

Evaluation of a needle-free injection device to prevent hematogenous transmission of porcine reproductive and respiratory syndrome virus

S. R. Baker, DVM, MPH; E. Mondaca, DVM, PhD; D. Polson, DVM, MS, PhD; S. A. Dee, DVM, MS, PhD

Summary

Objective: To evaluate the ability of a needle-free injection device (NFID) to prevent hematogenous transmission of porcine reproductive and respiratory syndrome virus (PRRSV).

Materials and methods: Eighty-eight 5-week-old gilts from a PRRSV-negative source were organized into five groups and individually housed by group in isolation rooms (four replicate trials, 22 pigs per trial). On Day 0, pigs in Group 1 (PRRSV source population) were inoculated with PRRSV isolate MN-184, and pigs in Group 4 (sham-inoculated group) were inoculated with virus-free medium. On Days 4, 5, and 6 post

inoculation, each pig in Groups 1, 2, and 3 was vaccinated with a *Mycoplasma hyopneumoniae* bacterin using the needle-syringe and the NFID. First, a needle-syringe and NFID were both used to vaccinate pigs in Group 1, and then the same needle-syringe and NFID were used to vaccinate pigs in Group 2 (needle-syringe) and Group 3 (NFID), respectively.

Results: On Day 11, all pigs in Group 2 tested positive for PRRSV RNA, suggesting that transmission of PRRSV had occurred between Groups 1 and 2 by repeated use of the same needle. On Day 21, all pigs in one replicate of Group 3 tested positive for PRRSV RNA, suggesting that transmission

of PRRSV had occurred between Groups 1 and 3 by repeated use of the same NFID.

Implications: Under the conditions of this study, hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle, and use of NFIDs does not prevent hematogenous transmission of PRRSV.

Keywords: swine, porcine reproductive and respiratory syndrome virus, needle-free injection devices, AcuShot

Received: June 6, 2011

Accepted: August 19, 2011

Resumen - Evaluación de un aparato de inyección sin aguja para prevenir la transmisión hematogena del virus del síndrome reproductivo y respiratorio porcino

Objetivo: Evaluar la habilidad de un aparato de inyección sin aguja (NFID por sus siglas en inglés) para prevenir la transmisión hematogena del virus del síndrome reproductivo y respiratorio porcino (PRRSV por sus siglas en inglés).

Materiales y métodos: Se organizaron ochenta y ocho hembras de 5 semanas de edad de una fuente negativa al PRRSV en cinco grupos y se alojaron individualmente por grupo en cuartos de aislamiento (cuatro réplicas, 22 cerdos por prueba). En el Día 0, los cerdos en el Grupo 1 (población fuente del PRRSV) fueron inoculados con el aisla-

miento MN-184 de PRRSV y los cerdos del Grupo 4 (grupo de inoculación simulada) fueron inoculados con medio libre de virus. Los días 4, 5, y 6 post inoculación, cada cerdo de los Grupos 1, 2, y 3 fueron vacunados con una bacterina de *Mycoplasma hyopneumoniae* utilizando la jeringa con aguja y el NFID. Primero, se utilizaron la jeringa con aguja y el NFID para vacunar a los cerdos del Grupo 1, luego la misma jeringa con aguja y el NFID se utilizaron para vacunar a los cerdos del Grupo 2 (jeringa con aguja) y del Grupo 3 (NFID), respectivamente.

Resultados: En el Día 11, todos los cerdos en el Grupo 2 resultaron positivos al RNA del PRRSV, sugiriendo que la transmisión del PRRSV había ocurrido entre los Grupos 1 y 2 por el uso repetido de la misma aguja.

En el día 21, todos los cerdos en una réplica del Grupo 3 resultaron positivos al RNA del PRRSV, sugiriendo que la transmisión del PRRSV había ocurrido entre los grupos 1 y 3 por el uso repetido del mismo NFID.

Implicaciones: Bajo las condiciones de este estudio, la transmisión hematogena de PRRSV puede ocurrir de cerdos infectados a cerdos susceptibles vía uso repetido de la misma aguja, y el uso de los NFID no previene la transmisión hematogena del PRRSV.

