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Brief communication

A one-night downtime period prevents the spread of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* by personnel and fomites (boots and coveralls)

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Summary

This paper summarizes observations recorded over a 4-year (1438-day) period regarding the ability of a 1-night period of downtime to prevent mechanical spread of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* between pig populations by personnel and fomites.

Keywords: swine, porcine reproductive and respiratory syndrome, downtime, personnel, fomites

Received: November 30, 2010
Accepted: March 14, 2011

Resumen - Un periodo de reposo de una noche previene la diseminación del virus del síndrome reproductivo y respiratorio porcino y del *Mycoplasma hyopneumoniae* a través del personal y fómites (botas y overoles)

Este artículo resume las observaciones registradas durante un periodo de 4 años (1438 días) con respecto a la habilidad de un periodo de reposo de una noche para prevenir la diseminación mecánica del virus del síndrome reproductivo y respiratorio porcino y del *Mycoplasma hyopneumoniae* entre poblaciones de cerdos a través del personal y fómites.

Résumé - Prévention de la dissémination du virus du syndrome reproducteur et respiratoire porcin et de *Mycoplasma hyopneumoniae* par le personnel et les objets contaminés (bottes et couvre-tout) suite à un temps de repos d'une nuit

Cet article résume les observations colligées pendant une période de 4 ans (1438 jours) sur la capacité d'un temps de repos d'une nuit à prévenir la dissémination mécanique du virus du syndrome reproducteur et respiratoire porcin et de *Mycoplasma hyopneumoniae* entre des populations de porcs par le personnel et des objets contaminés.

The practice of restricting personnel entry to swine farms for extended periods of time following contact with other pigs (downtime) is a highly controversial subject throughout the global swine industry. For many years, downtime periods ranging from 48 to 96 hours have been enforced to reduce the risk of pathogen introduction to farms by personnel, despite a lack of data supporting these periods of time. In the literature, Sellers et al[1] investigated the ability of people to harbor foot-and-mouth disease virus following exposure to infected swine. Results indicated the presence of one positive nasal swab from one person 28 hours post exposure, but not at 48 hours post exposure; however, attempts to replicate these results have not been successful.[2] Likewise, Amass et al[3] reported the recovery of porcine reproductive and respiratory syndrome virus (PRRSV) RNA from fingernail rinses and nasal swabs 5 and 24 hours, respectively, following contact with infected pigs; however, the viability of PRRSV in these samples was not confirmed and transmission to naive sentinels was not observed. Finally, Otake et al[4] recovered infectious PRRSV from the hands, boots, and coveralls of personnel following contact with infected animals and demonstrated transmission to naive sentinels. However, this study also demonstrated that basic sanitary interventions such as showering and changing clothing and footwear could prevent mechanical spread of PRRSV, irrespective of downtime.[4] Collectively, these data challenge the value of extended downtime periods; therefore, to better understand this risk, we summarized observations regarding the ability of a 1-night downtime period to prevent the mechanical spread of PRRSV and *Mycoplasma hyopneumoniae* (M hyo) from infected to susceptible populations by personnel and fomites.

Materials and methods

This study operated using protocols approved by the University of Minnesota Institute of Animal Care and Use Committee.

Facilities

Our observations were recorded in conjunction with a 4-year evaluation of area spread and biosecurity of PRRSV and M hyo conducted at the Swine Disease Eradication Center Production Region Model (St Paul, Minnesota) over the period 2006-2010.[5,6] For the purpose of this report, we will focus on activities that occurred in three facilities that were utilized throughout the 4-year area spread and biosecurity project: the source population, the on-site residence, and the high-level biosecurity building (Figure 1).

The source population contained three hundred 25- to 120-kg pigs that had been

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This article is available online at http://www.aasv.org/shap.html.

