RESEARCH REPORT
SWINE HEALTH

Title: Evaluation of the intranasal route of MLV vaccination for large scale applications – **NPB - #18-171**

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Industry Summary

Circulating strains of porcine reproductive and respiratory syndrome virus (PRRSV) are genetically diverse; while PRRSV-2, comprising nine genetic lineages, predominates in the US, a lower incidence (<2%) of the PRRSV-1 strain is also found. Current modified-live virus (MLV) vaccines against PRRSV are effective in mitigating the disease burden associated with PRRSV infection at the farm level, although they are deficient in their cross protection against infection by heterologous strains. The current PRRSV MLV vaccines that are administered by intramuscular injection have two major issues: (1) lack of cross protection and (2) shedding of the vaccine virus. The objective of this study was to evaluate the efficacy of a commercial MLV vaccine after delivery by the intranasal route utilizing a specially engineered prototype high-pressure device suitable for high throughput vaccination in farms. The obtained results were compared to the conventional intranasal vaccine administration via syringe and the intramuscular route. Fifty-four PRRSV free pigs were obtained and divided into five treatment groups: A group was vaccinated intranasally with a specially engineered prototype high-pressure device, which automates the vaccine administration process and results in a jet stream capable of distributing the vaccine virus deep into the nasal cavity (Device-VAC group, n=12). Another group was vaccinated intranasally with a syringe fitted with an MDA adaptor (IN-VAC group; n=12), and another group was vaccinated via the intramuscular route (IM-VAC; n=12). All vaccinated groups were challenged intranasally with a 2014 PRRSV field isolate 28 days post vaccination. In addition, there was a positive control group not vaccinated but challenged with PRRSV (POS-Controls, n=12) and a negative control group, which was not vaccinated or challenged (NEG-Controls; 6 pigs). Blood and nasal swabs were collected at regular intervals, all pigs were necropsied at day 10 post challenge (dpc) and gross and microscopic lesions were assessed. Prior to challenge most vaccinated pigs had seroconverted to PRRSV. Fever was significantly reduced in vaccinated groups compared to the POS-Control group at dpc 7 and 9. The IM-VAC and Device-VAC groups were not significantly different in regard to PRRSV viremia, seroconversion, and average daily weight gain, indicating a

comparable performance. In contrast, the IN-VAC group was often not different from the POS-Control group and in general appeared to have a reduced vaccine efficacy compared to the other two vaccine groups. The challenge virus selected for this study was a contemporary 1-7-4 field strain and, according to ORF5 sequencing, was 87.7% identical to the vaccine used. Based on the obtained data, the vaccine used in this study did not protect the pigs from developing gross and microscopic lesions regardless of the vaccine administration route. Future studies should include side-by-side trials of the Device-VAC and IM-VAC groups using additional heterologous PRRSV isolates to better assess differences in lung lesion reduction. Under the conditions of this study, nasal administration of a commercial PRRSV vaccine using a device designed for mass-vaccination worked well and data are comparable to those obtained after vaccination by the IM route. The device worked better and was easier to use compared to manual intranasal administration via a syringe. The obtained pilot study data indicate that the intranasal administration route may pose an alternative option for PRRSV vaccination on pig farms, regardless of size.

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Keywords: PRRSV; vaccination; pigs; intranasal versus intramuscular.

