RESEARCH REPORT
SWINE HEALTH

Title: Nanodisc-based mucosal DIVA vaccine for PRRS virus – **NPB #18-170**

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Date Submitted: September 30, 2020

Industry Summary: The deliverable generated by this project is the demonstration that the incorporation of PRRS virus envelope proteins into soluble nanoscale membrane assemblies called nanodiscs (NDs) constitute a safe and effective vaccine against PRRS virus. Notably, because these assemblies only incorporate transmembrane proteins, they would not incorporate the highly immunogenic nucleocapside protein. Consequently, this novel biologic can be used as a DIVA vaccine against PRRS. To generate this deliverable, we compared the level of protective immunity obtained in cohorts of weaner pigs that were vaccinated with a ND-based vaccine displaying PRRS virus envelope proteins (P-ND), administered either intranasally (IN) or intramuscularly (IM). As a negative control, a third cohort received NDs that were void of viral proteins, i.e. empty NDs (E-NDs), which were delivered by both routes; IN and IM. As a positive control, a cohort was vaccinated IM with an in-house inactivated whole virus (IWV) prepared with the same virus strain (G16X) that was used as the source of the viral envelope proteins used to prepare the NDs. These four groups received the same respective biologic twice at a 25-day interval. As requested by the NPB, an additional positive control consisted of a cohort that was immunized once with a commercial modified live virus (MLV) vaccine. A strict control cohort consisted of pigs that were neither vaccinated nor challenged. Forty days after the first immunization, all of the pigs in the trial were challenged IN with virulent virus strain 16244B which, based on the amino acid sequence of GP5, is <97% homologous to G16X. The level of protection stimulated by the vaccines was assessed using objective and subjective parameters indicative of protective immunity including: viremia, gross lung pathology, peripheral blood oxygen saturation (SpO_2), and weight gain. As compared to the non-vaccinated and unchallenged pigs in the strict control cohort, animals that received the E-NDs and were challenged with the virulent 16244B virus as a group exhibited: a three-fold decrease in their rate of weight gain; a significant level of lung dysfunction reflected by hypoxemia (oxygen saturation level of <90%); a significant area of the lung with gross lung pathology (45±9); and a sustained level of viremia (>3 $\log_{10}$ $TCID_{50}$/ml of serum) for 12 days. Pigs in the group that received P-NDs via the intranasal route were not protected from the virus challenge, as they exhibited the same extent of adverse events as those just described for the E-ND group. On the other hand, pigs in the cohort immunized intramuscularly with the P-ND vaccine exhibited a statistically significant ($p<0.05$) improvement in all of the protective immunity

parameters measured, which were not significantly different from the level of protective immunity afforded to pigs immunized with either the IWV or the MLV vaccine, with only one exception. Specifically, all three vaccines that were administered intramuscularly, namely the P-ND, the IWV, and the MLV vaccines, significantly improved the rate of weight gain after the virus challenge, eliminated the presence of hypoxemia, and substantially reduced the extent of gross lung pathology. The one exception consisted in the ability of the MLV to elicit protective immunity capable of terminating the viremia within 10 days after the virus challenge in every pig in this cohort. Nonetheless, the viremia in the cohort of pigs that received the IM P-ND vaccine was extinguished within 12 days after the challenge in 3 out of 8 pigs, with the other 5 pigs exhibiting a clear trend towards an impending viral elimination. Notably, there was no statistical difference in the rate of the extinction of viremia between the pigs immunized IM with the P-ND or with the IWV vaccine. The observations made in this project demonstrate that a vaccine based solely on viral envelope proteins incorporated into NDs are capable of providing a significant level of protective immunity against a virulent virus challenge. The only parameter of protective immunity measured in which the P-ND vaccine did not appear to be as effective as the MLV vaccine was the rate of cessation of viremia. Nonetheless, it was only a matter of days before the cessation of viremia in every pig immunized IM with either the P-ND or the IWV vaccines. Clearly, the notable advantage of P-ND and IWV vaccines would be their absolute safety. Unlike MLV vaccines, they possess no risk of reversion to virulence. Further, the P-ND vaccine can function as a DIVA vaccine. The use of this type of biologic could be used to effectively vaccinate pigs against PRRS virus while avoiding the generation of revertant vaccine viruses that continue to plague the industry as it strives to control and eliminate this pathogen.

Key Findings:

- A vaccine based solely on viral envelope proteins incorporated into nanoscale membrane assemblies called nanodiscs (NDs) administered intramuscularly is capable of providing a significant level of protective immunity against a virulent virus challenge.
- An inactivated whole virus (IWV) vaccine was also capable of providing a substantial level of protective immunity against PRRS virus.
- Both types of inert biologics (ND-based- and IWV-vaccine) were capable of eliciting protective immunity that was fairly comparable to that afforded by the MLV vaccine.
- Given the vastly superior level of safety offered by an inert biologic as compared to a MLV vaccine, the use of ND-based or IWV vaccine is desirable.
- Given the potential of ND-based vaccine to serve as a DIVA vaccine, further development of this type of biologic for commercial application is warranted.

