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Procedures for preventing transmission of foot-and-mouth disease virus (O/TAW/97) by people

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Abstract

The aim of this study was to determine personal hygiene protocols and animal avoidance periods needed to prevent transmission of FMDV (O/TAW/97). Forty-six, 9-week-old barrows free of FMDV were randomly allocated to five treatment groups and a control group. Investigators contacted and sampled FMDV-inoculated pigs for approximately 40 min and then contacted and sampled sentinel pigs after using no biosecurity procedures, washing hands and donning clean outerwear, or showering and donning clean outerwear. Personnel were sampled for nasal carriage of FMDV for 85.43 h. Contaminated personnel did not transmit FMDV to susceptible pigs after handwashing or showering, and donning clean outerwear. FMDV was transmitted when biosecurity procedures were not used. FMDV was not detected in nasal secretions of investigators. Thus, extended animal avoidance periods do not appear to be necessary to prevent transmission of FMDV (O/TAW/97) by people to pigs when organic material is removed through handwashing/showering and donning clean outerwear. This study supports similar findings in a previous publication using FMDV (O/UK/35/2001).
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1. Introduction

Foot-and-mouth disease virus (FMDV) is a non-enveloped, single-stranded RNA aphthovirus that generally causes highly contagious vesicular disease in nearly all cloven-footed livestock (House and

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House, 1999). Transmission has been reported to occur by direct contact with infected animals, aerosol, semen, food products, and fomites (House and House, 1999; Sellers and Parker, 1969; Callis, 1996). The 1997 foot-and-mouth disease outbreak in Taiwan was limited to porcine infections; still, economic losses attributable to the outbreak were estimated at US $1.6 billion (Yang et al., 1999). Depopulation of many infected farms was delayed up to 4 weeks because of a lack of manpower dollars (Yang et al., 1999). One caveat that prevents efficient use of essential personnel is the notion that personnel who have been on infected premises avoid susceptible livestock for a designated period due to risk of human transmission of FMDV.

This premise was established when FMDV was isolated from the nasal passages of one of eight people at 28 h, but zero of eight people at 48 h, after exposure to animals infected with FMDV (Sellers et al., 1970). Methods were not detailed in the manuscript and whether the FMDV detected was transmissible or contained an infectious dose was not determined. Despite shortcomings, government policies have been based on that report. DEFRA recommends that individuals avoid susceptible livestock for 7 days after being on an infected premises (Veterinary risk assessment no. 11, Foot-and-mouth disease, DEFRA, UK). Similarly, the USDA recommends that travelers originating from countries with FMDV avoid contact with susceptible animals for 5 days after entry to the United States (USDA/APHIS/VS). Animal avoidance periods are costly during an outbreak. The policy hinders the progress of an already limited number of essential personnel. The number of individuals needed to assess disease status increases, and when extra individuals are not available, the containment process is delayed. Moreover, recent research has demonstrated that animal avoidance periods were not necessary to prevent human transmission of FMDV (O/UK/35/2001) (Amass et al., 2003). That study proposed that extended animal avoidance periods would not be needed for other strains of FMDV. The objectives of this study were to determine personal hygiene protocols and animal avoidance periods needed to prevent transmission of a FMDV isolate from the 1997 outbreak in Taiwan.

2. Materials and methods

Forty-six, 9-week-old barrows originating from a herd in the United States free of FMDV, were transported to Plum Island Animal Disease Center and randomly allocated to one of six treatment groups: (1) 21 pigs which were inoculated with FMD virus; (2) 5 in-pen contact pigs; (3) 5 pigs which were exposed to people directly after they had been in contact with infected pigs; (4) 5 pigs which were exposed to people who had washed their hands and changed into clean outerwear after they had been in contact with infected pigs; (5) 5 pigs which were exposed to people who had showered and changed into clean outerwear after they had been in contact with infected pigs; and (6) 5 pigs which were not exposed to any source of FMD virus.

