Evaluation of the intranasal route for porcine reproductive and respiratory disease virus modified-live vaccination

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Tanja Opriessnig[a,b*], Gaurav Rawal[b], Lauren McKeen[d], Patricia Filippsen Favaro[c], Patrick G. Halbur[b], Phillip C. Gauger[b]

[a] *The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK*

[b] *Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA*

[c] *School of Medicine & Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA*

[d] *Department of Statistics, Iowa State University, Ames, Iowa, USA*

* Corresponding author.

Highlights

- Porcine reproductive and respiratory syndrome virus is important in pigs
- The virus commonly infects pigs via the respiratory system
- Vaccination is commonly administered via the intramuscular route
- A prototype nasal jet device that could be used for mass vaccination was investigated
- Intramuscular and intranasal vaccine efficacy was comparable in a pig challenge
- Pigs vaccinated intranasally had higher neutralizing antibody levels at challenge

ABSTRACT

Background: In pigs, modified live vaccines (MLV) against porcine reproductive and respiratory syndrome virus (PRRSV) are commonly used and administered by intramuscular (IM) injection. In contrast, PRRSV as a primary respiratory pathogen is mainly transmitted via the intranasal (IN) route. The objective of this study was to evaluate the efficacy of a commonly used commercial PRRSV MLV delivered by the IN compared to the IM route.

Methods: Fifty-four pigs were divided into five treatment groups. All vaccinated groups received the same vaccine but via different routes. Group IN-JET-VAC was vaccinated with an automated high pressure prototype nasal jet device (IN-JET-VAC, n=12), group IN-MAD-VAC was vaccinated with a mucosal atomization device (IN-MAD-VAC, n=12), group IM was vaccinated intramuscularly (IM-VAC; n=12) according to label instructions, group NEG-CONTROL (n=6) and a POS-CONTROL (n=12) were both unvaccinated. At 28 days post vaccination all vaccinated groups and the POS-CONTROL pigs were challenged with a pathogenic US PRRSV. Blood and nasal swabs were collected at regular intervals, and all pigs were necropsied at day 10 post challenge (dpc) when gross and microscopic lung lesions were assessed.

Results: Prior to challenge most vaccinated pigs had seroconverted to PRRSV. Clinical signs (fever, inappetence) were most obvious in the POSITIVE CONTROL groups from dpc 7 onwards. The vaccinated groups were not different for PRRSV viremia, seroconversion, or average daily weight gain. However, IN-JET-VAC and IN-MAD-VAC had significantly higher neutralizing antibody levels against the vaccine virus at challenge.

Conclusions: Comparable vaccine responses were obtained in IN and IM vaccinated pigs suggesting the intranasal administration route as an alternative option for PRRSV vaccination.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major economic burden to pork producers in the US [1] and PRRSV infection commonly manifests as reproductive and/or respiratory disease [2, 3]. The PRRSV, a member of the family *Arteriviridae* within the order Nidovirales belongs to the genus *Betaarterivirus* and can be classified into two species, *Betaarterivirus suid 1* (PRRSV-1, European) and *Betaarterivirus suid 2* (PRRSV-2, North American) [4]. The virus is known to have a high mutation rate often resulting in the evolution of new and more virulent strains on an ongoing basis; moreover, during natural infection, PRRSV has been found to exist as a quasispecies distribution of related genotypes [5, 6]. Both PRRSV-1 and PRRSV-2 are present in most pork producing regions except Australia and South America; however, PRRSV-2 is predominant in North America and Asia while PRRSV-1 strains are the predominant species composed of heterogeneous strains of variable virulence in Europe. PRRSV-2 is further subdivided into nine lineages [7-12].

Currently, seven commercial modified live virus (MLV) vaccines are available to protect pigs against PRRSV-2 infections in the US. The Ingelvac PRRS® MLV vaccine (Boehringer Ingelheim) is derived from lineage 5 strain VR2332, the Ingelvac PRRS® ATP vaccine (Boehringer Ingelheim) is derived from lineage 8 strain JA142, the Prevacent® PRRSV MLV vaccine (Elanco) is derived from a lineage 1Dß and related to strains MN184 and NC174, Prime Pac® PRRS RR (Merck) [13] is derived from parental strain NEB-1 [14], which belongs to lineage 7, and PRRSGard® (Pharmgate), a chimeric MLV, is composed of two lineage 1 isolates: a proprietary, highly attenuated PRRSV-2 strain as the vaccine backbone, and structural protein genes from the highly virulent contemporary field isolate MN184 [15]. Insufficient heterologous protection has been identified as an issue with the current PRRSV MLV vaccines

71 [16-18]. In addition to the MLV vaccines, inactivated, often autogenous vaccines, vectored
72 vaccines, and DNA vaccines have been experimentally tested to immunize pigs against PRRSV.
73 A study comparing a commercial MLV (Pyrsvac-183®; Syva labs, Leon, Spain) and an
74 inactivated vaccine (Progressis®, Merial Labs, Lyon, France) found that the MLV vaccine but
75 not the inactivated vaccine conferred protective immunity in sows against challenge with the
76 Lelystad PRRSV [16].