Résumé - Évaluation d'un appareil d'injection sans aiguille afin de prévenir la transmission hématogène du virus du syndrome reproducteur et respiratoire porcine

Objectif: Évaluer la capacité d'un appareil à injection sans aiguille (NFID) à prévenir la transmission hématogène du virus du syndrome reproducteur et respiratoire porcine (PRRSV).

Matériels et méthodes: Un total de 88 cochettes âgées de 5 semaines provenant d'un élevage négatif pour PRRSV ont été réparties en cinq groupes et hébergées par groupe dans des chambres d'isolement (quatre essais répétés, 22 porcs par essai). Au Jour 0, les porcs du Groupe 1 (population source

SRB: Saint Paul, Minnesota.

EM, DP: Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri.

SAD: Pipestone Veterinary Clinic, Pipestone, Minnesota.

Corresponding author: Dr Scott Dee, 1300 S Highway 75, Pipestone, MN 56164-3211; Tel: 507-825-4211; Fax: 507-825-3140; E-mail: sdee@pipevet.com.

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

Baker SR, Mondaca E, Polson D, et al. Evaluation of a needle-free injection device to prevent hematogenous transmission of porcine reproductive and respiratory syndrome virus. *J Swine Health Prod.* 2012;20(3):123-128.

du PRRSV) ont été inoculés avec l'isolat MN-184 du PRRSV, et les porcs du Groupe 4 (groupe faussement inoculé) ont été inoculés avec du milieu sans virus. Aux Jours 4, 5, et 6 post-inoculation, chaque porc dans les Groupes 1, 2, et 3 était vacciné avec une bactérine de *Mycoplasma hyopneumoniae* utilisant une seringue avec aiguille et le NFID. Premièrement, une seringue avec aiguille et le NFID ont tous les deux été utilisés pour vacciner les porcs dans le Groupe 1, et par la suite la même seringue avec aiguille et le NFID ont été utilisés pour vacciner les porcs dans le Groupe 2 (seringue avec aiguille) et le Groupe 3 (NFID), respectivement.

Résultats: Au Jour 11, tous les porcs dans le Groupe 2 se sont avérés positifs pour la

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease for the US swine industry, with annual losses estimated at approximately \$560 million.¹ The etiologic agent of PRRS is porcine reproductive and respiratory syndrome virus (PRRSV).² Controlling the disease with conventional methods such as vaccination and animal flow has had limited success, due in part to the multiple direct and indirect routes of transmission. PRRS virus has been directly transmitted from infected pigs to naive pigs horizontally via multiple porcine secretions like blood and semen.³ PRRS virus has also been indirectly transmitted from infected pigs to naive pigs by fomites. Boots and coveralls,⁴ needles,⁵ containers, and vehicles⁶ are objects at farms that have acted as fomites for PRRSV. The risk of other objects at farms acting as fomites needs to be evaluated to identify other indirect routes of transmission.

The risk of needle-free injection devices (NFIDs) acting as fomites for PRRSV transmission has not been evaluated. There has been a renewed interest in NFIDs for delivering vaccine or pharmaceuticals in the swine industry because they offer some advantages over conventional needle-and-syringe methods: elimination of broken needles, consistent vaccine delivery, reduction of injuries to on-farm personnel, elimination of needle disposal, and reduced pain and stress to pigs.⁷ The NFIDs deliver vaccine or pharmaceuticals directly through the skin (transdermally) by forcing it out of the device at such a high velocity (> 100 m per second) that it creates a small hole in the skin. Since it has been demonstrated

présence d'ARN du PRRSV, suggérant ainsi que la transmission du PRRSV est survenue entre les Groupes 1 et 2 par utilisation répétée de la même aiguille. Au Jour 21, tous les porcs d'une des réplifications du Groupe 3 ont testé positifs pour l'ARN du PRRSV, suggérant que la transmission du PRRSV s'est produite entre les Groupes 1 et 3 par utilisation répétée du même NFID.

Implications: Dans les conditions de la présente étude, la transmission hématogène du PRRSV d'un animal infecté à un animal susceptible peut se produire via l'utilisation répétée de la même aiguille, et l'utilisation d'un NFID ne prévient pas la transmission hématogène du PRRSV.

that hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle,⁵ another potential advantage of needle-free technology is reduction of transmission of PRRSV. Therefore, the objective of this study was to evaluate the ability of an NFID to eliminate the hematogenous transmission of PRRSV. The study was based on the hypothesis that the risk of hematogenous transmission of PRRSV from infected pigs to susceptible pigs is prevented through the use of an NFID.

