RESEARCH REPORT
SWINE HEALTH

Title: Viral structural components that enable vaccine-induced protective immunity against contemporary high morbidity and high mortality PRRS virus – **NPB #12-176**

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Date Submitted: September 18, 2014

Industry Summary.

The production of interferon (IFN)-α by an animal in response to a viral infection is known to be a principal determinant of the animal's ability to fight the infection. In addition, this substance is also known to play a major role in promoting the development of vaccine-induced adaptive immunity, thus acting akin to a vaccine adjuvant. Accordingly, the ability of a vaccine to stimulate an IFN-α response would be expected to have an impact on the strength of the protective immunity elicited by the vaccine. The goal of this project was to determine the strength of cross-protective immunity provided by two different PRRS live virus vaccines that have either a high (vaccine strain G16X) or low (vaccine strain Ingelvac PRRS MLV) capacity to provoke an IFN-α response in swine following their administration. The level of protective immunity elicited in grower pigs by either of these two vaccines was determined by challenging vaccinated animals with a genetically divergent (heterologous) and highly virulent PRRS virus, termed LTX1. While both PRRS live virus vaccines used for this project belong to the Type 2 North American (NA) lineage 5, the LTX1 strain, belongs to the "Canada-like" lineage 1. Type 2 PRRS viruses belonging to lineage 1 are highly virulent and were introduced within the last 10 years into in the U.S. from Canada. The LTX1 virus was selected as the challenge strain because it was responsible for a 2012 PRRS outbreak in a sow farm of the highest severity observed in the field by a group of veterinarians in Illinois. Two additional groups of pigs were not vaccinated and served as controls. Four weeks after vaccination, one of the unvaccinated groups and both of the vaccinated groups were challenged with the PRRS virus strain LTX1 and monitored for 14 days. The intensity and duration of the viremia following challenge, the amount of virus in the bronchoalveolar lavage fluid at 14 days post challenge, as well as body weight change were measured in all groups and used as parameters to evaluate cross-protective immunity. Pigs inoculated with the G16X vaccine exhibited a relatively high systemic IFN-α response within 4 days after vaccination. Although both vaccines were equally able to minimize the negative effect of the virus challenge on body weight gain, the G16X vaccine was more effective in providing protection as evidenced by a significant reduction in the peak level of viremia resulting from the virulent virus challenge as well as a faster elimination of the wild-type virus from both the blood stream and the lung. Similar results were obtained in a second experiment using another contemporary PRRS virus isolate also belonging to lineage 1. The PRRS virus vaccine G16X, elicits a sizable IFN-α response upon vaccination and provides effective cross-protective immunity as against virulent and genetically divergent (heterologous) type 2 (North American-like) PRRS viruses belonging to lineage 1, which is now a predominant lineage in pig farms in the American Midwest.

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Keywords: PRRS, heterologous immunity, vaccine, interferon, PRRSV

Scientific Abstract

The production of interferon (IFN)-α by an animal in response to a viral infection is known to be a principal determinant of the animal's ability to fight the infection. In addition, this substance is also known to play a major role in promoting the development of vaccine-induced adaptive immunity, thus acting akin to a vaccine adjuvant. Accordingly, the ability of a vaccine to stimulate an IFN-α response would be expected to have an impact on the strength of the protective immunity elicited by the vaccine. The goal of this project was to determine the strength of cross-protective immunity provided by two different PRRS live virus vaccines that have either a high (strain G16X) or low (strain Ingelvac PRRS MLV) capacity to provoke an IFN-α response in swine following their administration. The level of protective immunity elicited in grower pigs by either of these two vaccines was determined by challenging vaccinated animals with a genetically divergent (heterologous) and highly virulent PRRS virus, termed LTX1. While both PRRS live virus vaccines used for this project belong to the Type 2 North American (NA) lineage 5, the LTX1 strain, belongs to the "Canada-like" lineage 1. Type 2 PRRS viruses belonging to lineage 1 were introduced gradually during the last 10 years into in the U.S. from Canada and now represent the predominant Type 2 PRRS lineage in the Midwestern United States. A vaccination and challenge study was conducted with two groups (n=6) of 7 week-old pigs, which were immunized with either of the two PRRS virus vaccines. Two additional groups of pigs were not vaccinated and served as controls. Four weeks after vaccination, one of the unvaccinated groups and both of the vaccinated groups were challenged with the PRRS virus strain LTX1 and monitored for 14 days. Pigs in the mock-vaccinated group had a significant (>50%) reduction in weight gain during the observation period as compared to the strict control group that was not challenged. The mock vaccinated and challenged pigs also exhibited high levels of viremia throughout the 14 days of observation and at the end of the study (14 days post-challenge) all 6 pigs also had a high level of virus in their lungs. Pigs inoculated with the G16X vaccine exhibited a relatively high systemic IFN-α response within 4 days after vaccination. Immunization of the animals with either vaccine (G16X or Ingelvac PRRS MLV) similarly counteracted the negative effect of challenge with LTX1 virus in their growth as measured by body weight gain and at 14 days after challenge was not different from that of the strict control group. Vaccination with G16X resulted on a lower peak viremia at 7 days after challenge and also promoted the elimination of the challenge virus from the serum by 10 days post challenge in 80% (4 of 5) of the pigs vaccinated with G16X as compared to a higher level of viremia and only 16% (1 of 6) virus elimination in the group vaccinated with the Ingelvac PRRS MLV. Vaccination with G16X also resulted in 4 orders of magnitude reduction in the amount of virus present in the lung 14 days after challenge as compared to the amount of virus that was present in the lung of the mock-vaccinated and challenged control pigs. In contrast, the average reduction of challenge virus in the lungs the Ingelvac PRRS MLV-vaccinated animals was less than 3 orders of magnitude. In a second study, the G16X vaccine was also found to be able to also provide cross-protective immunity against another lineage 1 virus. The results of this study indicate that the level of IFN-α response to immunization with a PRRS MLV vaccine can be used as a predictive parameter of the potential effectiveness (potency) of PRRS virus vaccine and that the use of this biological property of this virus as selection criteria for vaccine strain selection will aid in the development of a more effective PRRS virus vaccine. In summary, the G16X vaccine was found capable of providing cross-protection from disease resulting from either of two different type 2 (North American-like) PRRS viruses belonging to lineage 1, which is now a predominant lineage in commercial swine farms in the American Midwest.