77 Development of improved vaccines and vaccination protocols against heterologous
78 PRRSV strains are urgently required. While it is known that local respiratory mucosal immunity
79 is extremely important to fight airborne infections [19-21], previous findings demonstrated that
80 there is no significant difference between routes of administration and the severity of clinical
81 manifestation [22]. Similar development of a robust immunity to PRRSV when utilizing
82 intranasal vaccination with MLV vaccine strains has also been observed by other groups [23-25].
83 Using the intranasal (IN) route for vaccination, provided complete protection against a low
84 virulent African swine fever virus (ASFV) when compared to intramuscular (IM) administration
85 of the vaccine [26]. In that study, the IN vaccinated pigs had low to undetectable levels of ASFV
86 viremia and lack of lesions upon necropsy. In a PRRSV study using experimental virus-like
87 particles (VLPs) and VLPs plus the 2', 3'-cGAMP VacciGrade™ adjuvant vaccines in two doses
88 2 weeks apart via the IN route appeared to exacerbate PRRSV viremia after challenge [27]. A
89 higher level of interferon-α production, but not interferon-γ and IL-10, is correlated with
90 enhanced virus replication [27]. In contrast, in mice, both mucosal and systemic immunity were
91 observed after IN vaccination of a recombinant *Lactococcus lactis* expressing open-reading
92 frame (ORF) 6 of PRRSV [28]. Hence, IN vaccination of pigs using MLV vaccines may lead to
93 priming the local mucosal immunity and the regional lymph nodes. In contrast, priming of the

mucosal immunity by the IM administration of the MLV vaccine may be less efficient.

Despite the possible benefits of IN immunization of pigs, practical difficulties in vaccinating pigs IN in commercial farms on a large scale is a major hurdle to field adaptation of the technique. Experimental IN vaccinations in pigs are currently performed with mucosal atomization devices (MAD) fitted to a syringe to generate mist or spray by manual pressure, which results in particle sizes of 30-100μm. This is a time-consuming procedure, requiring at least two people: one restraining the pig and the other filling the syringe, fitting a new MAD, and vaccinating the pig. In order to overcome this, we evaluated a prototype engineered high-pressure nasal jet (JET) capable of delivering the vaccine to the distal nasal passage and tonsil in the form of a focused jet spray actuated by high pressure, allowing repeatable and rapid intranasal vaccination of pigs from an inserted vaccine bottle, without the need of reloading the syringe for each pig. The objective of this study was to evaluate and compare the IN (JET or MAD) to the IM route of vaccination, side-by-side, using a commercial MLV PRRSV vaccine in a growing pig PRRSV challenge model.

2. Methods

2.1. Pig source, approvals, and experimental design

The study design was approved the Iowa State University Institutional Animal Care and Use Committee (approval number IACUC-19-022) and the Institutional Biosafety Committee (approval number IBC-19-009). 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 of 6 pigs (NEG-CONTROL) or 12 pigs (all other groups) as outlined in Table 1. The groups were distributed into nine rooms of 6 pigs each. At three