Materials and methods

The study protocol was approved by the University of Minnesota Institutional Animal Care and Use committee.

Animals and housing

A total of 88 five-week-old gilts were purchased from a farm known to be PRRSV-naive on the basis of 6 years of diagnostic data and absence of clinical signs. The pigs were divided into four replicates of five groups. Each group was housed in a separate room in an isolation facility at the University of Minnesota. The isolation rooms were separately ventilated and drained. Strict biosecurity protocols were followed when entering each room to minimize the risk of contamination between groups. After exposure to a given group of pigs, the investigator showered and changed scrubs, gloves, hairnet, and boots before entering the next room.⁶

Experimental design and study timeline

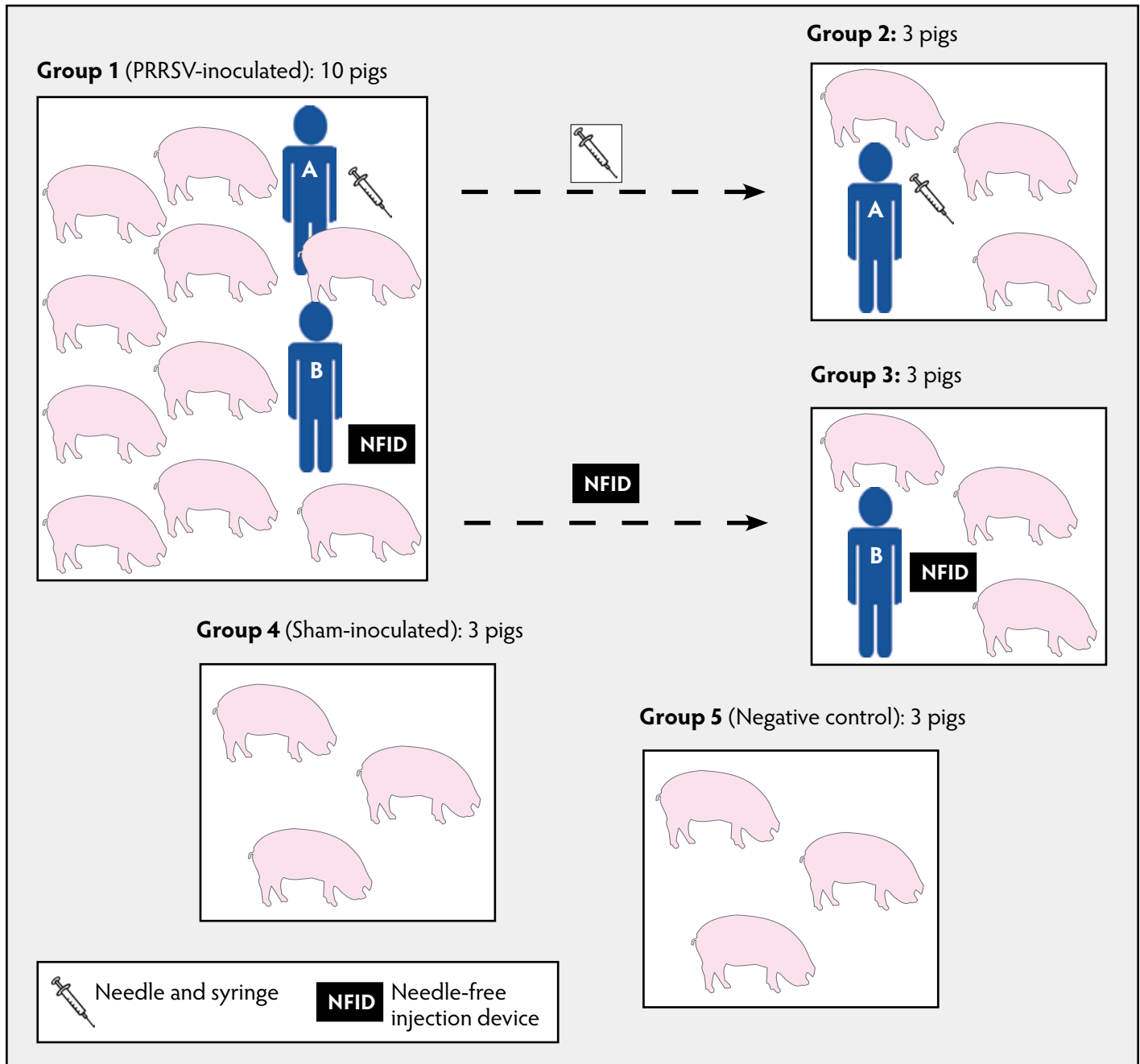
There were five groups in this study. Group 1 pigs were inoculated with PRRSV and consisted of 10 pigs per replicate. Groups 2, 3, 4, and 5 each consisted of three pigs per replicate.