Keywords: PRRS virus, vaccine, protective immunity, nanodiscs, inactivated vaccine, killed vaccine, modified live virus vaccine, DIVA.

Scientific Abstract: This project examined the level of protective immunity attained in weaner pigs (23±2 days of age) vaccinated either intranasally (IN) or intramuscularly (IM) with a nanodisc (ND)-based vaccine displaying PRRS virus envelope proteins (P-ND). The efficacy of this biologic was compared to that of an in-house inactivated whole virus (IWV) vaccine that was prepared using the same virus (G16X) used as the source of the viral proteins to assemble the P-NDs. For IN immunization the P-NDs were adjuvanted with a whole cell lysate of the *Mycobacterium smegmatis*. For IM immunization, the P-NDs were mixed with the adjuvant Montanide ISA 201 VG (Seppic). As a negative control, a pig cohort received NDs that were void of viral proteins, i.e. empty NDs (E-NDs), which were delivered to the same pigs via both routes (IN/IM) and adjuvanted equally as the P-NDs according to their route of delivery. These four groups (each group n=8) received a booster immunization with the same respective biologic twice at a 25-day interval. To further assess the level of vaccine efficacy, an additional pig cohort (n=5) was immunized once, at the same time as the other cohorts received their first immunization, with a commercial modified live virus (MLV) vaccine. A strict control cohort consisted of pigs (n=3) that were neither vaccinated nor challenged. Forty days after the first immunization, all of the pigs in the trial were challenged IN with virulent virus strain 16244B which, based on the amino acid sequence of GP5, is <97% homologous to G16X. The level of protection stimulated by the vaccines was assessed using objective and subjective parameters indicative of protective immunity including: viremia, gross lung pathology, peripheral blood oxygen saturation (SpO_2), and weight gain. As compared to the non-vaccinated and unchallenged pigs in the strict control cohort, animals that received the E-NDs and were challenged with the virulent 16244B virus as a group exhibited: a three-fold decrease in their rate of weight gain; a significant level of lung dysfunction reflected by hypoxemia (oxygen saturation level of <90%); a significant area of the lung with gross lung pathology (45±9); and a sustained level of viremia (>3 $\log_{10}$ $TCID_{50}$/ml of serum) for the entire period of monitoring (12 days). Pigs in the group that received the P-NDs IN were not protected from the virus challenge, as they exhibited the same extent of adverse events as those observed in the E-ND group. On the other hand, pigs in the cohort immunized IM with the P-NDs exhibited a statistically significant ($p<0.05$) improvement in all of the protective immunity parameters measured, which were not significantly different from the level of protective immunity afforded to pigs immunized with either the IWV or the MLV vaccine, with only one exception. Specifically, all three vaccines delivered IM, namely P-ND, IWV, and MLV, significantly improved the rate of weight gain after the virus challenge, eliminated the presence of hypoxemia, and substantially reduced the extent of gross lung pathology. The one exception consisted of the ability of the MLV to elicit protective immunity capable of terminating the viremia within 10 days after the virus challenge in every pig in this cohort. Nonetheless, the viremia in the cohort of pigs that received the IM P-ND vaccine was extinguished within 12 days after the challenge in 3 out of 8 pigs, with the other 5 pigs exhibiting a clear trend towards an impending viral elimination. Notably, there was no statistical difference in the rate of viremia extinction between the pigs immunized IM with the P-ND or with the IWV vaccine. The lack of protective immunity afforded by the P-ND biologic delivered IN was most likely due to a lack of sufficient amount of viral antigen in the vaccine. The level of protective immunity attained by the P-ND vaccine delivered IM based solely on viral envelope proteins incorporated in their natural configuration into NDs was impressive.

Introduction: The use of efficacious inactivated PRRS virus vaccines represents a powerful tool to empower efforts to control and eventually eradicate this troublesome virus. Since the early 1990s, researchers have been attempting to develop inactivated PRRS virus vaccines. However, most of the candidates have failed to elicit protective immunity even against homologous virus challenge. Recent research findings relating to both inactivated and subunit candidate PRRS virus vaccines have shown promise. Although, they need to be pursued further to improve their efficacy and cost-effectiveness before considering commercialization. Success in this area of PRRS vaccinology requires exploring novel strategies which may help to develop new vaccine concepts or improve existing technologies.