Additionally, ten, 10-month-old wethers, originating from a herd in the United States free of FMDV, were used to confirm that (O/TAW/97) did not infect ovines (Yang et al., 1999; Dunn and Donaldson, 1997). Five sheep were placed in the pen with infected pigs and five sheep were not exposed to any source of FMD virus.

2.1. Facilities, environment and diet

The groups of pigs were maintained in rooms of two different sizes, 5.3 m × 3.6 m or 3.5 m × 3.1 m. A larger room housed both pigs and sheep that were not exposed to FMD virus in pens 2.0 m × 1.8 m at opposite sides of the room, away from a central drain. The animals in the smaller rooms were not penned. Pig pens/rooms had a 1.21 m long stainless steel nursery feeder, a rubber mat, a plastic play ball, and one nipple waterer. Pigs were fed a commercial diet (Plum Island 14 hog feed/P grower/finisher feed for pigs, Agway, Inc., Syracuse, IN) ad libitum. Sheep pens/rooms had a tub of water and a pan containing a commercial diet (Dehydrated alfalfa pellets, OB of PA, Inc., York, PA), filled according to body weight. All rooms were HEPA exhaust filtered and had negative (relative to the hallways) pressure ventilation.

2.2. Study design and personnel procedures

The pigs to be inoculated were sedated and inoculated intradermally at four sites in each of the hind heel bulbs with a total dose of 400 pig heel bulb infectious dose 50% (Burrows, 1966) of FMD virus

(O/TAW/97), three pigs passages away from the original field isolate. Two days after they had been inoculated, when they had lesions, in-pen contact animals were moved into the room with them. Simultaneously, four investigators whose nares had been sampled prior to entry, entered the room, and came into contact with the first seven inoculated pigs. Investigators wore disposable coveralls, nitrile gloves, and room-designated rubber boots. During contact, all four people performed gross physical examinations of each pig. Physical examinations consisted of handling all four feet and prepuce, manually opening the mouth of each pig, examining the tongue and oral mucosa, and handling the snout while all but the first person took turns manually restraining the pigs by sitting each pig on its hind-quarters such that each examiner was snout to face with each pig. The people took turns taking the rectal temperature of each pig using a separate thermometer for each group of seven inoculated pigs. The third person primarily, and the second person infrequently manually restrained each pig in dorsal recumbancy on the floor while the fourth person collected blood and nasal swab samples from each pig. One swab was used in each nostril to sample each animal. Immediately after having contact with the inoculated pigs, the investigators had similar contact with pigs of group 3. First, nasal swabs were collected by the fourth person with the third person and occasionally the second person manually restraining the pig. Second, physical exams were performed as described above. Third, blood samples were collected by the fourth person with the third person and occasionally the second person manually restraining the pig.

The protocol was repeated for each treatment group. People contacted and sampled the next seven FMD virus-inoculated pigs, washed all visible organic material from their hands with soap and water (1.5–2.5 min), donned new outerwear and gloves and then contacted and sampled the pigs of group 4. The people then showered and ate for approximately 30 min. Next, investigators contacted and sampled the last seven FMD virus-inoculated pigs, showered for 4–4.5 min, donned new outerwear and gloves and contacted pigs in group 5. People remained in each room for approximately a 40 min contact period. In-pen contact sheep and in-pen contact pigs were placed with FMDV-inoculated pigs for the period of time that the human exposure treatments took place and then moved back into their respective isolation rooms.

The investigators then entered a central contaminated hallway for approximately 1 h during which the 21 FMD virus-inoculated pigs were humanely euthanased and lesions recorded. The investigators showered out of the contaminated hallway. Nasal swabs were collected from each person and then each person showered out of containment. The investigators had no further contact with FMD virus, and nasal swabs were collected from them daily for 4 consecutive days. For the next 14 days, individual, room-designated caretakers who had been free from FMDV contact for at least 5 days cared for sentinel and negative control animals.