117 weeks of age, all piglets were vaccinated by the IM or IN routes using a commercial PRRSV
118 MLV vaccine. For the IM route, a needle and syringe was used. For the IN route, the vaccine
119 was administered utilizing an atomization device fitted on a syringe (IN-MAD-VAC group) or
120 using a prototype JET (IN-JET-VAC). The pigs were challenged with a pathogenic PRRSV
121 isolate at 7 weeks of age. All pigs were weighed at vaccination, challenge, and necropsy while
122 blood was collected on a weekly basis. Nasal swabs were collected from all pigs from challenge
123 to necropsy every other day. The pigs were monitored for clinical signs for 10 days post PRRSV
124 challenge (dpc), euthanized, and necropsied. The experimental timeline is summarized in Fig. 1.
125
126 *2.2. Vaccination*
127 For this study, the Ingelvac PRRS® MLV vaccine (Boehringer-Ingelheim Vetmedica,
128 Inc.) was used (Serial number 2451274B, expiration date: 05-Mar-2020). The vaccine was
129 reconstituted immediately prior to planned vaccination and each pig in the IN-JET-VAC, IN-
130 MAD-VAC and IM-VAC groups received 2 ml of the vaccine as recommended by the
131 manufacturer. Vaccination was done IM into the right neck area with a hypodermic needle (23
132 gauge × 1/3 inch) for the IM-VAC group as recommended by the manufacturer or IN either with
133 a syringe fitted with a mucosal atomization device (MAD; IN-MAD-VAC group) or with a
134 prototype pressurized gas actuated JET in excess of 5 psi at one-half inch distance from the tip of
135 the device (kindly provided by Pulse NeedleFree Systems, Inc.; Lenexa, KS, USA; IN-JET-VAC
136 group) at 3 weeks of age (Supplementary material). Specifically, using the prototype high-
137 pressure JET, the vaccine administration process results in a jet stream. The main differences
138 between the JET and the MAD are that the JET delivery is (a) automated, mechanically
139 generated pressure (instead of the plastic syringe that depends upon how much force the user

140 squeezes the syringe) and (b) that the atomization tip is multi-use and durable. It is recommended
141 by the manufacturer to replace the plastic disposable MAD after each usage, but it is sometimes
142 used for a lower number of applications before it wears and must be replaced. The JET's
143 atomization tip is stainless steel and can be cleaned and sterilized.
144
145 *2.3. PRRSV challenge*
146 At 7 weeks of age, 28 days post vaccination, pigs in all groups were IN challenged with 5
147 ml of the contemporary lineage 1A PRRSV-2 strain ISU-5 (also known as
148 USA/IN/65239S/2014; GenBank accession number MF326992) at a concentration of 10^5 50%
149 tissue culture infectious dose ($TCID_{50}$) per ml from our collection. The PRRSV challenge strain,
150 with an ORF5 restriction fragment length polymorphism (RFLP) pattern of 1-7-4 was isolated
151 from a breeding herd with abortions and respiratory disease in young pigs and was previously
152 shown to induce severe disease and lesions in pigs [29]. Each pig was inoculated by slowly
153 dripping 2.5 ml of the inoculum in each nostril for a total of 5 ml inoculum per pig.
154
155 *2.4. Clinical assessment*
156 All pigs were weighed at arrival, challenge, and necropsy. To evaluate disease after
157 challenge, rectal temperatures were obtained from all pigs every other day and the pigs were
158 assessed for presence of respiratory disease using a respiratory score as described [2] on dpc 1, 3,
159 5, 7, and 9.
160
161 *2.5. Necropsy, gross lesions, microscopic lesions and PRRSV immunohistochemistry*
162 All pigs were euthanized at dpc10 by pentobarbital overdose and necropsied. The

severity of macroscopic lung lesions was scored as a percentage of the lung surface affected by lesions by a pathologist (PCG) blinded to the treatment status of the pigs and recorded. Tissues (lungs, tonsil and tracheobronchial lymph nodes) were collected in 10% neutral buffered formalin for histopathology and lungs scored for severity of interstitial pneumonia ranging from 0 (normal) to 6 (diffuse, severe) as described [2]. Assessment of PRRSV antigen load in lung tissues was done using immunohistochemistry [31] on lung sections with scores ranging from 0 (no PRRSV present) to 3 (large levels of antigen diffusely distributed).

2.6. Sample collection

Blood samples were collected weekly until challenge and at dpc 3, 6 and 9 (Fig. 1). Nasal swabs were collected one day before challenge and at dpc 1, 3, 5, 7 and 9 (Fig. 1).

2.7. Serology

Serum samples were tested by a commercial indirect PRRSV enzyme-linked immunosorbent assay (IDEXX PRRS X3 Ab Test; IDEXX Inc). A sample was considered positive when the sample-to-positive (S/P) value was equal or greater than 0.4. A fluorescent focus neutralization (FFN) assay was performed on serum samples collected on dpc 0 from all pigs for the detection of neutralizing antibodies, based on Iowa State University Veterinary Diagnostic Laboratory standard operating procedures. Specifically, two PRRSV strains were tested: the Ingelvac PRRSV® MLV vaccine strain VR2332 (lineage 5) and the challenge strain ISU-5 (lineage 1A). These two strains are 87.4% homologous based on ORF5.