This notion is supported by the fact that the PRRS virus envelope proteins are known targets for a protective humoral immune response. Nanodisc (**ND**) particles are soluble, stable, and reproducibly prepared discoid shaped nanoscale structures that contain a discrete lipid bilayer bound by two amphipathic scaffold proteins. Because of this arrangement, ND particles permit the functional reconstitution of membrane/envelope proteins into the ND lipid bilayer in their native like configuration, including normal folding and native ligand binding (Civjan et al., 2003). Thus, these nanoscale structures can be used to prepare vaccines that incorporate the integral transmembrane proteins expressed by enveloped viruses like PRRS virus. Viral envelope proteins span the entirety of the biological membrane to which they are permanently attached. Notably, when ND are fabricated, they will naturally exclude virus proteins that are not associated with the viral envelope, namely the nucleocapside (N) protein. Exclusion of the N protein from NDs incorporating virus envelope proteins should provide a marker antigen, and thus a ND-based PRRS virus vaccine would serve as the basis for a **DIVA** (Differentiating Infected from Vaccinated Animals) vaccine for this virus. Notably, the IDEXX HerdChek PRRS, which has become the industry standard for the detection of antibodies against PRRS virus, is an ELISA test based on the detection of antibodies against the PRRS virus N protein.

Objective: The objective of this project was to demonstrate that the incorporation of PRRS virus envelope proteins into nanodiscs (ND-PRRS) constitutes a safe and effective mucosal PRRS virus vaccine that can elicit an adequate level of protective immunity and can function as a DIVA vaccine.

Materials & Methods

Viruses. The PRRS virus strain G16X was derived from the isolate 89-46448-40 (Zuckermann patent) and belongs to type II PRRS lineage 5. The virulent type II PRRS virus strain 16244B (GenBank accession no. AF046869) was isolated in 1997, from a piglet originating from a farm wherein sows experienced severe reproductive failure (Allende et al., 1999), and is likely to represent a virulent derivative of the commercial vaccine Ingelvac PRRS® MLV (Allende et al., 2000). Stocks of G16X and 16244B were grown in MARC-145 cells. The virus stocks of G16X used to assemble nanodiscs (NDs) incorporating PRRS virus envelope proteins, as well as the inactivated whole virus (IWV) vaccine, were concentrated approximately 10-fold by density ultracentrifugation (67,000 *g* for 2.5 h) through a 15% (w/v) iodixanol cushion. The concentrated G16X virus typically yields a titer of 10^9 $TCID_{50}$/ml. The commercial MLV vaccine (Prevacent® PRRS, Elanco) was purchased from a veterinary supply company and used following the manufacturer's instruction.

Inactivated whole virus (IWV) vaccine. The IWV was prepared using purified stocks of the PRRS virus strain G16X which was inactivated using BEI following standard procedures. One dose of the IWV vaccine consists of the equivalent of 10^8 $TCID_{50}$ before inactivation of concentrated virus. Further, to enable a direct comparison of the IWV vaccine with the ND vaccine, and given that the viral envelope glycoprotein 5 (GP5) is considered to be a major protective antigen for PRRS virus, this antigen was used to determine the amount of viral antigen in the IWV vaccine. This was accomplished using a semiquantitative Western blot in which the presence of GP5 was detected using a pool of serum from pigs that had been immunized with the G16X virus. This procedure is described below. The IWV vaccine dose was based upon the amount of GP5 and was estimated to be 0.5 mcg. The IWV was adjuvanted by mixing the inactivated virus suspension at a 50:50 (w:w) ratio with the adjuvant Montanide ISA 201 VG (Seppic).

Assembly of Nanodiscs incorporating PRRS virus envelope proteins (P-NDs). The process of nanodisc (ND) assembly has been previously described (Denisov et al., 2004). Briefly, endotoxin free *E. coli* derived-polyhistidine N-terminal tagged membrane scaffold protein (MSP) preparation will be obtained by extensively washing the MSP protein bound to a Ni-NTA column with aseptically prepared 1% Triton X-100 in PBS, followed by 10 bed volumes of sterile buffer without detergent. After elution with 0.3 M imidazole, the buffer will be exchanged using an Amicon 15 centrifugal ultrafiltration concentrator. The sample was filtered-sterilized and the endotoxin level determined using PYROGENT-5000 assay (Lonza). No detectable levels of endotoxin were found. Palmitoyloleoyl phosphatidylcholine (POPC) from Avanti Polar Lipids (Alabaster, AL) was dissolved in chloroform. The dried phospholipid (POPC) was reconstituted with PRRSV-disc buffer (30 mM Tris-HCl [pH 7.5], 0.3 M NaCl) containing 0.5% triton X-100. MSP from stock solution (200 to 400 μM) was combined with the POPC-triton X-100 mixture and incubated on ice with gentle agitation. Detergent-solubilized PRRS virions (typically $>10^9$ $TCID_{50}$/ml) were added to the MSP-POPC-triton X-100 mixture with phospholipid at a previously determined amount that optimize self-assembly. The mixtures were incubated for 1 h on ice. Self-assembly was initiated by the addition of 500 mg wet SM-2 Bio-Beads (Bio-Rad, Hercules, CA) per ml of solution. Supernatant was removed from the Bio-Beads by centrifugation and the ND assemblies were first purified by Ni^{2+} affinity chromatography. Following column washing, NDs were eluted from the IMAC column. The eluate was concentrated by centrifugal ultrafiltration and the resulting material was fractionated by size exclusion chromatography (SEC) in order to separate empty nanodiscs (**E-NDs**) from nanodiscs incorporating PRRS envelope proteins (**P-ND**). Fractions were collected every 1 min and peak elution was monitored at 254 nm. Following IMAC and SEC separations, samples of the flow through and eluent from the IMAC, as well as a pool of the size exclusion fractions expected to have ND that incorporated envelope glycoproteins, were analyzed by Western blot. The PRRS virus proteins were revealed in the Western blot using a pool of polyclonal anti-PRRSV serum derived from PRRS immune pigs. As a control of the Western blot analysis, a sample of the virus suspension used to prepare the P-NDs was also analyzed.