Scientific Abstract

Circulating strains of porcine reproductive and respiratory syndrome virus (PRRSV) are genetically diverse; while PRRSV-2, comprising nine genetic lineages, predominates in the US, a lower incidence (<2%) of the PRRSV-1 strain is also found. Current modified-live virus (MLV) vaccines against PRRSV are effective in mitigating the disease burden associated with PRRSV infection at the farm level, although they are deficient in their cross protection against infection by heterologous strains. The current PRRSV MLV vaccines that are administered by intramuscular injection have two major issues: (1) lack of cross protection and (2) shedding of the vaccine virus. The objective of this study was to evaluate the efficacy of a commercial MLV vaccine after delivery by the intranasal route utilizing a specially engineered prototype high-pressure device suitable for high throughput vaccination in farms. The obtained results were compared to the conventional intranasal vaccine administration via syringe and the intramuscular route. Fifty-four PRRSV free pigs were obtained and divided into five treatment groups: A group was vaccinated intranasally with a specially engineered prototype high-pressure device, which automates the vaccine administration process and results in a jet stream capable of distributing the vaccine virus deep into the nasal cavity (Device-VAC group, n=12). Another group was vaccinated intranasally with a syringe fitted with an MDA adaptor (IN-VAC group; n=12), and a group was vaccinated via the intramuscular route (IM-VAC; n=12). All vaccinated groups were challenged intranasally with a 2014 PRRSV field isolate 28 days post vaccination. In addition, there was a positive control group not vaccinated but challenged with PRRSV (POS-Controls, n=12) and a negative control group, which was not vaccinated or challenged (NEG-Controls; 6 pigs). Blood and nasal swabs were collected at regular intervals, all pigs were necropsied at day 10 post challenge (dpc) and gross and microscopic lesions were assessed. Prior to challenge most vaccinated pigs had serovonverted to PRRSV. Fever was significantly reduced in vaccinated groups compared to the POS-Control group at dpc 7 and 9. The IM-VAC and Device-VAC groups were not significantly different in regard to PRRSV viremia, seroconversion, and average daily weight gain, indicating a comparable performance. In contrast, the IN-VAC group was often not different from the POS-Control group and in general appeared to have a reduced vaccine efficacy compared to the other two vaccine

groups. The challenge virus used for this study was a contemporary 1-7-4 field strain and, according to ORF5 sequencing, was 87.7% identical to the vaccine used. Based on the obtained data, the vaccine used in this study did not protect the pigs from developing gross and microscopic lesions regardless of the vaccine administration route. Future studies should include side-by-side trials of the Device-VAC and IM-VAC groups using additional heterologous PRRSV isolates to better assess differences in lung lesion reduction. Under the conditions of this study, nasal administration of a commercial PRRSV vaccine using a device designed for mass-vaccination worked well and data are comparable to those obtained after vaccination by the IM route. The device worked better and was easier to use compared to manual intranasal administration via a syringe. The pilot study data indicate that the intranasal administration route may pose an alternative option for PRRSV vaccination on pig farms, regardless of size.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major economic burden to pork producers in the US (Linhares et al., 2016). Respiratory and reproductive diseases are the main manifestation of PRRSV infection affecting pigs of all age groups (Halbur et al., 1996; Mengeling et al., 1996). PRRSV is genetically highly diverse and can be classified into two species, PRRSV-1 and PRRSV-2. The virus is known to have a high mutation rate, resulting in the evolution of new and more virulent strains in the field on an ongoing basis (Rowland et al., 1999). While PRRSV-1 is present in North America, PRRSV-2 strains are the predominant species (>98%) with numerous heterogeneous strains of variable virulence.

Currently, five commercial modified live virus (MLV) vaccines are available to protect pigs against PRRSV infections in the U.S. The Ingelvac PRRS® MLV vaccine (Boehringer Ingelheim) is derived from PRRSV-2 prototype strain VR2332, the Ingelvac® PRRS ATP vaccine (Boehringer Ingelheim) is derived from atypical PRRSV-2 strain JA142, Fostera® PRRS vaccine (Zoetis) is derived from the PRRSV-2 prototype strain US P129 strain (Nan et al., 2017), and Prevacent® PRRSV (Elanco) is derived from lineage 1 and related to MN184 and NC174. Insufficient heterologous protection has been identified as an issue with the current PRRSV MLV vaccines (Murtaugh et al., 2002; Okuda et al., 2008; Zuckermann et al., 2007). In addition to the MLV vaccines, inactivated (often autogenous) vaccines, vectored vaccines, and DNA vaccines have been experimentally tested to immunize pigs against PRRSV. A study comparing commercial MLV and inactivated vaccines found that the MLV vaccine but not an inactivated vaccine conferred protective immunity in sows (Zuckermann et al., 2007). Development of improved vaccines and vaccination protocols against heterologous PRRSV strains are urgently required. In recent experiments by our group with other attenuated vaccine candidates of PRRSV, we found that intranasal administration of the vaccine elicits a better cross-protective immune response than the intramuscular vaccination route (unpublished observations). A similar development of a robust immunity to PRRSV has been observed in other studies utilizing intranasal vaccination with MLV vaccine strains (Binjawadagi et al., 2011; Dwivedi et al., 2011; Zhang et al., 2007). We hypothesized that intranasal vaccination of pigs with PRRSV MLV vaccines leads to virus replication in the nasal turbinate epithelial cells and other permissive cells, which primes local mucosal immunity and the regional lymph nodes. In contrast, priming of the mucosal immunity by the intramuscular administration of the MLV vaccine may be less efficient. Our previous experiments demonstrated that intranasal vaccination could efficiently prevent PRRSV infection at its initial point of entry into the pigs.