2.8. RNA extraction and RT-real-time PCR

Nucleic acids were extracted from serum samples and nasal swabs using the MagMAX™ Pathogen RNA/DNA kit (Thermo Fisher Scientific) and a Kingfisher Flex instrument (Thermo Fisher Scientific) following the instructions of the manufacturer. For each sample, 100 µl were used for extraction, and nucleic acids were eluted into 90 µl of elution buffer as described [15]. A quantitative reverse transcription PCR (qRT-PCR) using the Path-IDTM Multiplex One-Step RT-PCR kit (Thermo Fisher Scientific), was used in the study to screen serum samples and nasal swabs. The PRRSV screening PCR targets conserved genomic regions i.e., ORF6 and ORF7. Briefly, 2.5 µl of 10× Multiplex Enzyme Mix (Thermo Fisher Scientific), 12.5 ul of 2× Multiplex RT-PCR Buffer (Thermo Fisher Scientific), 2.5 µl of 10× PRRSV Primer Probe Mix V2 (Thermo Fisher Scientific), 0.5 µl nuclease-free water (Thermo Fisher Scientific), and 7 µl nucleic acid extract were included in a final 25 µl PCR reaction. Amplification reactions were performed on an ABI 7500 Fast instrument (Thermo Fisher Scientific) using the standard mode with the following conditions: one cycle of 48°C for 10 min, one cycle of 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 45 s. The analysis was done using an automatic baseline, NA PRRSV detector (FAM) at the threshold of 0.1, EU PRRSV detector (VIC) at the threshold of 0.05 and XIPC detector (Cy5) at the threshold of 10% of the sigmoid amplification curve's maximum height. A cycle threshold (C_T) of <37 was considered positive, and C_T ≥37 was considered negative for both PRRSV species. All samples collected after challenge were also tested for presence of the Ingelvac PRRSV® MLV vaccine strain to determine if the virus load was due to the vaccine or the challenge strain. Specifically, the primers and probe used in the Ingelvac PRRS® MLV vaccine specific RT-qPCR were as previously described [32]. The Ingelvac PRRS® MLV PCR forward primer sequence is 5′- TGGCGCCGGCTCTTTT-3′, the reverse primer sequence is 5′-CATTGGCGCGCTATTTAAATTA-3′, and the probe sequence is

5-FAM-ACCGATTTGCCGCCTTCAGATG-BHQ1-3′. This Ingelvac PRRS® MLV RT-qPCR assay targets the non-structural protein 2 (NSP2) gene [32].

2.9. PRRSV ORF5 Sanger and *CLAMP sequencing*

For all challenged pigs, selected samples collected at dpc 9 (one sample per room corresponding to two samples per group) were further investigated by ORF5 Sanger sequencing and a PRRSV CLAMP sequencing assay to determine if the PRRSV detected at dpc 9 was the vaccine or challenge strain (https://vetmed.umn.edu/sites/vetmed.umn.edu/files/shmp_2019l205_sequencing_wild-type_prrs_in_vaccinated_herds-sciencepage.pdf). The PRRSV ORF5 Sanger and CLAMP sequencing assays were conducted at the Iowa State University Veterinary Diagnostic Laboratory per standard operating procedures. The PRSV CLAMP sequencing technology uses a modified bridged nucleic acid oligonucleotide ("clamp") to block Ingelvac PRRSV® MLV vaccine virus ORF5 amplification and preferentially amplify a wild-type (challenge strain) ORF5.

2.10. Statistical analysis

The statistical software used for analysis were JMP Pro 14 and SAS Version 9.4. Summary statistics were calculated for continuous variables from all groups to assess the overall quality of the data. The rejection level for the null hypothesis was 0.05. Generalized linear mixed effect models were fit with fixed "treatment" effects and a random "room" effect (nested within treatment). In the case of repeated measures, fixed effects corresponded to "day" and "treatment*day interaction", and a random "subject identifier" effect. If the time-by-group interaction was not significant, then the group effect was assessed. Otherwise, the data were