P-ND vaccine formulation. The amount of viral envelope proteins incorporated into the P-ND assemblies was based on the amount of the envelope glycoprotein 5 (GP5), using a chemiluminescent Western blot

assay. GP5 was selected for quantitation based on being highly immunogenic and abundantly present in the viral envelope. In this assay, the PRRS viral proteins present in a sample were revealed using a porcine anti-PRRSV serum, and the density of the resulting GP5 band measured using a FluorChem R system (Protein Simple). The relative concentration of GP5 was calculated by plotting the density of the resulting GP5 band present in the P-ND assemblies against a standard curve generated from the density of three standards consisting of decreasing concentrations of recombinant GP5. The P-NDs and the standards were analyzed by running them in the same blot. One dose of P-ND consisted of a 2 ml volume of the assemblies diluted to yield a 0.25 μg /ml of GP5 protein. For mucosal immunization, the vaccine was adjuvanted by mixing the P-NDs with 200 μg of whole cell lysate (WCL) of the saprophytic *Mycobacteria smegmatis*. The mixture was administered intranasally (**IN**) as a spray in one nostril with the aid of a nasal sprayer (Prima Mist Sprayer, Neogen). As a quality control, the pro-inflammatory activity of the mycobacteria WCL used as the mucosal adjuvant was confirmed with a bioassay. This assay demonstrated that the product was able to efficiently stimulate the production of TNF-alpha in porcine macrophages, namely ZMAC cells, with a similar specific activity as the commercial product Lipoarabinomannan from *Mycobacterium smegmatis* (InVivoGen), which is a TLR-2 ligand. For parenteral immunization, the P-NDs were mixed at a 50:50 (w:w) ratio with the adjuvant Montanide ISA 201 VG (Seppic) following the manufacturer's instructions. Montanide ISA 201 VG is a water/oil/water (W/O/W) emulsion adjuvant approved by the USDA for use in pigs. The volume of the adjuvanted P-NDs vaccine was 4 ml and administered IM using a hypodermic needle and a syringe. As a negative control, empty NDs (E-NDs) displaying no viral antigen were also prepared.

Experimental design. Nine-day-old pigs free of PRRS virus, PCV2 and *Mycoplasma* were randomly assigned to six treatment groups (see Table 1). Two groups (each n=8) were immunized either intranasally (IN) or intramuscularly (IM) with P-NDs. Two additional groups were immunized intramuscularly with either the IWV vaccine (n=8) or with a commercial (Prevacent PRRS, Elanco) MLV vaccine (n=5). A fifth group (n=8) received the E-NDs both IN and IM. Twenty-five days later, pigs immunized with the P-ND by either route or with the IWV vaccine received a booster immunization with the same vaccine formulation and by the same mode of delivery used for their first vaccination. A sixth group of pigs (n=3) was untreated and unchallenged and served as the strict control.

Assessment of vaccine efficacy. Forty days after the first immunization (fifteen days after the booster immunization with P-NDs or IWV) pigs were challenged intranasally with a virulent lineage 5 PRRS virus strain 16244B, which is heterologous (≤96% homology) to the G16X virus used to prepare the P-NDs and the IWV vaccine. Afterwards, the animals were monitored for 12 days for clinical signs of pneumonia. The body weight of the animals was recorded the day before challenge. Blood samples were collected to determine the extent of viremia and virus-specific immunity. Pulse oximetry was used to objectively assess lung function. Thirteen days after virus challenge, the animals were euthanized and their lungs scored for the percent of gross lung lesions by a pathologist blinded to the treatment of the animals. To objectively assess the extent of pneumonia, the density of the dissected lung lobes was determined by measuring their mass (g) divided by their volume (cm^3). The lung volume was measured using the water

displacement method. The extent of viremia was determined in serum samples collected at 7, 10, and 12 days after the virus-challenge by exposing alveolar macrophages (ZMAC cell line) to 10-fold serial dilutions of serum and scored by cytopathic effect (cell death). The method of Reed and Muench was used to calculate the $TCID_{50}$/ml.