Group 2 pigs were vaccinated by needle-syringe, Group 3 pigs were vaccinated with an NFID, Group 4 pigs were sham-inoculated, and Group 5 pigs were not treated.

On Day 0, pigs in Group 1 were inoculated intramuscularly with 2 mL of PRRSV isolate MN-184 at a concentration of 1×10^5 median tissue culture infectious doses. PRRS virus isolate MN-184 was used for this project because of its classification as highly pathogenic, which is based on high levels of viral shedding, a high viral load in blood, and severe clinical signs.⁸ All pigs in Group 4 were intramuscularly inoculated with 2 mL of virus-free minimal essential medium.

The experimental design is summarized in Figure 1. On Days 4, 5, and 6 post inoculation, pigs in Group 1 were vaccinated with 4 mL of *Mycoplasma hyopneumoniae* bacterin (Ingelvac MycoFlex; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri) via the traditional needle and syringe system (2-mL adjustable bottle-mount Vaccinator Prima Tech; Think Livestock, Huntly, Australia) and a gas-powered NFID (AcuShot Needle-Free; AcuShot Inc, Winnipeg, Manitoba, Canada). Vaccination with *M hyopneumoniae* bacterin was used in the study because nursery pigs are commonly vaccinated against this bacterium. All pigs in Group 1 were vaccinated in the right side of the neck with 2 mL of vaccine administered intramuscularly via the same needle and syringe. These pigs were then vaccinated with 2 mL of vaccine transdermally in the left side of the neck via the same NFID. Swab samples were taken of the surfaces of the needle and syringe and the NFID on Days 5 and 6 (excluding the needle and the tip of the NFID), without use of a template or a standard defined area of the device, to validate the absence of PRRSV.⁹ One swab was collected from each of the two devices in each of the four replicates on Days 5 and 6 for a total of 16 swabs. Swabs from the two surfaces were put into individual sterile tubes (Falcon, Franklin Lakes, New Jersey) containing 2 mL of phosphate buffered saline. The surfaces of the needle and syringe and NFID, excluding the needle and the tip of the NFID, were wiped with an iodine solution. The needle and syringe and NFID were placed into sealed containers and transferred to rooms that housed Group 2 and Group 3, respectively. The same needle and syringe and NFID that were used to vaccinate the infectious group were used to vaccinate Groups 2 and 3, respectively. On Day 21, all pigs in each group were euthanized.

Figure 1: Designation of groups and personnel movement between a group of pigs (Group 1) inoculated with porcine reproductive and respiratory syndrome virus (PRRSV) and two groups of PRRSV-naïve sentinel pigs (Groups 2 and 3). Group 4 pigs (PRRSV-naïve) were inoculated with virus-free medium (sham-inoculated group). Group 5 pigs were PRRSV-naïve untreated controls. Each group was housed in isolation. Days 4, 5, and 6 post inoculation of Group 1, Person A vaccinated the pigs in Group 1 with a *Mycoplasma hyopneumoniae* bacterin using a needle and syringe (right neck), and Person B vaccinated the same pigs with the same bacterin (left neck) using a needle-free injection device (NFID). The needle and syringe used to vaccinate Group 1 pigs was transferred to the room housing Group 2 pigs and the NFID was transferred to the room housing Group 3 pigs. Person A vaccinated Group 2 pigs with the same needle and syringe used to vaccinate Group 1 pigs, and Person B vaccinated Group 3 pigs with the same NFID used to vaccinate Group 1 pigs. Four replicates were performed for a total of 88 five-week-old gilts.



