less than 25% of experts identified "finished products to distributing" and "fresh products to point of service," as having a high risk of disease spread. More specifically, 19% and 23% of experts, respectively, said the movements carried a high risk of disease spread, indicating the majority actually rated the disease spread as low. These movements were also perceived to critically impact business within 2–7 days, if stopped. This initial assessment would indicate that, in the event of an FMD outbreak, these two movements should be allowed to continue so as not to prevent finished products from reaching consumers and thus avoid interruption of the pork meat supply.

Conversely, there were no movements that were perceived by experts to have both a long time to critical business impact and a high risk of disease spread. However, two movements, which at least 75% of experts identified as having a high risk of disease spread, "off size and cull pigs, sows, and boars to sale barn/buying

station" (84%) and "feeder pigs from sale barn to production site" (82%), were reported as having a time to critical business impact between 14 and 21 days. As described by Taylor and Rushton (8), sites where animals are comingled from multiple sources and subsequently transported back to another farm have the potential to spread the disease further. This combination of high perceived risk of disease spread and low time criticality may indicate that these movements are logical candidates for immediate restriction in the event of an FMD outbreak.

While this study provides an initial assessment of movements that would benefit from proactive risk assessments, there are several limitations that future research should address. First, the study did not assess the specific expertise or experience of respondents, which could have been used to weigh responses for given movements based on the level of familiarity/expertise each respondent had in each movement. Second, the expertise sample was predominantly veterinarians and those in allied industry. Future work should specifically target more experts from the producer and harvest industries.

CONCLUSION

This work represents a preliminary descriptive analysis of the major pork supply chain movements, and the extent to which experts agree these movements may contribute to both FMD disease spread and how movement restrictions may critically impact business. While preemptive planning and risk assessment is underway to prepare for a potential FMD outbreak in the United States, it is important to consider whether the benefits of restricting movement (thereby reducing the size or duration of the outbreak) actually outweigh the costs (interrupting business continuity or causing animal welfare concerns). This analysis helps to provide some context for the determination of managed movements within the swine industry, while considering potential consequences of disease spread paired with time sensitivity.

A recent analysis conducted by Paarlberg et al. (11), on the potential cost of an FMD outbreak in the United States, across all livestock sectors, estimated a decrease of $14 billion (9.5%) in United States farm income. Losses in gross revenue for live swine were estimated at a 34% reduction, and pork products at a 24% reduction (11). Given the severe economic losses, which would result in the event of an FMD outbreak in the United States, it is important to consider options, which may help to limit the size and scope of an outbreak, as well as support the continuity of low-risk business operations in order to safeguard industry vitality.

To ensure its economic viability, the pork industry must place a high priority on the development of criteria and the facilitation of agreements to allow specific movements of live swine, industry personnel, and pork products during all phases and types an FMD outbreak. This work provides an initial step to guide emergency planning, as it reveals movements that are critical to business vitality, and should thus be the primary focus of proactive risk assessments in order to minimize disruption of these movements.

As these results show movements pertaining to basic swine husbandry as well as the movement of weaned pigs off of sow farms, pose both a high risk of disease spread paired with a short window of time before severe economic or animal welfare concerns are realized. Effectively managing these movements will therefore require careful consideration of the cost to benefit ratio when issuing movement permits. The information of this study can also be used to help determine which movements are of little consequence if they are temporarily restricted in an effort to contain the outbreak (such as the movement of cull animals), as well as those that would have severe economic consequences without contributing much to the containment of disease spread were they to be restricted (movement of pork products to the consumers).

AUTHOR CONTRIBUTIONS

GP – primary author. FS – contributing author. AM and TL – survey design and data analysis. TS, PD, and TG – contributing authors.

ACKNOWLEDGMENTS

The authors of this study would like to acknowledge the support and guidance of the University of Minnesota USDA APHIS Cooperative Agreement 15-1900-1493-CA, the National Pork Board, and AASV for their contributions to this study. Their inputs are greatly appreciated.

REFERENCES

1. USDA. Ready reference guide – quarantine, movement control, and continuity of business. *Foot-and-Mouth Disease (FMD) Response*. USDA APHIS Veterinary Services (2015). Available from: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/fmd_rrg_cob_qmc_plan.pdf
2. USDA. NAHEMS guidelines: continuity of business. *Foreign Animal Disease Preparedness and Response Plan*. USDA APHIS Veterinary Services (2013). Available from: http://www.cfsph.iastate.edu/pdf/fad-prep-nahems-guidelines-continuity-of-business
3. Wagstrom E, Johnson K, Goldsmith TJ, Farnham M, Bedford P, Mahero M, et al. The role of proactive risk assessments in ensuring business continuity in the swine industry during an FMD outbreak. *136th International Conference on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork*; Washington DC (2011). p. 392–4.
4. Reynolds B, Seeger MW. Crisis and emergency risk communication as an integrative model. *J Health Commun* (2005) 10(1):43–55. doi:10.1080/10810730590904571
5. Bargen LL, Whiting TL. Time to critical overcrowding of Manitoba swine barns in the event of restriction on animal movement. *Can Vet J* (2002) 43(11):855–62.
6. Patterson GR, Hofelich Mohr A, Snider T, Lindsay TA, Davies P, Goldsmith T, et al. Risk prioritization of pork supply movements during an FMD outbreak in the US – data and Materials. *Data Repository for the University of Minnesota*. (2016). Available from: http://hdl.handle.net/11299/181833
7. USDA. Swine industry manual. *Foreign Animal Disease Preparedness and Response Plan*. USDA APHIS Veterinary Services (2011). Available from: https://www.aasv.org/documents/swineindustrymanual.pdf
8. Taylor N, Rushton J. *A Value Chain Approach to Animal Diseases Risk Management: Technical Foundations and Practical Framework for Field*

Application. FAO, Rome: FAO Animal Production and Health Guidelines, No 4 (2011).
9. PIC. *Wean to Finish Manual*. (2013). Available from: http://www.pic.com/Images/Users/1/salesportal/literature/manuals/WeanToFinishManual_April2013_small%5B1%5D.pdf
10. McKean JD. Good production practices: vehicle for enhanced pork safety. *Proceedings of the 32nd Annual Meeting of the American Association of Swine Veterinarians*; Nashville, TN (2001).
11. Paarlberg PL, Lee JG, Seitzinger AH. Potential revenue impact of an outbreak of foot-and-mouth disease in the United States. *J Am Vet Med Assoc* (2002) 220(7):988–92. doi:10.2460/javma.2002.220.988

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