Table 1. Experimental design to assess the efficacy of PRRS virus vaccines based on either viral envelope proteins incorporated into nanodiscs (ND), inactivated whole virus (IWV) of modified live virus (MLV).

Group designation (vaccine formulation)	Route of vaccination and schedule (days)	Estimated dose of viral antigen (based on GP5)	Adjuvant	Challenge virus strain	Number of pigs per group
P-ND IN (source of envelope glycoproteins-strain G16X)	IN d0 and d25	0.5 mcg	M. smegmatis	16244B	8
P-ND IM (source of envelope glycoproteins-strain G16X)	IM d0 and d25	0.5 mcg	VGA205	16244B	8
IWV (source of envelope glycoproteins-strain G16X)	IM d0 and d25	0.5 mcg	VGA205	16244B	8
E-ND (without viral antigen)	IN/IM d0 and d25	-	M. smegmatis (IN) VGA205 (IM)	16244B	8
MLV (Prevacent PRRS, ELANCO)	IM d0	$4x10^4$ $TCID_{50}$ (estimated dose)	-	16244B	5
Strict control (unvaccinated/unchallenged)	-	-	-	None	3

IN=intranasal; IM=intramuscular.

Statistical analysis. Statistical comparisons were performed using GraphPad Prism 8.4.2 (San Diego, CA). Where appropriate, data were log-transformed and assumed to have a normal distribution. Depending on the study design, a Student's t-test or an ANOVA (one- or two-way, depending on the experimental design) followed by Bonferroni corrected t-test were applied to identify differences between treatments. Only significant differences ($p<0.05$) are reported on the graphs.

Results: A Western blot analysis of the NDs incorporating PRRS envelope glycoproteins (P-NDs) used to immunize pigs for this study is shown in Fig. 1. The lane labeled as PRRS virus shows four bands characteristic of PRRS virus. The two top bands correspond to the envelope glycoproteins **GP2** and **GP5**, followed by two bands of smaller size corresponding to the matrix (**M**) protein and the nucleocapsid (**N**). As expected, NDs incorporating the virus-envelope proteins (P-NDs) show that the GP2, GP5 and M proteins are present, while the viral nucleocapsid (N) protein is not. These results demonstrate that PRRS virus envelope proteins GP2, GP5 and M were incorporated into the E-NDs while the N protein was excluded.

Evidence that the challenge with virulent 16244B virus resulted in a syndrome consistent with PRRS-induced disease is made evident by comparing the observations made in pigs belonging to the strict control group (unvaccinated/unchallenged) with the pigs belonging to the group which received the E-NDs and challenged with the virulent virus. Pigs in the latter group exhibited a three-fold decrease in their rate of weight gain (Fig. 2); a significant level of lung dysfunction reflected by hypoxemia as indicated by oxygen

saturation level of <90% (Fig. 3); a significant area of the lung with gross lung pathology with a group mean of 45±9 (Fig. 4); and a sustained level of viremia (>3 $\log_{10}$ $TCID_{50}$/ml of serum) for 12 days (Fig. 5). Similarly, pigs in the group that received P-NDs via the IN route were also unprotected from the virus challenge, as they exhibited the same adverse events as those just described in the E-ND group in equal extent. On the other hand, pigs in the cohort immunized intramuscularly with the P-ND vaccine exhibited a statistically significant ($p<0.05$) improvement in all of the protective immunity parameters measured as compared to the E-ND-immunized pigs (statistically significant differences between the E-ND vaccinated group and all other vaccinated groups are noted in each figure by asterisks above the respective data). Notably, the improvement in the protective immunity parameters measured was not significantly different from the level of protective immunity afforded to pigs immunized with either the IWV or the MLV vaccine, with only one exception. Specifically, P-ND administered IM, the IWV and the MLV vaccines, significantly improved the rate of weight gain after the virus challenge (Fig. 2); eliminated the presence of hypoxemia (Fig. 3); and substantially reduced the extent of gross lung pathology (Fig. 4). The one exception consisted in the ability of the MLV to elicit protective immunity capable of terminating the viremia within 10 days after the virus challenge for every pig in this cohort (Fig. 5). Nonetheless, the geometric mean of the viremia exhibited at 12 days after challenge by the animals vaccinated IM with either the P-NDs or the IWV vaccines was >10-fold lower than viremia exhibited by the negative control pigs immunized with the E-NDs or by the animals in the cohort dosed IN with the P-NDs (Fig. 5). Furthermore, the viremia in the cohort of pigs that received the IM P-ND vaccine was extinguished within 12 days after the challenge in 3 out of 8 pigs, with the other 5 pigs exhibiting a clear trend towards an impending viral elimination. Notably, there was no statistically significant difference in the rate of the extinction of viremia between the pigs immunized IM with the P-ND or the IWV vaccine (Fig. 5).