Despite the possible benefits of intranasal immunization of pigs, practical difficulties in vaccinating pigs in commercial farms via the intranasal route on a large scale is a major hurdle to field adaptation of the technique. Experimental intranasal vaccinations, including our experiments, are currently performed with atomizers fitted to a syringe to generate mist or weak spray by manual pressure. In order to overcome this, in this study we evaluated the intranasal vaccination route by directly comparing individual intranasal administration of a commercially available MLV vaccine with a syringe or with a specially engineered high-pressure device designed for high throughput intranasal vaccination (provided by Pulse Needlefree Systems, Lenexa, Kansas). The prototype device used in this experiment has been engineered to deliver the vaccine to the distal nasal passage and tonsil in the form of a focused jet spray. The prototype device, actuated by pressure, allowed repeatable and rapid intranasal vaccination of pigs.

Objective

The objective of this study was to systematically evaluate the intranasal route of vaccination with a commercial MLV PRRSV vaccine and to develop a practical method for intranasal vaccination utilizing a specially engineered prototype high-pressure device suitable for large scale vaccination under experimental conditions. Results were compared side-by-side to the intranasal route using a syringe and a MAD adaptor and to the intramuscular route of vaccination.

Materials and Methods

Pigs and experimental design. Experimental design: Fifty-four, 2.5-week-old, PRRSV-free piglets were purchased from a PRRSV naïve breeding herd, transported to the research facility at Iowa State University, and randomly assigned to five groups and rooms as outlined in Table 1. At three weeks of age, all piglets were vaccinated by the intramuscular or intranasal routes using a commercial PRRSV MLV vaccine. For the intranasal route, the vaccine was administered utilizing an atomization device fitted on a syringe (IN-VAC group) or using a prototype intranasal vaccination device (Device-VAC). For the intramuscular route, a needle was used. The pigs were challenged at 7 weeks of age. All pigs were bled and weighed at vaccination, challenge and necropsy. They were monitored for clinical signs for 10 days, euthanized, and necropsied. The experimental timeline is summarized in Fig. 1.

Table 1. Experimental Groups.

Group	Pig #	Vaccination	Vaccination route	Challenge
Device-VAC	12	MLV	Intranasal-High pressure device	PRRSV
IM-VAC	12	MLV	Intramuscular	PRRSV
IN-VAC	12	MLV	Intranasal-MAD syringe adaptor	PRRSV
NEG-Control	6	None	Not applicable	None
POS-Control	12	None	Not applicable	PRRSV

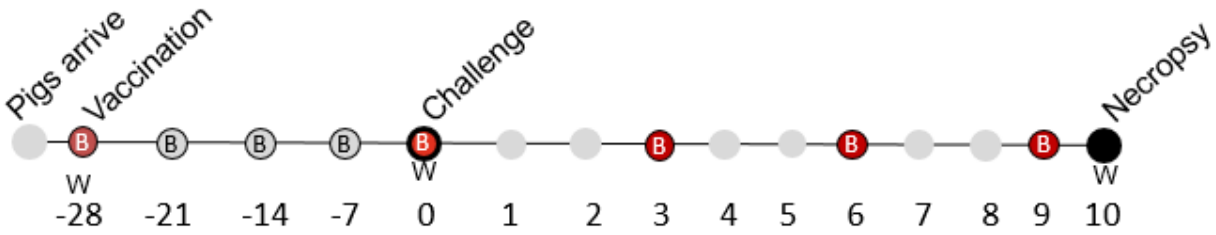

Fig. 1: Experimental timeline. The day post challenge (dpc) 0 corresponds to PRRSV challenge and necropsy was done on dpc 9. B=Blood collection; W=Weight collection.