232 analyzed cross-sectionally to determine at which time points the group means are different using
233 analysis of variance (ANOVA) followed by pair-wise comparison performed by Tukey-Kramer
234 adjustment to identify the groups that were different. A non-parametric ANOVA (Kruskal-
235 Wallis) was used for non-normally distributed data or when group variances were dissimilar, and
236 pair-wise comparisons were done using Wilcoxon rank sum test.
237
238 **3. Results**
239 *3.1. Humoral antibody responses*
240 A significant variation due to room was not detected for serology results. There was a
241 significant time-by-group effect ($P < 0.001$). At arrival at the research facility, none of the pigs
242 had detectable antibodies against PRRSV and NEG-controls remained negative for the duration
243 of the study (Table 2). At challenge, 11/12 IN-JET-VAC, 11/12 IN-MAD-VAC pigs, and 12/12
244 IM-VAC had seroconverted; however, IN-MAD-VAC pigs had significantly lower levels of
245 antibodies compared to IM-VAC pigs. The IN-JET-VAC group was not different from either of
246 the other two vaccine groups. By dpc 9, all challenged pigs had seroconverted including the non-
247 vaccinated POS-Control group; however, all vaccinated groups had significantly higher serum
248 antibody levels (Table 2).
249
250 *3.2. Presence of neutralizing antibodies*
251 At the time of challenge, in vaccinated pigs none of the pigs had FFN titers against the
252 challenge strain while 12/12 IN-JET-VAC pigs (log_{10} mean±SEM; 1.2±0.1), 10/12 IN-MAD-
253 VAC pigs (1.2±0.2) and 7/12 IM-VAC pigs (0.6±0.2) had titers against the Ingelvac PRRS®
254 MLV vaccine strain. The two groups receiving IN vaccination had significantly higher ($P <$

0.0073) group means of neutralizing antibodies compared to the pigs vaccinated via the IM route.

3.3. Clinical disease

There was no significant variation due to room in any of the clinical disease variables. Clinical signs of respiratory disease were not observed in any of the pigs before PRRSV challenge. There was a significant time-by-group effect ($P < 0.001$). All challenged pigs developed increased rectal temperatures starting with dpc 3 and the NEG-control group had significantly lower group means on dpc 3, 5 and 7 compared to all other groups (Fig. 2). POS-control pigs had significantly increased rectal temperatures compared to all other groups at dpc 7 and 9. When a cut-off for 40.7°C was used, the average days of a pig with a fever was 2.9±0.3 for IN-JET-VAC pigs, 2.7±0.3 for IN-MAD-VAC pigs, 3.3±0.2 for IM-VAC pigs, 4.3±0.2 for POS-CONTROL pigs, and 0.6±0.2 for the NEG-CONTROL pigs. The average fever days was significantly highest ($P < 0.001$) for the POS-CONTROL pigs, lowest for the NEG-CONTROL pigs, and in between these groups for all vaccinated groups. The POS-CONTROL pigs had loss of appetite by dpc 5 and for the remainder of the study. These pigs were also mildly lethargic and commonly remained recumbent when people entered the room for observations. A mild increase in respiratory scores (score of 1 or 2) was observed by 7 dpc in all challenged groups regardless of vaccination status without any difference among groups. The ADG (in g ± SEM) of the pigs from the time of PRRSV challenge and the necropsy is summarized in Table 3. There was a significant difference in ADG between POS-control and NEG-control groups ($P = 0.0009$).

3.4. PRRSV RNA in serum

A significant room effect was not detected for PRRSV RNA viremia. There was a significant time-by-group effect ($P < 0.001$). NEG-control pigs were negative for PRRSV RNA in serum samples throughout the study. At dpv 7, 7/12 IN-JET-VAC pigs, 2/12 IN-MAD-VAC pigs and 12/12 IM-VAC pigs were viremic. By dpv 14 11/12 IN-JET-VAC pigs, 7/12 IN-MAD-VAC pigs and 12/12 IM-VAC pigs were viremic. By dpv 21, 11/12 in the IN-JET-VAC and IM-VAC groups and 10/12 IN-MAD-VAC groups were viremic. By dpv 28 each vaccinated group had 11/12 viremic pigs. After challenge all vaccinated pigs and all POS-CONTROLs were viremic at dpc 3, 6 and 9. Group mean levels of log10 PRRSV genomic copy numbers in serum are summarized in Fig. 3.

After challenge, the presence of vaccine virus versus challenge virus was assessed and is summarized in Fig. 4. At dpc 3, vaccine virus was found in 11/12 IN-JET-VAC pigs (log_{10} mean±SEM; 4.1±0.4), in 11/12 IN-MAD-VAC- pigs (3.7±0.4) and in 10/12 IM-VAC pigs (2.7±0.5) (Fig. 4). At dpc 6, vaccine virus was found in 6/12 IN-JET-VAC pigs (1.6±0.5), in 7/12 IN-MAD-VAC- pigs (1.9±0.5) and in 4/12 IM-VAC pigs (1.2±0.6). Finally, at dpc 9, vaccine virus was found in 3/12 IN-JET-VAC pigs (0.6±0.3), in 3/12 IN-MAD-VAC- pigs (0.8±0.4) and in 1/12 IM-VAC pigs (0.4±0.4). After challenge, there was no significant difference in amount of vaccine PRRSV RNA among the vaccinated groups at any time point. Vaccine virus was never detected in any POS-CONTROL group pig. PRRSV PCR clamping on selected dpc 9 serum samples confirmed the presence of the challenge strain in all samples analyzed.