Animal sampling

Blood samples were collected from all pigs on arrival (Day 0) to ensure that they were PRRSV-negative. Samples were collected from the pigs in Group 1 on Days 1 and 4 to determine if inoculation of PRRSV had infected all of them. Samples were collected from all pigs in Groups 2 and 3 on Days 6, 11, 18, and 21 to determine if PRRSV was transmitted from the infectious group to the Group 2 and 3 pigs via the needle and syringe and NFID, respectively. Samples were collected from all pigs in Groups 4 and 5 on Day 21. Sera were separated from blood samples by centrifugation.

Diagnostic analysis

Serum samples were tested for the presence of PRRSV RNA by polymerase chain reaction (PCR) using the TaqMan PCR assay (Perkin-Elmer Applied Biosystems, Foster City, California).¹⁰ Sera were tested for PRRSV antibodies by Idexx 2XR enzyme-linked immunosorbent assay (ELISA) (Idexx Laboratories, Westbrook, Maine).¹¹ An ELISA sample-to-positive (S:P) ratio

≥ 0.4 was considered positive. All PRRSV-seropositive results were confirmed with the PRRSV immunofluorescent assay (IFA). The IFA tested against four strains of PRRSV: a North American field strain, VR-2332, a European field strain, and Lelystad. A positive PRRSV PCR from each replicate was submitted to the University of Minnesota Veterinary Diagnostic Laboratory for sequencing of the open reading frame (ORF) 5 region. The sequence results were compared to the ORF5 from the original inoculum (PRRSV wild-type isolate MN-184).

Statistical analysis

Statistical analyses of the PCR and ELISA results on different study days of each group were performed using Minitab software (Minitab Inc, State College, Pennsylvania) applying Fisher's exact test. A value of $P < .05$ indicates a significant difference between the results within a group or the results between treatment groups.

Results

PCR and ELISA testing pigs

All pigs were PRRSV-negative by PCR (Table 1) and seronegative by ELISA (S:P ratio < 0.4) on arrival (Table 2). All pigs in Groups 4 and 5 were PRRSV-negative (Table 1) and seronegative when tested on Day 21 (Table 2).

All Group 1 pigs in each replicate were PRRSV-positive by PCR on Day 1 (Table 1) and seropositive on Day 21 (Table 2). Clinical signs of anorexia and lethargy were observed in several pigs. Ten pigs died or were euthanized due to respiratory distress during the experiment. The nucleic acid in the ORF5 region of one sample from each replicate was 99.8% homologous with the PRRSV inoculum isolate nucleic acid.

In Group 2, at least two pigs in each replicate were PRRSV-positive on Day 6 (2 days post exposure; Table 1). All pigs in each replicate were PRRSV-positive both on Day 11 and Day 18 (7 and 14 days post exposure; Table 1) and further PCR testing was not

Table 1: Results of PCR for PRRSV among groups of 5-week-old gilts either inoculated with PRRSV and vaccinated with *Mycoplasma hyopneumoniae* bacterin (Group 1) or vaccinated with the same needle and syringe (Group 2) or NFID (Group 3) used to vaccinate Group 1*

Groups		No. of pigs PCR-positive for PRRSV / no. of pigs tested†							
		Day 0*	Day 1	Day 4	Day 6	Day 11	Day 18	Day 21	
Group 1 Infected	Pigs	0/40 ^a	39/39 ^b	39/39 ^b	NT	NT	NT	NT	
	Replicates	0/4	NT	NT	4/4	4/4	4/4	NT	
Group 2 Needle-syringe	Pigs	0/12 ^a	NT	NT	9/12 ^b	12/12 ^b	12/12 ^b	NT	
	Replicates	0/4	NT	NT	4/4	4/4	4/4	NT	
Group 3 NFID	Pigs	0/12 ^a	NT	NT	0/12 ^a	2/12 ^a	3/12 ^a	3/12 ^a	
	Replicates	0/4	NT	NT	0/4	1/4	1/4	1/4	
Group 4 Sham inoculation		Pigs	0/12 ^a	NT	NT	NT	NT	NT	0/12 ^a
Group 5 Negative control		Pigs	0/12 ^a	NT	NT	NT	NT	NT	0/12 ^a