Pitkin A, Otake S, Dee S. A one-night downtime period prevents the spread of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* by personnel and fomites (boots and coveralls). *J Swine Health Prod*. 2011;19(6):345–348.

experimentally inoculated with PRRSV MN-184 during years 1 to 4 and with M hyo strain 232 during years 2 to 4.[5,6] This was a continuous-flow population; therefore, the facility was never emptied during the entire 4-year study. New animals, approximately 6 to 8 weeks in age, entered this facility every 2 to 4 weeks depending on the experimental design of the current study.[5,6] New animals were placed in pens adjacent to existing animals, allowing for nose-to-nose contact and circulation of pathogens from infected to naive animals. Throughout the course of the study, clinical signs of respiratory disease and shedding of PRRSV and M hyo within the population were documented, and a 10% to 12% annual mortality was recorded.[5,6]

The on-site residence was a house on the Swine Disease Eradication Center premises that served as a shower-in, shower-out facility for personnel and a site for washing farm-specific clothing and disinfection of farm-specific footwear.

Finally, the high-level biosecurity building was a swine facility which contained 10 animals naive for PRRSV and M hyo throughout the project. This facility was operated using all-in, all-out pig-flow principles; therefore, new batches of animals entered every 2 weeks during year 1 (a total of 26 batches of pigs during year 1) and every 4 weeks during years 2 to 4 (a total of 39 batches of pigs across years 2 to 4). The biosecurity program for this facility consisted of a series of scientifically validated interventions for all direct and indirect routes of PRRSV and M hyo transmission, including an air filtration system designed to prevent introduction of PRRSV- and M hyo-contaminated bioaerosols.[5-7]

Time periods and activities

Again, for the purpose of this report, we will focus on three specific activity periods that occurred daily throughout the 4-year area spread and biosecurity project: the contamination period, the sanitation and downtime period, and the monitoring period. The contamination period occurred from approximately 2 PM to 4 PM Central Standard Time (CST) each day. The purpose of this period was to expose study personnel to PRRSV- and M hyo-infected animals. During the contamination period, personnel entered all 11 pens in the source-population facility, where they fed animals, cleaned pen floors, treated sick pigs, removed dead animals, and completed necessary repair work. On an average working day, one or two people would have entered each facility. On a monthly basis, personnel also marketed finished animals and sampled the population to monitor its PRRSV and M hyo status. Following completion of the contamination period each day, personnel left the facility and initiated the sanitation and downtime period.

The sanitation and downtime period occurred immediately after the contamination period and ran from approximately 4 PM to 6 AM CST. The purpose of this period was to neutralize the effects of the contamination period. During this period, personnel moved from the source population to the on-site residence where they removed farm clothing and footwear, took a shower, donned street clothing and footwear, and refrained from pig contact from approximately 4 PM that afternoon until approximately 6 AM the next morning. Farm-specific clothing was washed and footwear was disinfected using 7% glutaraldehyde and 26% quaternary ammonium chloride (Synergize; Preserve International, Atlanta, Georgia). At approximately 5:30 AM CST on the following day, personnel took another shower, donned clean farm-specific clothing and footwear, and entered the high-level biosecurity building for initiation of the monitoring period.