3.5. PRRSV RNA in nasal swabs

A significant room effect was not detected PRRSV RNA shedding. NEG-control pigs were negative for PRRSV RNA in nasal swabs over time. There was no significant time-by-group effect (P = 0.1218). In nasal swabs, PRRSV RNA was only detected sporadically in challenged groups at different dpc (Table 4). The detected RNA was exclusively challenge strain. At dpc 9, POS-control pigs shed significantly more PRRSV RNA via nasal secretion compared to all other challenged groups**.**

3.6. Macroscopic and microscopic lesions and PRRSV antigen in tissue sections

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

4. Discussion

PRRSV control continues to be an issue in most pork producing regions. While there are several commercial vaccines available, all are being administered via the IM route. PRRSV as a primary respiratory virus, is mainly transmitted by the nasal route and utilizing the IN route of vaccination could likely improve upper respiratory tract immunity and protection by reducing or preventing initial virus uptake. It is also thought that respiratory vaccines induce lung resident memory cells, which are potentially important for protective immunity [33-35]. Pork producers and pig veterinarians likely would switch to the IN route if proven to be more effective, but more importantly, a new vaccination route needs to be practical and cost effective. The objective of

this study was to evaluate and compare the efficacy of a widely used commercial MLV PRRSV vaccine administered via the IM route compared to the IN route, either via JET or via MAD. In this study, pigs were challenged with a contemporary US field isolate (ISU-5) 28 days post vaccination.

It has been determined that droplets larger than 10 μm predominantly deposit in the upper respiratory tract by inertial impaction, while droplets of less than 5 μm diameter are capable of reaching the lower respiratory tract including the trachea, bronchial, and bronchiolar regions, as well as alveolar spaces [33]. It has also been suggested that the distribution of drugs administered intranasally varies based on the delivery device used [36]. Specifically, anatomically correct nasal models of 2-, 5-, and 50-year-old subjects were developed, and regional nasal delivery of suspensions investigated. It was found that nasal sprays are not adequate delivery devices for pediatric populations, due to the narrower nasal passage and greater anterior deposition (~60%). MAD atomizer resulted in significantly less anterior deposition (~10%-15%) compared to the nasal pumps, but there was ~30% run off to the throat of 30-100 μm in size [36]. With this in mind, and as nebulizers or nasal sprays are not practical for pig vaccination, two IN administrations methods were compared in the current study. Vaccination using the JET in the IN-JET-VAC group was easy, quick and effective and was preferred by the personnel administering the vaccine in this trial. The JET 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-MAD-VAC group, syringes had to be re-filled and a new MAD adaptor had to be attached after each pig. However, while more time consuming, it is possible, that a single person vaccinates a pig IN with a syringe and a MAD whereas the JET requires a

minimum of two people, a holder, and a person to deliver the vaccine. Nevertheless, switching the MAD after each pig adds considerably to the overall vaccination cost for a farm.

In this study the challenge strain (lineage 1A) and the vaccine strain (lineage 5) were not similar. The particular challenge strain used was 87.4% identical to the commercial vaccine strain 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. 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), POS-control pigs had significantly higher rectal temperatures than all other groups and essentially stopped eating, which was not observed in the vaccinated groups, indicating that the vaccination had a protective effect regardless of administration route. Moreover, POS-control pigs had the lowest average daily gain from challenge to necropsy (240.9g ±30.0g) followed by all vaccinated groups (475.9g ± 44.1g for IN-JET-VAC, 411.7g ± 382g for IN-MAD-VAC, and 425.8g ±36.5g for IM-VAC) with the non-challenged NEG-control pigs having the highest ADG (616.5 ±g ± 57.9g). Interestingly, the ADG in the IN-JET-VAC group was not different from the NEG-CONTROL. In this study the endpoint was dpc 10. This was done based on our previous studies that determined that the peak of macroscopic and microscopic lung lesions occurs between 10 and 12 days post challenge and existing lesions resolve quickly thereafter [2, 30, 37]. In future studies, the long-term impact of different vaccination routes on viral shedding, PRRSV transmission and average daily gain should also be assessed.