Discussion: In this study, several objective and subjective parameters of protective immunity were used to ascertain the ability of a ND-based vaccine, displaying PRRS virus envelope proteins (P-ND) in their natural configuration, to elicit protective immunity in weaner pigs against an experimental challenge with PRRS virus. All of the parameters measured indicated that the IM administration of the ND-based vaccine elicited significant levels of protective immunity that were equal to the level of protective immunity elicited by an IWV vaccine. When compared to the protective immunity elicited by the commercial PRRS MLV vaccine, the only parameter of protective immunity measured in which the P-ND vaccine did not appear to be as effective as the MLV vaccine was the rate of cessation of viremia. Nonetheless, it was only a matter of days before the cessation of viremia in every pig that was immunized IM with either the P-ND or the IWV vaccines. The lack of protective immunity afforded by the ND-based biologic delivered IN was most likely due to a lack of a sufficient amount of viral antigen in the vaccine. Clearly, the notable advantage of P-ND and IWV vaccines would be their absolute safety. Since unlike an MLV vaccines, they possess no risk of reversion to virulence. Further, the P-ND vaccine can function as a DIVA vaccine. The use of this type of biologic could be used to effectively vaccinate pigs against PRRS virus while avoiding the generation of revertant vaccine viruses that continue to plague the industry as it strives to control and eliminate this troublesome pathogen. The results also indicate that a properly formulated inactivated whole virus (IWV) vaccine is capable of providing a substantial level of protective

immunity against PRRS virus. The level of protective immunity afforded by the P-NDs and the IWV vaccines were fairly comparable to that afforded by the MLV vaccine. Given the vastly superior level of safety offered by an inert biologic, such as the inactivated virus vaccine, as compared to a MLV vaccine, the use of ND-based or IWV vaccine is highly desirable.

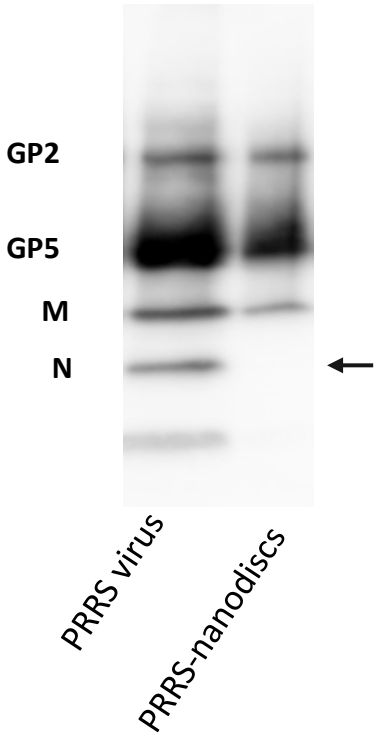

Figure 1. Western blot analysis of nanodiscs (ND) assemblies incorporating PRRS virus envelope proteins prepared for the vaccination studies. Detergent-solubilized PRRS virions were used to prepare NDs incorporating PRRS virus envelope proteins. A sample of the resulting NDs, were separated by electrophoresis in a 15% SDS-PAGE gel and subsequently transferred onto a 0.2 micrometer PVDF membrane for Western blot analysis. The membrane was probed with a pool of serum obtained from pigs immunized against PRRS virus. The presence of the structural viral proteins reacting with the anti-PRRS virus antibodies in the pig serum was revealed by reacting the membrane with a horseradish peroxidase (HRP)-conjugated rabbit anti-pig IgG reagent. After further processing, the membrane was exposed to a chemiluminescent reagent and the resulting bands visualized using a FluorChemR instrument. The location of the viral structural proteins, including the viral proteins that are present in the viral envelope (GP2, GP5 and M) as well as the nucleocapside (N) protein, are labeled on the left side of the figure. The arrow on the right indicates the location where the N protein would appear had it been incorporated into the NDs or if the preparation was contaminated with non-membrane associated proteins. The absence of the N protein into the NDs indicates that viral proteins not associated with a lipid bilayer were excluded from the ND assemblies.