The monitoring period was the third and final period pertaining to this report and occurred in the high-biosecurity building from approximately 6 AM to 8 AM CST each day. The purpose of this period was to measure the efficacy of the practices utilized during the sanitation and downtime period. Specifically, two outcomes were measured: detection of PRRSV and M hyo on personnel and the PRRSV-M hyo status of sentinel animals housed in the facility. Upon completion of the sanitation and downtime period, personnel moved immediately from the on-site residence to the high-level biosecurity building, a distance of approximately 100 m. Upon entry into the facility-specific anteroom, a swabbing protocol, designed to determine if PRRSV RNA or M hyo DNA was present on personnel, clothing, and footwear, was conducted.[5,6] For collection of samples, Dacron swabs (Fisher Scientific, Hanover Park, Illinois) were immersed in minimal essential medium (MEM) supplemented with 3% fetal calf serum (Difco, Detroit, Michigan) and swiped using a zigzag pattern across each person's head, face, neck, torso, hands, arms, and legs. Swabs were then stored in MEM at -20°C to maintain viral integrity and maximize testing sensitivity until laboratory analysis could be conducted 24 to 48 hours post collection. Following sampling, facility-specific boots, coveralls, and gloves were donned and boots were disinfected using 7% glutaraldehyde and 26% quaternary ammonium chloride. Personnel then entered the animal air space, fed pigs, and cleaned pens. In addition, on a weekly basis, a diagnostic assessment of the health of this 10-pig population was conducted, involving visual observation of clinical signs of respiratory disease and blood testing and nasal swabbing of all 10 pigs. Immediately following collection, all samples from personnel, fomites, and animals were tested for PRRSV RNA and M hyo DNA by polymerase chain reaction (PCR) at the University of Minnesota Veterinary Diagnostic Laboratory (MN VDL, St Paul, Minnesota).[8,9]

Controls

In addition to the daily sampling of animals, people, and inanimate objects, two sets of control protocols, the PRRSV Spread Control and the PRRSV Downtime Control, were conducted. These controls did not involve M hyo because they were conducted during year 1 of the study, when PRRSV was the only pathogen previously inoculated in the source population. The objective of the Spread Control was to document whether PRRSV spread to naive sentinels had occurred by personnel and fomites following contact with infected animals (Figure 2). For 5 consecutive days, one person contacted infected animals in the source population and then immediately contacted naive sentinels (n = 10 pigs 6 to 8 weeks of age) in the high-level biosecurity building in the absence of basic sanitary measures (showering, washing hands, changing coveralls and boots). The objective of the Downtime Control was to document whether PRRSV transmission by personnel and fomites could be prevented by basic sanitary measures (Figure 3). For 5 consecutive days, one person moved from the source population to the high-level biosecurity building, which contained 10 naive sentinel pigs 6 to 8 weeks of age. Before entering the high-level building, this person took a shower and changed coveralls and boots between groups, but did not observe a designated period of downtime. The control groups were housed separately to prevent direct and indirect contact. Daily animal care was practiced as described, which allowed for extensive physical contact between animals and personnel in each group. In addition,

Figure 1: Diagram of facilities and daily movement of personnel between buildings. Each day, study personnel showered and donned clean clothing and footwear in the on-site residence. Personnel then entered the high-level biosecurity (filtered) facility where they donned clean clothing and boots and washed hands prior to entering the animal air space which contained pigs naive for porcine reproductive and respiratory syndrome virus (PRRSV) and *Mycoplasma hyopneumoniae* (M hyo). Prior to animal contact, swabs from hands, coveralls, and boots were collected and tested for PRRSV RNA and M hyo DNA by polymerase chain reaction. Following completion of animal-care duties, personnel then entered the source population facility, which contained 300 pigs infected with PRRSV and M hyo. Following completion of animal care, personnel then re-entered the on-site residence where they showered, donned clean clothing, and remained away from animal contact for 1 night. The next morning, the process was repeated. This pattern of personnel movement was repeated for 1438 days.