Vaccination. For this study, the Ingelvac® PRRS MLV vaccine (Boehringer-Ingelvac Vetmedica) was used (Serial number 2451274B, expiration date: 05-Mar-2020). Each pig received 2 ml of the vaccine as recommended by the manufacturer. Vaccination was done intramuscularly into the right neck area with a hypodermic needle for the IM-VAC group as recommended by the manufacturer or intranasally either with a syringe fitted atomization device (IN-VAC group) or with a specially engineered pressurized gas actuated device (provided by Pulse Needlefree Systems, Lenexa, Kansas; Device-VAC group) at 3 weeks of age. Specifically, the prototype high-pressure device automates the vaccine administration process and results in a jet stream thought capable of distributing vaccine virus deeper into the nasal cavity compared to using a regular syringe approach.

Challenge. At 7 weeks of age, 28 days post vaccination, pigs in all groups were intranasally challenged with 5 ml of a contemporary PRRSV-2 strain (USA/IN/65239S/2014) at a concentration of 10^5 50% tissue culture infectious dose ($TCID_{50}$) per ml from our collection. The PRRSV challenge strain, with an ORF5 RFLP pattern of 1-7-4, was isolated from a breeding herd with abortions and respiratory disease in young pigs and was previously shown to induce disease and lesions in pigs (van Geelen et al., 2018). Each pig was inoculated by slowly dripping 2.5 ml of the inoculum in each nostril for a total of 5 ml inoculum per pig.

Clinical assessment. All pigs were weighed at arrival, challenge and necropsy. To evaluate disease after challenge, rectal temperatures were obtained from all pigs and the pigs were assessed for presence of respiratory disease as described (Halbur et al., 1995) on dpc 1, 3, 5, 7, and 9.

Necropsy. All pigs were necropsied at dpc10. The severity of gross lesions was scored and recorded by a pathologist blinded to the treatment status of the pigs. Tissues (lungs, tonsil and tracheobronchiolar lymph nodes) were collected for histopathology and scored for severity of interstitial pneumonia as described (Halbur et al., 1995). In addition, PRRSV IHC (Halbur et al., 1994) was done on lung sections to assess the amount of PRRSV replication in the pigs.

Sample analysis. Blood samples were collected weekly until challenge and at dpc 3, 6 and 9. Nasal swabs were collected at challenge and at dpc 1, 3, 5, 7 and 9. Serum samples were tested by a commercial PRRSV ELISA (IDEXX PRRS X3 Ab Test; IDEXX Inc). A fluorescent focus neutralization (FFN) assay was performed on serum samples collected dpc 0 from all pigs for the detection of neutralizing antibodies, using a previously described method (Wu et al., 2001). Specifically, three different PRRSV strains were tested: the vaccine strain (VR2332), the challenge strain USA/IN/65239S/2014, and strain VR2385 which has been extensively tested in our pig model (Halbur et al., 1995) and clusters with the vaccine virus used in this study. Serum samples and nasal swabs were tested by a quantitative PRRSV real-time RT-PCR as described (Gerber et al.,

2014).

Statistical analysis: Summary statistics were calculated for continuous variables from all groups to assess the overall quality of the data. Analysis of variance (ANOVA) was used to detect significant differences among treatment groups and pair-wise comparison was performed by least significant difference. The rejection level for the null hypothesis was 0.05 followed by pairwise testing using the Tukey-Kramer adjustment to identify the groups that are different. A non-parametric ANOVA (Kruskal-Wallis) was used for non-normally distributed data or when group variances were dissimilar, and pair-wise comparisons were done using Wilcoxon rank sum test.

Results

Humoral responses. At arrival at the research facility, none of the pigs had detectable antibodies against PRRSV and NEG-Controls remained negative for the duration of the study **(**Fig. 2). At challenge, 11/12 Device-VAC, 12/12 IM-VAC and 11/12 IN-VAC pigs had seroconverted; however, IN-VAC pigs had significantly lower levels of antibodies compared to IM-VAC pigs. However, the Device-VAC group was not different from either of the other two vaccine groups (Fig. 2). By dpc 9, all challenged pigs had seroconverted including the non-vaccinated POS-Control group; however, all vaccinated groups had significantly higher serum antibody levels (Fig. 2).