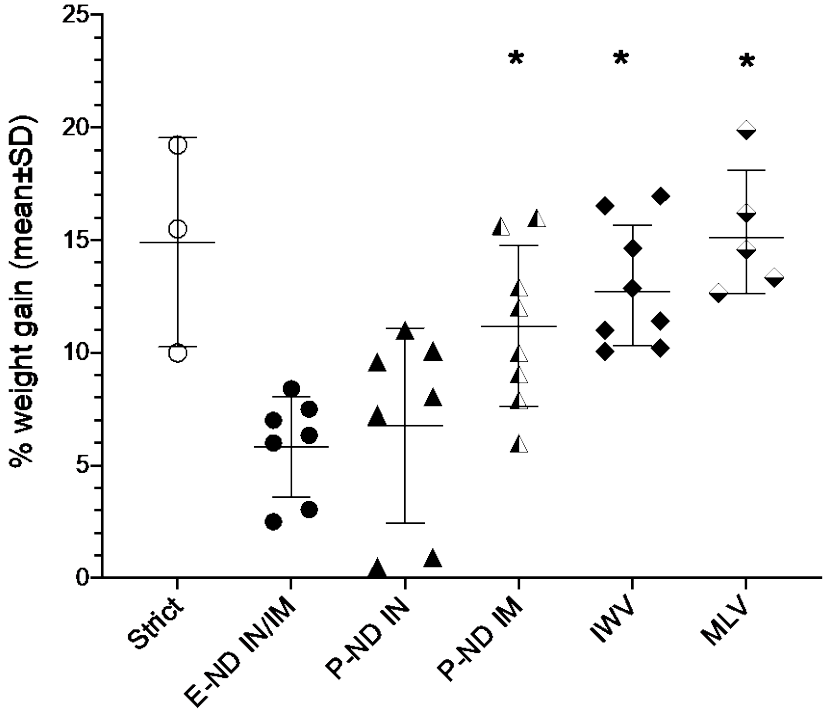

Fig 2. Rate of weight gain after PRRS virus challenge. Animals were weighed at 7 and 12 days after being challenge with the virulent PRRSV isolate 16244B. Unchallenged and unvaccinated animals (strict controls) (n = 3) were also weighed at these two time points. The change in body weight during this interval was determined on an individual basis. Each symbol represents the value obtained from each pig and are grouped according to their treatment. The mean % weight change ± SEM for each group is indicated. Statistically significant differences ($p < 0.05$) between the E-ND vaccinated group and the other groups indicated by an asterisk above the respective group.

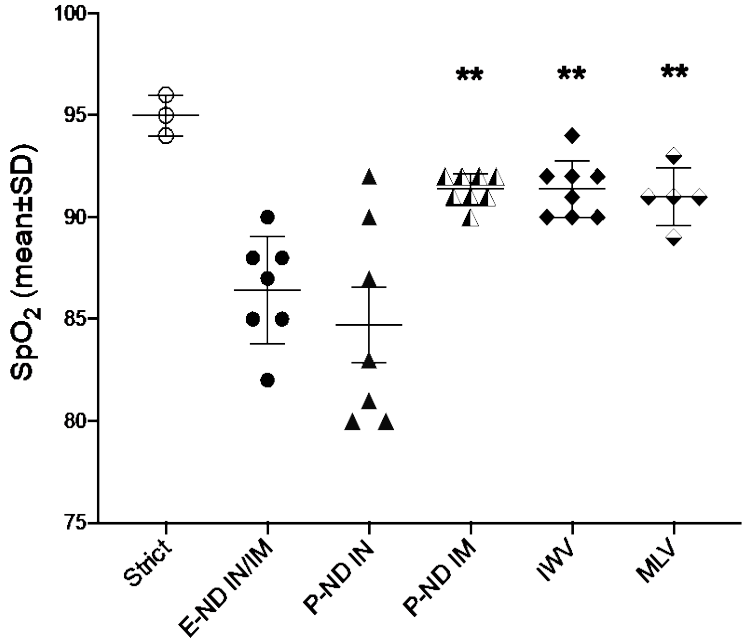

Fig 3. Lung function after PRRS virus challenge. The capillary blood oxygenation was measured using a pulse oximeter. Each symbol represents the SpO_2 obtained from each pig at 9 days after the PRRS virus challenge. The mean±SD of each group is indicated. Statistically significant differences ($p < 0.01$) between the E-ND vaccinated group and the other groups indicated by the double asterisk above the respective group.

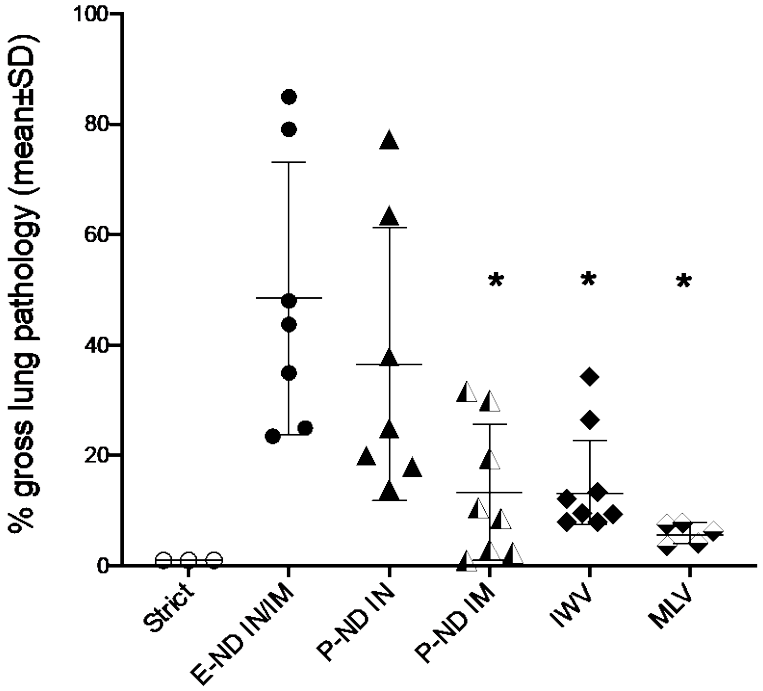

Fig 4. Extent of gross lung pathology. After euthanasia (13 days after virus challenge), the lungs were harvested and the extent of gross lung pathology scored by a board-certified pathologist, who was blinded to the animal treatment. Each symbol represents the % gross pathology scored for each pig. The mean ± SD for each group is graphed. Statistically significant differences ($p < 0.05$) between the E-ND vaccinated group and the other groups indicated by an asterisk above the respective group.