Seroconversion rates were more rapid in IM-VAC and IN-JET-VAC compared to IN-MAD-VAC with most pigs seroconverting between dpv 14 and 21. At challenge, IN-MAD-VAC pigs still had significantly lower mean S/P ratios compared to the IM-VAC group whereas IM-VAC and IN-JET-VAC were not different indicating a slight delay in systemic humoral immunity. However, both IN vaccinated groups had significantly higher neutralizing antibodies against the vaccine compared to the IM-VAC group.

After vaccination, vaccine viremia was highest in IM-VAC followed by IN-JET-VAC followed by IN-MAD-VAC, and only at dpv 28 were all groups similar. When assessing PRRSV viremia after challenge, the IM-VAC and IN-JET-VAC groups behaved similarly and different from the POS-control group. In contrast, the IN-MAD-VAC group followed a pattern similar to the POS-control group.

The gross lesions in the challenged pigs were severe for most pigs as evidenced by mean gross lung lesions scores of 55-65%. Similarly, the microscopic lesions were severe and PRRSV antigen could be demonstrated by PRRSV IHC in essentially all infected pigs without differences. It would be important to repeat a portion of this study (IN-JET-VAC, IN-MAD-VAC and POS-CONTROLS) with another vaccine, perhaps more compatible to the challenge strain.

5. Conclusions

Under the conditions of this study, nasal administration of a commercial PRRSV vaccine using an experimental JET designed for larger scale IN vaccination worked well and obtained data are comparable to those obtained after vaccination of the pigs by the IM route, as recommended by the manufacturer. It appears, the JET vaccine administration worked well and

was easy and fast for each pig compared to IN administration via MAD. This study data indicates that the IN administration route via the JET may be a viable option for PRRSV vaccination on pig farms. This technology can immediately be used for rapid mass vaccination on larger 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, IN vaccination with a PRRSV MLV vaccine using an experimental JET 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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Declaration of Competing Interests

The authors declare they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplemental material

Images of the vaccination tools used for each of the vaccinated groups.

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533 **Table 1.** Experimental groups. Abbreviations used: MLV = modified live virus; n/a = not applicable.

Group name	Number of pigs	Vaccine	Tools used	Vaccination route	Challenge
IN-JET-VAC	12	MLV	Automated pressurized gas actuated delivery device (JET) with a prototype multi-use atomization tip	Intranasal	PRRSV
IN-MAD-VAC	12	MLV	Syringe fitted with a single-use mucosal atomization device (MAD) adaptor	Intranasal	PRRSV
IM-VAC	12	MLV	Syringe fitted with a needle	Intramuscular	PRRSV
POS-CONTROL	12	None	n/a	n/a	PRRSV
NEG-CONTROL	6	None	n/a	n/a	n/a

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Table 2. Prevalence of ELISA positive pigs per group (mean group ELISA S/P ratios ± SEM) at vaccination (dpv 0), at the day of challenge (dpv 28 or dpc 0), and at necropsy (dpc 9).

Group name	**Vaccination**				**Challenge**	
	dpv 0	**dpv 7**	**dpv 14**	**dpv 21**	**dpv 28/dpc 0**	**dpc 9**
IN-JET-VAC	0/12 (0.0±0.0)^{A,1}	0/12 (0.0±0.0)^{A}	9/12 (0.7±0.2)^{A}	11/12 (1.3±0.2)^{A}	11/12 (1.4±0.1)^{A,B}	12/12 (1.7±0.1)^{A}
IN-MAD-VAC	0/12 (0.0±0.0)^{A}	0/12 (0.0±0.0)^{A}	1/12 (0.2±0.1)^{B}	10/12 (0.9±0.1)^{A}	11/12 (1.2±0.1)^{A}	12/12 (1.7±0.0)^{A}
IM-VAC	0/12 (0.0±0.0)^{A}	0/12 (0.0±0.0)^{A}	10/12 (0.9±0.2)^{A}	12/12 (1.7±0.1)^{B}	12/12 (1.6±0.1)^{B}	12/12 (1.8±0.1)^{A}
POS-CONTROL	0/12 (0.0±0.0)^{A}	0/12 (0.0±0.0)^{A}	0/12 (0.0±0.0)^{B}	0/12 (0.0±0.0)^{C}	0/12 (0.0±0.0)^{C}	12/12 (1.3.9±0.0)^{B}
NEG-CONTROL	0/12 (0.0±0.0)^{A}	0/12 (0.0±0.0)^{A}	0/12 (0.0±0.0)^{B}	0/12 (0.0±0.0)^{C}	0/6 (0.0±0.0)^{C}	0/6 (0.0±0.0)^{C}

[1]Different superscripts on a treatment day (^{A,B,C}) indicate significant differences among group mean S/P ratios ($P < 0.05$) at a given time point.