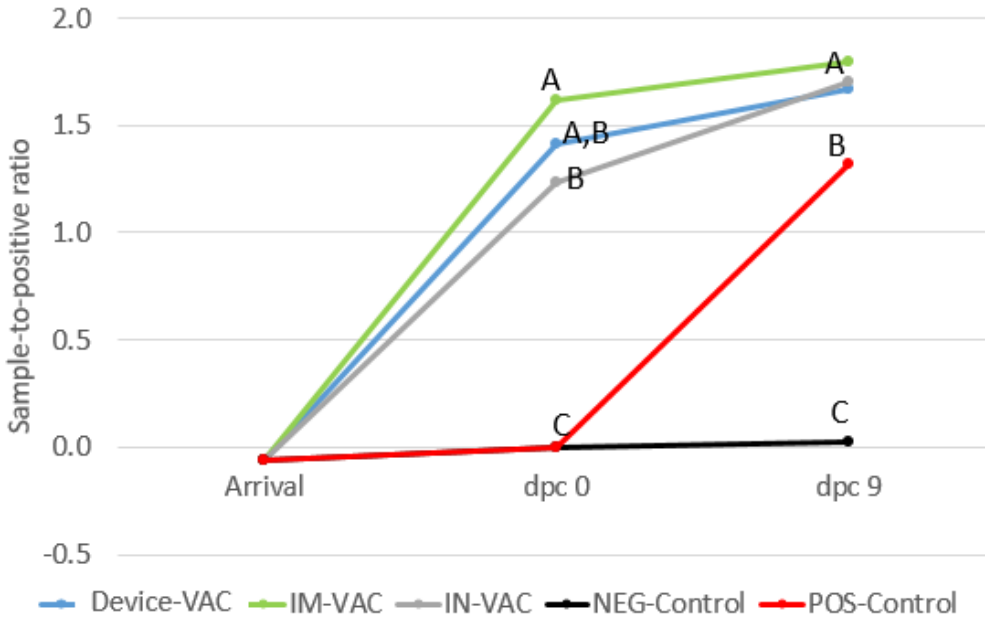

Fig. 2: Mean group ELISA S/P ratios in vaccinated and non-vaccinated pigs at arrival, day of challenge (dpc 0) and at necropsy (dpc 9). Different superscripts on a treatment day (^{A,B,C}) indicate significant differences among groups ($P<0.05$).

To check for neutralizing antibodies, a FFN assay was used with either the challenge strain, the vaccination strain or PRRSV strain VR2385. None of the pigs had FFN titers against the challenge strain while 3/12 Device-VAC and 3/12 IM-VAC pigs had titers against the vaccine strain and VR-2385.

Clinical disease. Clinical signs of respiratory disease were not observed in any of the pigs before PRRSV challenge. The ADG (in g ± SEM) of the pigs between the time of PRRSV

challenge and the necropsy was 411.5±76.0 for the Device-VAC group, 425.8±32.8 for the IM-VAC group, 411.7±38.2 for the IN-VAC group, 616.5±57.9 for the NEG Control group, and 240.9±30.0 for the POS-CONTROL group. There was a significant difference in ADG between POS-Controls and NEG-Controls (p=0.0009). All challenged pigs developed increased rectal temperatures and the NEG-Control group had significantly lower group means on dpc 3, 5 and 7 compared to all other groups. POS-Control pigs had significantly increased rectal temperatures compared to all other groups at dpc 7 and 9. A mild increase in respiratory scores (score of 1 or 2) was observed by 7 dpc in all challenged groups regardless of vaccination status.