* Experimental design and vaccination protocol described in Figure 1. Blood samples were collected from all pigs on Day 0, from Group 1 pigs on Days 1 and 4, from Group 2 and 3 pigs on Days 6, 11, 18, and 21, and from Group 4 and 5 pigs on Day 21. Sera were tested for PRRSV RNA by TaqMan PCR assay (Perkin-Elmer Applied Biosystems, Foster City, California). Sera from Group 2 pigs on Day 21 were not tested. A replicate was considered PRRSV-positive when at least one pig was positive. One pig in Group 1 died of respiratory distress on Day 0.

† Shaded areas of the table indicate days when pigs were PRRSV-positive.

^{ab} Values with different superscripts within a row differ significantly ($P < .05$; Fisher's exact test). Statistical analysis compared numbers of PRRSV-positive and PRRSV-negative individual pig samples, not the replicates.

PCR = polymerase chain reaction; PRRSV = porcine reproductive and respiratory syndrome virus; NFID = needle-free injection device; NT = not tested.

conducted. All Group 2 pigs were seropositive on Day 21 (17 days post exposure; Table 2). The nucleic acid in the ORF5 region of one sample from each replicate was 99.8% homologous with the PRRSV inoculum isolate nucleic acid.

In Group 3, all pigs in one replicate were PRRSV-positive on Day 18 (14 days post exposure; Table 1) and were seropositive on Day 21 (17 days post exposure; Table 2). The nucleic acid in the ORF5 region of one representative sample was 99.2% homologous with the PRRSV inoculum isolate nucleic acid.

The proportion of PRRSV PCR-positive animals was significantly lower within the NFID group than within the needle-syringe group ($P < .05$; Fisher's exact test).

PCR testing syringes and the NFID

All four swabs taken from the surface of the syringe before transferring it from Group 1 to Group 2 tested PRRSV-negative on Day 5. One of the four swabs taken from the surface of the syringe tested PRRSV-positive on Day 6. All eight swabs taken from the surface of the NFID before transferring it from Group 1 to Group 3 tested PRRSV-negative on Days 5 and 6.

Discussion

The study was based on the hypothesis that the risk of hematogenous transmission of PRRSV from infected pigs to susceptible pigs is prevented through the use of NFIDs. The results indicated that use of the NFID reduced but did not prevent hematogenous transmission of PRRSV. A possible explanation is that blood was occasionally observed at the site of vaccination. If the high velocity of the vaccine delivery by the NFID ruptured blood vessels near site of injection, blood from PRRSV-infected pigs could have contacted the tip of the NFID and then been injected into a PRRSV-negative pig. Therefore, NFIDs like AcuShot have the potential to act as fomites for PRRSV transmission. The possibility of a breach in biosecurity is unlikely because the negative controls in the study remained negative for PRRSV throughout the study.

Overall, the study had several strengths. For each study group there were multiple replicates, multiple diagnostic methods to validate the results (PCR, ELISA, sequencing, and IFA), and multiple control groups. The needle-and-syringe group (Group 2)

Table 2: Results of PRRSV ELISA testing among groups of 5-week-old gilts either inoculated with PRRSV and vaccinated with *Mycoplasma hyopneumoniae* bacterin (Group 1) or vaccinated with the same needle and syringe (Group 2) or NFID (Group 3) used to vaccinate Group 1*

Groups		No. of pigs seropositive / no. of pigs tested†	
		Day 0	Day 21
Group 1	Pigs	0/40 ^a	30/30 ^b
Infected			
Group 2	Pigs	0/12 ^a	12/12 ^b
Needle-syringe	Replicates	0/4	4/4
Group 3	Pigs	0/12 ^a	3/12 ^a
NFID	Replicates	0/4	1/4
Group 4	Pigs	0/12	0/12
Sham inoculated			
Group 5	Pigs	0/12	0/12
Negative control			

* Experimental design and vaccination protocol described in Figure 1. Blood sampling schedule described in Table 1. During the study, 10 Group 1 pigs died of respiratory distress or were euthanized for that reason.