Table 3. Average daily gain (ADG) in g ± SEM from challenge to necropsy and macroscopic and microscopic lesions and PRRSV antigen presence as determined by IHC on lung tissues at necropsy.

Group	ADG	Gross lesions[1]	Microscopic lesions[2]	PRRSV IHC[3]
IN-JET-VAC	475.9±44.1^{A,B,4}	52.6±6.2^{A}	4.5±0.3^{A}	2.9±0.1^{A}
IN-MAD-VAC	411.7±38.2^{B}	65.3±4.9^{A}	5.0±0.3^{A}	3.0±0.0^{A}
IM-VAC	425.8±36.5^{B}	51.9±5.3^{A}	4.5±0.3^{A}	2.8±0.2^{A}
POS-CONTROL	240.9±30.0^{C}	65.2±3.9^{A}	5.3±0.2^{A}	3.0±0.0^{A}
NEG-CONTROL	616.5±57.9^{A}	0.0±0.0^{B}	0.8±0.2^{B}	0.0±0.0^{B}

[1] Percentage of lung surface affected by visible lesions ranging from 0-100%.

[2] Score range from 0=normal to 6=severe, diffuse

[3] Score range from 0=no PRRSV antigen detected to 3=large amount of PRRSV antigen diffusely distributed.

[4] Different superscripts within a column (^{A,B,C}) indicated significant ($P < 0.05$) group mean differences.

549 **Table 4**. Nasal swab PRRSV RNA positive pigs/total pigs per group (mean group log_{10} PRRSV genomic copies ± SEM) in pigs

550 challenged with PRRSV at different days post challenge (dpc).

Group	1 dpc	3 dpc	5 dpc	7 dpc	9 dpc
IN-JET-VAC	1/12 (0.4±0.4)^{A,1}	3/12 (1.1±0.6)^{A}	6/12 (2.1±0.6)^{A}	2/12 (0.7±0.5)^{A}	0/12 (0.0±0.0)^{A}
IN-MAD-VAC	0/12 (0.0±0.0)^{A}	5/12 (1.6±0.6)^{A}	3/12 (0.9±0.5)^{A}	5/12 (1.5±0.5)^{A}	1/12 (0.2±0.2)^{A}
IM-VAC	0/12 (0.0±0.0)^{A}	1/12 (0.2±0.2)^{A}	3/12 (0.6±0.3)^{A}	2/12 (0.7±0.5)^{A}	1/12 (0.2±0.2)^{A}
POS-CONTROL	0/12 (0.0±0.0)^{A}	5/12 (1.8±0.7)^{A}	3/12 (0.9±0.5)^{A}	6/12 (1.0±0.7)^{A}	4/12 (1.4±0.6)^{B}

551 [1] Different superscripts for treatment group means (^{A,B}) indicate significant ($P < 0.05$) differences at a given dpc.

552 **Figure legends:**

553 **Fig. 1.** Experimental timeline. Abbreviations used: B=Blood collection; W=Weight assessment;
554 NS=Nasal swab collection; dpv=day post vaccination; dpc=day post challenge.

555

556 **Fig 2.** Mean group rectal temperature in the different treatment groups after challenge. Different
557 superscripts at a given day post challenge (^{A,B,C}) indicate significant ($P < 0.05$) differences
558 among group mean rectal temperatures.

559

560 **Fig. 3.** Mean group PRRSV viremia (log_{10} genomic copies) in pigs over time. The viremia from
561 7 to 28 days post vaccination corresponds to vaccine virus whereas the viremia from day post
562 challenge (dpc) 3 to 9 after challenge corresponds to a mix of vaccine and challenge strain.
563 Different superscripts at a given day (^{A,B,C,D}) indicate significant ($P < 0.05$) differences among
564 group mean log_{10} PRRSV genomic copies.

565

566 **Fig. 4.** Comparison of the mean group amount of vaccine virus (checkerboard pattern) versus
567 challenge virus (solid fill) at 3, 6 and 9 days post challenge (dpc) which corresponds to 31, 34
568 and 37 days post vaccination in the different treatment groups.

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