PRRSV RNA in serum and nasal swabs. NEG-Control pigs were negative for PRRSV RNA in serum samples and nasal swabs on all days tested. PRRSV RNA positive serum samples were identified at 3 dpc in 11/12 Device-VAC pigs, 6/12 IM-VAC pigs, 12/12 IN-VAC pigs and 12/12 POS-Control pigs. By 6 dpc, PRRSV RNA was present in 100% of the challenged pigs regardless of vaccination status. By 9 dpc, 8/12 Device-VAC, 7/12 IM-VAC, 10/12 IN-VAC and 11/12 POS-Control pigs were PRRSV RNA positive on serum. Group mean levels of PRRSV genomic copy numbers are summarized in Fig. 3. In nasal swabs, PRRSV was only detected sporadically in single pigs at dpc 3 (2/12 Device-VAC, 3/12 IN-VAC and 1/12 POS-Controls) and 6 (1/12 POS-Controls) and all pigs were PRRSV-RNA negative on nasal swabs by dpc 9 (data not shown).

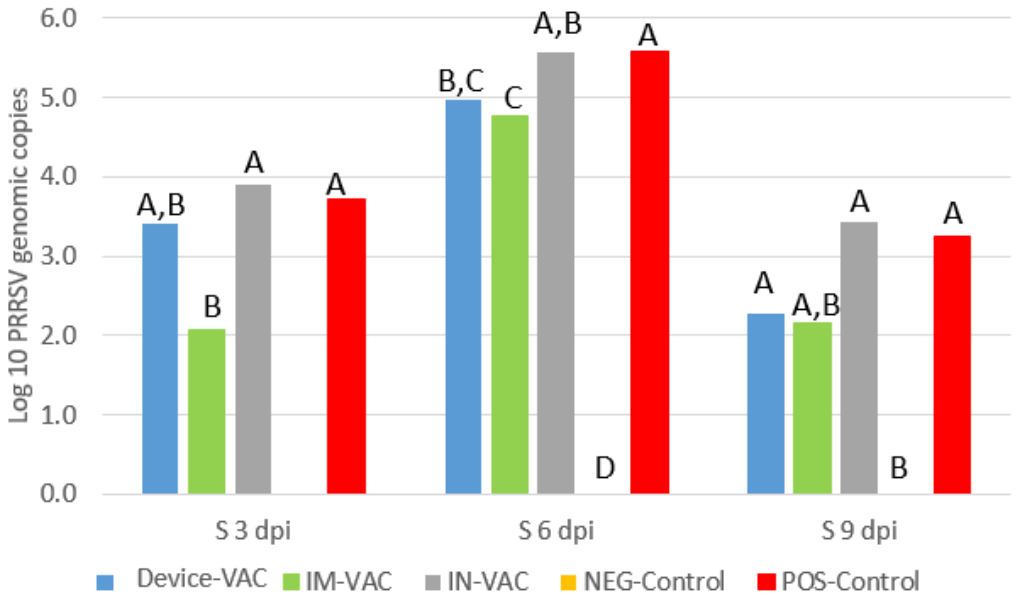

Fig. 3: Mean group PRRSV genomic copies in serum samples vaccinated and non-vaccinated pigs at 3, 6 and 9 days post challenge Different superscripts on a treatment day (^{A,B,C,D}) indicate significant differences among groups ($P<0.05$).

Lesions and PRRSV antigen in tissue sections. Lesions ranged from moderate to severe, and were characterized by multifocal to diffuse tan consolidation of the lung surface. There were no significant differences among challenged pigs. Microscopically, most lungs had focal to diffuse mild to severe interstitial pneumonia. PRRSV antigen was demonstrated by IHC staining in all treatment groups except NEG-CONTROL pigs. Detailed results are provided in Table 2.

Table 2. Gross and microscopic lesions and PRRSV antigen presence as determined by PCR.

Group	Gross lesions (range: 0-100%)	Microscopic lesions (range: from 0-6)	PRRSV IHC (range: from 0-3)
Device-VAC	52.6 ± 6.2^{A}	4.5 ± 0.3^{A}	2.9 ± 0.1^{A}
IM-VAC	51.9 ± 5.3^{A}	4.5 ± 0.3^{A}	2.8 ± 0.2^{A}
IN-VAC	65.3 ± 4.9^{A}	5.0 ± 0.3^{A}	3.0 ± 0.0^{A}
NEG-Control	0.00 ± 0.0^{B}	0.8 ± 0.2^{B}	0.0 ± 0.0^{B}
POS-Control	65.2 ± 3.9^{A}	5.3 ± 0.2^{A}	3.0 ± 0.0^{A}