2.3. Diagnostic evaluation

The caretakers recorded FMD lesions (vesicles on feet, snout/nose, mouth, and/or tongue) in each animal daily. Animals with unambiguous lesions consistent with FMD were removed from the room and humanely euthanased. Samples were collected from animals that did not have lesions at the end of the trial, 14 days after exposure. Blood and nasal swabs were collected for serology and/or virus detection either after they were euthanased or at the end of the 14-day observation period for the clinically normal animals.

2.4. Sample handling

Heparinized blood and nasal swabs were frozen at −70 °C. Blood was frozen without treatment, but nasal swab extracts (collected by inserting one polyester-tipped applicator 2–4 cm into each nostril, and then combining in 2 ml of 0.2% (w/v) bovine serum albumin in tissue culture media containing 20 mM HEPES buffer and antibiotics) were clarified for 30 min at 12,600 × *g* prior to freezing. The nasal swab samples were collected from the investigators by inserting a single polyester-tipped applicator about 1 cm into each nostril, and processing them as described above.

2.5. Detection of FMDV in samples

For animal samples, multi-well plates containing 2 cm^2 monolayers of BHK-21 cells (passage level

62–66; ATCC) were used to detect FMDV in duplicate 10 and 10^{-1} dilutions of thawed heparinized blood or nasal swab samples (sample volumes of 200 μl). Plates were monitored for cytopathic effect (cpe) during 3 days. For human samples, the inoculation was done in a proportional way but in T25 flasks. All samples without cpe were frozen/thawed and passaged two more times as described above to confirm absence of infectivity. Specificity of CPE was determined by identification of virus in freeze-thawed extracts from the multi-well plate, using an indirect ELISA assay similar to one used for virus isolation (OIE, 2000).

2.6. Detection of FMDV-specific antibodies in samples

An indirect ELISA was used to detect blood immunoglobulin M (IgM) and G (IgG) in pig samples only. Known positive, known negative, and unknown samples (prepared as 4-fold dilutions, starting with a dilution of 1/25) were incubated in virus- and mock-coated wells on 96-well-plates (virus was captured using a rabbit-anti-FMD virus type 01 Manisa; Pirbright Laboratory), and bound IgM was then detected with peroxidase conjugates prepared in goats against pig IgM, pig IgG, and 2,2′-azino-di(3-ethylbenzthiazoline-6-sulphonate). The same positive control serum were added to each 96-well-plate to minimise the differences between assays and to determine "percentage of positivity" (PP) values. Endpoint dilutions were determined using a PP value of 15%, selected on the basis of extensive screening of blood from non-immune and immune animals (Crowther, 1995).

3. Results

3.1. Control sheep

In-pen contact control sheep and negative control sheep did not exhibit clinical signs consistent with FMD, had no gross lesions of FMD, and FMDV was not detected in blood or nasal swab samples at the end of the study.

3.2. Confirmation of FMD virus in the inoculated pigs

All 21 of the inoculated pigs developed gross lesions consistent with FMD by the day of human exposure. Foot-and-mouth disease virus was detected in 21 of 21 (100%) blood and nasal swab samples from the FMDV-Inoculated pigs. IgM to FMDV was not detected in any of these pigs and indirect ELISA for IgG was not performed.

3.3. In-pen contact pigs (group 2)

All five of the in-pen contact pigs developed gross lesions consistent with FMD and FMD virus was detected in all the samples of blood and the nasal swabs collected when they were euthanased 1–2 days after their exposure. In-pen contact pigs were seronegative to FMD virus.

3.4. Direct human exposure group (group 3)

All five of the pigs developed gross lesions consistent with FMD, and FMD virus was detected in all the samples of blood and the nasal swabs collected when they were euthanased 2–4 days after their exposure. Only one of the pigs had specific IgM antibodies to FMD virus. All five pigs were seronegative for IgG to FMD virus.