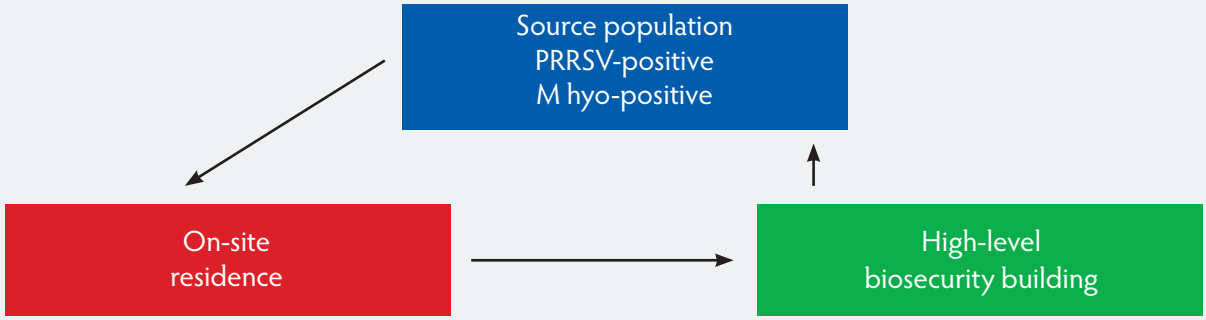
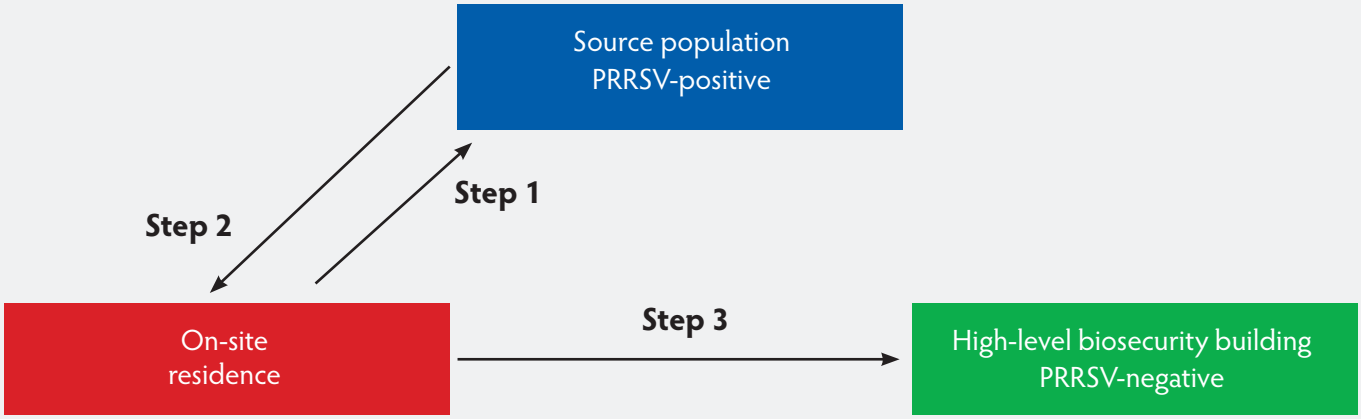


Figure 2: Diagram summarizing the movement of personnel in the porcine reproductive and respiratory syndrome virus (PRRSV) Spread Control protocol. Facilities described in Figure 1. The purpose of this protocol was to prove that spread of PRRSV could occur from infected to naive animals by personnel and fomites in the absence of the specified biosecurity protocols. The control protocol was conducted for 5 consecutive days, and each day, swabs were collected from hands, coveralls, and boots upon entry into the high-level facility and tested for PRRSV RNA by polymerase chain reaction.

Figure 3: Diagram summarizing the movement of personnel in the porcine reproductive and respiratory syndrome virus (PRRSV) Downtime Control protocol. Facilities described in Figure 1. The purpose of this protocol was to document if PRRSV spread via personnel and fomites could be prevented by basic sanitary measures. The control protocol was conducted for 5 consecutive days, involving personnel moving from the PRRSV-positive source population directly to the high-level facility following showering and changing clothing and footwear. Upon entry into this latter facility, swabs from hands, coveralls, and boots were collected and tested for PRRSV RNA by polymerase chain reaction.



daily personnel swabs and fomite swabs were collected at designated points (Figures 2 and 3) as previously described. Furthermore, sentinel animals in both control groups were blood tested on Day 0 (on arrival at the sentinel facility), on Day 5 (at the end of the 5-day contact period) and on Day 20 (15 days after completion of the contact period). Blood samples were tested for the presence of PRRSV RNA by PCR at the MN VDL.