† Sera were tested by ELISA for PRRSV (Idexx 2XR ELISA; Idexx Laboratories, Westbrook, Maine), with a sample-to-positive ratio ≥ 0.4 considered positive. A replicate was considered seropositive if one pig in that replicate was seropositive. Shaded areas identify seropositives.

^{ab} Values with different superscripts within a row differ significantly ($P < .05$; Fisher's exact test). Statistical analysis compared numbers of seropositive and seronegative individual pig samples, not the replicates.

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; NFID = needle-free injection device.

was the positive control. This study supports a previously published study which reported that hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle.⁵ The sham-inoculated group (Group 4) was a negative control for inoculation. The negative control group (Group 5) demonstrated that PRRSV was not transmitted between rooms via personnel or air.

Besides its strengths, the study has two acknowledged limitations. First, the mechanism for PRRSV transmission by the NFID was not identified. Future studies are needed to identify the mechanisms of PRRSV transmission by NFID. Another limitation of this study was that the experiment was performed in isolation barns. Further evaluation of NFIDs under field conditions is warranted to ensure their suitability for use in swine production.

Despite these limitations, it was possible to demonstrate that NFIDs are a new technology that could reduce hematogenous transmission of PRRSV within a pig herd during vaccination. However, there is still a risk, albeit a smaller risk, of NFIDs acting as fomites for PRRSV transmission. The results of this study are important to the swine industry because it evaluated the risk of NFIDs as fomites for PRRSV. Swine veterinarians can explain to producers that NFIDs may reduce but do not eliminate hematogenous transmission of PRRSV.

Implications

- Hematogenous transmission of PRRSV can occur from infected pigs to susceptible pigs via repeated use of the same needle.
- Needle-free injection devices do not prevent hematogenous transmission of PRRSV.

Acknowledgments

The authors would like to thank Drs Cesar Corzo, Andrea Pitkin, Alejandrina Da Silva, Alejandra Pinto, Abigail L. Redalen, and Matt Allerson for their help during this study. Also, the authors would like to thank Boehringer Ingelheim for their financial support.

References

1. Neumann E, Kleibenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *JAVMA*. 2005;227:385–392.
2. Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorcyca D, Chladek D. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). *J Vet Diagn Invest*. 1992;4:127–133.
3. Cho JG, Dee SA. Porcine reproductive and respiratory syndrome virus. *Theriogenology*. 2006;66:655–662.
4. Otake S, Dee SA, Rossow KD, Deen J, Joo HS, Molitor TW, Pijoan C. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J Swine Health Prod*. 2002;10:59–65.
5. Otake S, Dee SA, Rossow KD, Joo HS, Deen J, Molitor TW, Pijoan C. Transmission of porcine reproductive and respiratory syndrome virus by needles. *Vet Rec*. 2002;150:114–115.
6. Dee SA, Deen J, Otake S, Pijoan C. An experimental model to evaluate the role of transport vehicles as a source of transmission of porcine reproductive and respiratory syndrome virus to susceptible pigs. *Can J Vet Res*. 2004;68:128–133.
7. Chase CL, Daniels CS, Garcia R, Milward F, Nation T. Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. *J Swine Health Prod*. 2008;16:254–261.
8. Cho JG, Dee SA, Deen J, Murtaugh MP, Joo HS. An evaluation of isolate pathogenicity on the transmission of porcine reproductive and respiratory syndrome virus by aerosols. *Can J Vet Res*. 2007;71:23–27.
9. Pitkin A, Deen J, Dee SA. Further assessment of fomites and personnel as vehicles for mechanical transport and transmission of porcine reproductive and respiratory syndrome virus. *Can J Vet Res*. 2009;73:298–302.
- *10. Molitor TW, Tune KA, Shin J, Collins J, Kapur V. Applications of TaqMan™ PCR in the detection of porcine reproductive and respiratory syndrome virus. *Proc AD Leman Swine Conf*. 1997;173–175.
- *11. Snyder ML, Mermer B, Anderson PR, Wensvoort G, Hill HT. Evaluation data for an immunological ELISA for porcine reproductive and respiratory syndrome virus. *Proc 2nd Int Symp PRRSV*. 1995;15.

* Non-refereed references.