3.5. Handwash and clean outerwear human exposure group (group 4)

The pigs did not show clinical signs consistent with FMD, had no gross lesions of FMD virus, and no FMD virus was detected in nasal swabs or blood taken from them at the conclusion of the experiment. These pigs did not show an antibody response to FMD virus at the end of the study 14 days after they had been exposed.

3.6. Shower and clean outerwear human exposure group (group 5)

The pigs did not show clinical signs consistent with FMD, had no gross lesions of FMD virus, and no FMD virus was detected in nasal swabs or blood taken from them at the conclusion of the experiment. These pigs

did not show an antibody response to FMD virus at the end of the study 14 days after they had been exposed.

3.7. Negative control group (group 6)

The pigs did not show clinical signs consistent with FMD, had no gross lesions of FMD virus, and no FMD virus was detected in nasal swabs or blood taken from them at the conclusion of the experiment. These pigs did not show an antibody response to FMD virus at the end of the study 14 days after they had been exposed.

3.8. Human samples

No FMD virus was detected in the nasal swab samples taken from the investigators before they had contact with the inoculated pigs. Moreover, FMDV was not detected in any of the human nasal swab samples collected immediately after contact with the inoculated pigs or at 13.82 h, 37.65 h, 61.43 h, and 85.43 h after they had been exposed to FMDV.

4. Discussion

To test the effectiveness of two biosecurity procedures in preventing mechanical transmission of FMDV (O/TAW/97) a positive control group (direct human exposure), a negative control group, and two biosecurity procedures (handwash and clean outerwear, and shower and clean outerwear) were compared. In each group investigators were exposed to FMD virus-inoculated pigs for approximately 40 min before each procedure was applied. The inoculated pigs were actively shedding FMD virus, as evidenced by the transmission of the virus to in-pen contact pigs housed in the same pen as the inoculated pigs during the human exposures. Moreover, FMDV (O/TAW/97) infection under these experimental conditions acted the same as under Taiwan 1997 outbreak conditions in that FMDV (O/TAW/97) did not appear to infect sheep in this study. However, we were unable to determine if sheep were sub-clinically infected because we did not complete serological evaluation of sheep.

These results provide further evidence that people can mechanically transmit FMD virus when moving from groups of infected to susceptible pigs. Moreover, mechanical transmission occurred under conditions of animal handling that would routinely be used by foreign animal disease diagnosticians.

Our results supported those of previous studies (Amass et al., 2003) and provided further evidence that washing hands and donning clean outerwear or showering and donning clean outerwear was sufficient to prevent mechanical transmission of two different strains of FMDV by personnel to pigs. Failure of the handwash and clean outerwear human exposure pigs and shower and clean outerwear human exposure pigs to seroconvert to FMD virus suggests that these pigs were not infected, or that the experiment was terminated before seroconversion could occur. Previous studies have shown that seroconversion to this same isolate of FMDV can be detected, with the same ELISA performed in this study, in pigs within 4 days following direct inoculation, or within 4–5 days following a 4 h contact transmission period (Pacheco and Mason, unpublished). Therefore, pigs in this study had adequate time to seroconvert indicating that hand washing or showering and clothing changes were sufficient to reduce the dose of FMDV on investigators to a level unable to infect pigs.

Our result that this strain of FMDV could not be detected in exposed investigators in conjunction with lack of transmission of FMDV when handwashing or showering and clothing changes were implemented, but no animal avoidance periods were utilized, suggests that animal avoidance periods are not needed if personnel remove all visible organic material from their body surfaces and don clean outerwear. These results are consistent with a previous study with FMD virus (O/UK/35/2001) in which the FMD virus was only detected in one of four investigators immediately after exposure to animals infected with FMD virus (O/UK/35/2001) but not 12.75 h, thereafter (Amass et al., 2003).