Discussion

PRRSV control continues to be an issue in many pork producing regions. While there are several commercial vaccines available, all are being administered via the intramuscular route. As PRRSV is a virus that is mainly transmitted by the nasal route, there are thoughts that intranasal immunity and protection could be improved by using the intranasal route for vaccinating the pigs. The objective of this study was to evaluate and compare the efficacy of the intranasal route of vaccination with a commercial MLV PRRSV vaccine and to develop a practical method for intranasal vaccination, utilizing a specially engineered prototype high-pressure device suitable for large scale vaccination under field conditions. In this study we compared three vaccine administration routes: IM, manual IN with an MDA adaptor/syringe and automated IN using a high-pressure device designed for mass vaccination of pigs using the intranasal route. Pigs were challenged with a contemporary US field isolate 28 days post vaccination using the intranasal route.

When comparing the two intranasal route methods, vaccination in the Device-VAC group appeared to be easy, quick and effective and was preferred by the personnel administering the vaccine in this trial. The device dispersed the vaccine into a fine mist and the procedure was overall very quick as the pigs just needed to be lifted up and held by a person while a second person carrying the device walked from pig to pig and administered the vaccine. In contrast, in the IN-VAC group, syringes had to be re-filled and a new MDA adaptor had to be attached after each pig by a person. Another person held the pig while a third person performed the vaccination. However, while more time consuming it is possible that a single person vaccinates a pig intranasally with a syringe and adaptor whereas the device requires a minimum of two people, a holder and a person to deliver the vaccine.

Clinical disease after challenge was characterized by mild respiratory signs and increased rectal temperatures. After challenge, most challenged pigs regardless of vaccination status developed fever and the average rectal temperature was significantly different from the NEG-Control pigs. In the later stages of this trial (dpc 7 and 9); however, POS-Control pigs had significantly higher rectal temperature than all other groups, indicating that the vaccination had some protective effect regardless of administration route. Moreover, POS-Control pigs had the lowest average daily gain from challenge to necropsy (240g) followed by all vaccinated group (411g, 425g, 411g) with the non-challenged NEG-Control pigs reaching the highest ADG (616g). The only significant difference in ADG was between NEG-Control and POS-Control groups.

Seroconversion rates and mean ELISA S/P ratios in the IM-VAC and Device-VAC groups were essentially identical, with slightly lower S/P ratios in the IN-VAC group. Also when looking at PRRSV viremia, the IM-VAC and Device-VAC groups behaved very similarly and differently from the POS-Control group. In contrast, the IN-VAC group followed a pattern similar to the POC-Control group in the early days (dpc 3 and 6) after PRRSV challenge.

In this study, a 2014 field isolate PRRSV was chosen to challenge the pigs. This particular virus was 87.4% identical to the commercial vaccine strain used in this study based on ORF5 sequencing. Challenge strain selection was done in an attempt to enhance disease lesions, which could enable recognition of true differences among groups. The gross lesions in the challenged pigs were severe for most pigs as evidenced by mean gross lung lesions scores of 55-65% of the lung surface affected. Similarly, the microscopic lesions were severe and PRRSV antigen could be demonstrated by PRRSV IHC in essentially all infected pigs without significant differences. It would be important to repeat a portion of this study (Device-VAC and IM-VAC and POS-Control) with another, more vaccine compatible challenge strain.

Under the conditions of this study, nasal administration of a commercial PRRSV vaccine using a device designed for mass-vaccination worked well and data are comparable to those obtained after vaccination of the pigs by the IM route, as recommended by the manufacturer. Of note, the device worked better and was easier to use compared to intranasal administration via a syringe. This pilot study data indicate that the intranasal administration route may be a viable option for PRRSV vaccination on pig farms. This technology can immediately be used for rapid mass vaccination on U.S. pig farms, with the additional advantage of safety and possible reduced operational cost of vaccination. In addition, this technique could be readily adapted for other vaccines. In summary, intranasal vaccination with a PRRSV MLV vaccine using a device engineered for optimal delivery and suitability for mass vaccinations has a high chance of introducing an incremental but valuable development to the current field practices in PRRSV control.

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