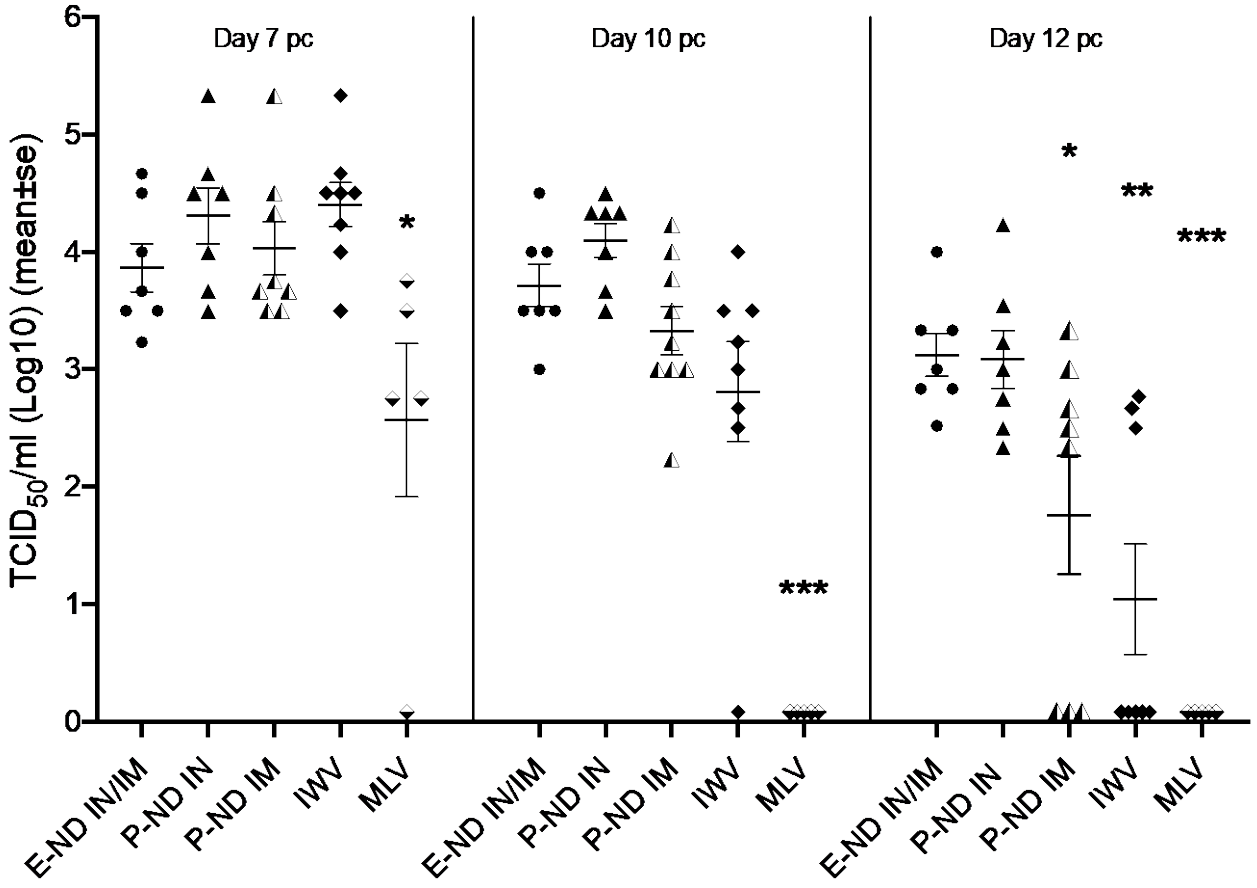

Fig. 5. Extent and frequency of viremia after PRRS virus challenge. The level of viremia was determined at the indicated time after the PRRS virus challenge. Each symbol represents the level of viremia detected in the serum from a single pig, which are grouped by their treatment. No PRRS viremia was present in any of the experimental animals before challenge, including the strict control pigs (not shown). The mean ± SD for each group is graphed. Statistically significant differences ($p < 0.01$) between the E-ND vaccinated group and the other groups indicated by a either a single ($p < 0.05$) or a double ($p < 0.01$) asterisk above the respective group.

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