The results of this study appear to be inconsistent with previous reports using FMD virus strains O_1 Swiss, A_5, O_2, and/or C Noville (Sellers et al., 1970). In these studies, FMDV was isolated from one of eight people at 28 h but not at 48 h after exposure. Additionally, showering did not prevent human transmission of O_1 BFS 1860 and/or C Noville FMDV to one of four steers when people exhaled, coughed, and sneezed directly onto the muzzle of the steers for 2.5 min (Sellers et al., 1971). One possibility for the

varying results is that different viral strains were used. Alternatively, the extent of contact between people and animals in the latter study was unnaturally excessive. The investigators in our study did not take extraordinary measures to transmit FMDV. Instead, routine procedures consistent with protocols for veterinarians investigating an outbreak were performed. Moreover, negative controls were not utilized in the 1971 study so the findings are suspect. In our study, we utilized low-passage BHK-21 cells to detect virus, whereas earlier studies utilized other methods of virus detection. Although there is some evidence to indicate that cell lines may not be as sensitive as primary cell systems for assaying animal-derived FMDV (House and House, 1989), we have found that carefully maintained low-passage cultures of BHK cells are just as sensitive as other methods utilized for evaluation of pig-derived viruses. Specifically, in our hands, BHK-21 cells (passage 62–66), IBRS2 cells (passage 117–122), and primary fetal porcine kidney-derived cells gave nearly identical titers with all of the viruses we inoculated into animals, including the isolates that had never been in cell culture (Pacheco and Mason, unpublished data). Another widely used primary cell culture system, BTY cells, has been reported to be more sensitive than other systems (OIE Manual). However, this cell type does not support the growth of O/TAW/97 (Dunn and Donaldson, 1997), so we did not employ BTY cells in our studies. We did not undertake more sensitive methods of virus detection, e.g., PCR or other genetic methods, since the point of our studies was the detection of live, infectious virus, not non-infectious degradation products.

In summary, the strain of FMDV that we have utilized in this study is considerably different from the O/UK/2001 PanAsian strain that was utilized in an earlier study from our group (Amass et al., 2003). In particular, O/TAW/97 is more virulent in pigs (unpublished data) and cannot infect cattle (Dunn and Donaldson, 1997), so we thought it was particularly important to pursue its ability to be transmitted upon man. Surprisingly, we saw no transmission to pigs by either O/UK/2001 or O/TAW/97, when minimal biosecurity precautions were implemented (Amass et al., 2003). These results suggest that pigs are unlikely to be efficiently infected if minimal biosecurity is employed. Our studies complement other studies, demonstrating that large doses of FMDV exhaled by infected pigs are required to infect naive pigs (A&D, E&I 128, p313). If the results of Alexander and Donaldson's studies are predictive on field transmission, then ability to transmit virus to pigs in an epizootic is likely to require physical contact of naive and infected animals, or contact transmission by workers, making our studies especially relevant. However, some ruminants, such as sheep, can have a higher susceptibility to infection from certain strains of FMDV, as previously reported (Donaldson et al., 2001). Thus certain strains of FMDV could require application of more rigorous biosecurity procedures in other more susceptible species. In the case of the O/TAW/97 isolate, for example, the genetic differences associated with its inability to infect cattle (Beard and Mason, 2000; Pacheco et al., 2003) could contribute to its inefficiency in being spread by aerosol route, or trapped in the nasal cavity of our ‘‘emergency responders". However, as indicated above, even in the case of the O/UK/2001 virus (which lacked significant genetic differences to other type O viruses; Mason et al., 2003), we only detected virus in nasal secretions of 1 four emergency responders (Amass et al., 2003).

The effectiveness of biosecurity interventions tested in this study may not reflect their efficacy under field conditions. Although this study supports data from a previous study, caution should still be taken in interpretation as this study was a single replication under controlled laboratory conditions. Large populations of animals, susceptibility of these animals to infection, increased contact among personnel and animals, lack of compliance by personnel, large pathogen loads, and sub-optimal facility sanitation can all confound the efficacy of biosecurity procedures in the field. Still, the findings of the latest studies regarding FMD transmission by people support a revision of policy in which lengthy animal avoidance periods are replaced by implementation of strict personal hygiene procedures and outerwear changes.

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