Results

Over the course of the 1438-day study period, a total of 25 people were involved in movements and activities between the aforementioned facilities. A total of 7174 and 4833 personnel and fomite samples, respectively, were tested for PRRSV RNA and M hyo DNA. All swabs were PCR-negative and all animals (n = 480) housed in the high-level biosecurity facility remained PRRSV-naive and M hyo-naive throughout the entire 4-year study period. The approximate duration of the sanitation and downtime period was 14 to 16 hours each day. Mechanical spread of PRRSV secondary to contact with contaminated personnel and fomites was observed during the Spread Control protocol. Five of 10 sentinels were PRRSV-positive on Day 5 post exposure, while 10 of 10 sentinels were PRRSV-positive on Day 20 post exposure. In addition, PRRSV RNA was detected on 40 of 45 swabs collected from personnel and fomites upon entry into the high-biosecurity facility following contact with the infected animals in the source population. In contrast, PRRSV transmission to sentinels was prevented during the Downtime Control protocol, as all sentinels remained PRRSV-negative over the testing period. While PRRSV RNA was detected on 42 of 45 swabs from personnel and fomites collected upon exiting the source population across all 5 exposure days, all swabs (45 of 45) collected upon entry into the sentinel facility following a shower and coverall-boot change were PCR-negative. The time required to shower and change coveralls and boots between groups was approximately 45 minutes.

Discussion

Under the conditions utilized to compile this report, a downtime period of 1 night (14 to 16 hours in duration) prevented the spread of PRRSV and M hyo by personnel and fomites between infected and susceptible populations. While the information is strictly observational in nature and was collected under model conditions, it challenges the validity of extended downtime periods. Furthermore, while data describing the ability of fomites and personnel to mechanically transmit PRRSV, along with intervention strategies to reduce these risks, have been previously published,[4,5] this new study is novel and rigorous, as the observations were collected over an extended period of time, involved numerous people, employed an extensive antigen-based sampling procedure, and included a set of control protocols based on PRRSV. Unfortunately, due to study-design limitations, a larger number of controls, as well as a set of M hyo controls, could not be included. In addition, this study did not require the collection of nasal swabs from personnel. This decision was based on the fact that in a previous study,[3] recovery of PRRSV RNA from human nasal samples was limited to a single sample collected 48 hours post exposure. It was the authors' opinion that if this was indeed a true result, we would have expected that the frequency of detection would have been greater, that the initial detection would have occurred at an early period(s) post exposure, and that virus would have been detected in a larger number of study personnel. Finally, due to the length of our study period and the invasiveness of the procedure, it would have been impossible to enforce compliance over such a large number of participants.

In conclusion, while these observations are intriguing and have the potential to improve the efficacy of the personnel downtime policies within the industry, more research is clearly needed to investigate and clarify questions which still exist surrounding this issue. For example, since these observations are applicable only to PRRSV and M hyo, we do not know whether they would be repeatable across other pathogens such as porcine circovirus type 2 or swine influenza virus. Nor have we conclusively determined the minimum amount of downtime required to prevent pathogen spread. On the basis of these observations regarding PRRSV and M hyo, any amount of downtime could be considered "wasted time." However, since our controls did not include M hyo, we cannot draw any affirmative conclusions without more data. Ideally, if future studies could answer these questions, and an organized discussion could take place across decision makers throughout the industry, perhaps agreement across industry leaders could be reached, resulting in the development of a national program of downtime. Until then, it is hoped that the observations from this report will initiate discussions across early adaptors, guide decision making, and promote change across systems owned and operated by those who are already convinced by these data.

Implications

- Under the conditions of this study, mechanical spread of PRRSV and M hyo by personnel and fomites (boots and coveralls) is prevented by basic sanitation procedures.
- On the basis of these observations, extended downtime periods are not required to reduce the risk of mechanical spread of these two pathogens by personnel and fomites.
- These results should not be extrapolated across other pathogens such as porcine circovirus type 2 or swine influenza virus until further research can be conducted.

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