Introduction.

Porcine reproductive and respiratory syndrome (PRRS) first appeared in the late 1980's, and has since become a major disease of swine causing significant economic loses to the pork industry worldwide. In the nearly 20 years since the identification of PRRS virus, the goal of developing a broadly protective vaccine against this pathogen

has not been achieved. The constant diversification of the PRRS virus RNA genome, which gives rise to significant antigenic variation, is perceived as the main problem in PRRS vaccinology, and is commonly attributed as the culprit for the lack of cross-protective (a.k.a. heterologous) immunity. The inability of a given vaccine to provide adequate protection against a genetically dissimilar field virus is often referred as a lack of "heterologous protection." To address this issue, our laboratory has examined the biological properties of several wild-type and attenuated PRRS virus strains and shown that a common property of North American PRRS virus isolates, including the two commercially available live attenuated vaccine strains, is their ability to suppress the main anti-viral response and function of plasmacytoid dendritic cells (pDC), namely to secrete copious amounts of interferon (IFN)-α upon activation. Notably, the secretion of IFN-α by this important cell of the innate immune system is known to play a central role in regulating the development of anti-viral adaptive immunity.

Our preliminary data strongly suggests a critical connection between the effect of PRRS virus on the ability of pDC to produce IFN-α and the development of protective adaptive immunity to PRRS virus. Using a panel of attenuated PRRS virus strains, we have previously observed that the ability or lack thereof of a PRRS virus live vaccine to suppress the IFN-α secreting capacity of pDCs, rather than its genetic similarity to the challenge virus, appears to determine its ability to elicit adequate protective immunity from challenge with a genetically dissimilar and virulent PRRS virus isolate. In our studies we found that a plaque-purified variant (G16X) derived from a stock prepared from a virus that was isolated from a clinical sample of an ancient (1991) case of PRRS, and exhibited a negligible ability to either produce disease or suppress the ability of porcine pDC to produce IFN-α was able to stimulate considerable protective immunity against the genetically dissimilar "atypical PRRS" NADC-20 strain isolated in 1998.

Hypothesis: The goal of this project is to test the hypothesis that a live attenuated virus vaccine exhibiting a negligible IFN-α suppressing activity on pDC will be able to elicit an effective protective immunity against genetically dissimilar contemporary (2001 and 2007 isolates) PRRS virus strains associated with high morbidity and mortality. Furthermore, we propose a PRRS live virus vaccine capable of stimulating the production of IFN-α will lead to the development of adequate cross-protective immunity.

Objective

Determine the efficacy of PRRS live virus vaccines with different capacities to suppress the IFN-α responsiveness of pDC in affording protection to pigs from challenge with genetically dissimilar present-day highly virulent PRRS virus isolates.

Materials and Methods

The efficacy of two PRRS vaccine viruses was tested using standard vaccination and virus challenge studies. One group of animals was vaccinated with the vaccine candidate G16X, developed by our research group at the University of Illinois, which we have shown has a negligible capacity to inhibit the IFN-α response of pDCs *in vivo*. A second group of pigs was vaccinated with the commercially available Ingelvac PRRS MLV, which we have previously shown has significant ability to inhibit the IFN-α response of pDCs *in vivo* (Zuckermann et al., unpublished). The experiment was done as a blinded, placebo controlled study. To achieve masking, all personnel involved in daily observations, clinical scoring, assessment of gross and microscopic lung pathology and the processing of samples and interpretation of laboratory results remained masked throughout the experimental phase study. Twenty-four 6-weeks old pigs were purchased from the University of Illinois Veterinary Research Farm. The herd of swine at this farm is known to be free of all major swine pathogens including PRRS virus, influenza, mycoplasma and circovirus. The negative status for PRRS antibodies of the study animals was confirmed by serology prior to the start of the study. All 24 animals were ear tagged and randomly assigned to a treatment group (four groups and 6 pigs per group) and then transferred to a BSL2 animal containment facility. All of the pigs allocated to the same treatment group (6 pigs) were penned together. After a 7-day period of acclimation each group of pigs was vaccinated according to their treatment allocation as follows: **Group 1-** each pig in the mock vaccine was injected intramuscularly with 2 ml of vaccine

diluent. **Group 2-** each pig in this group received one dose of Ingelvac PRRS MLV (Serial No. 245-D45). The vaccine was reconstituted and administered intramuscularly according to the manufacturer instructions (titration of the inoculum indicated that the total dose administered was $4x10^4$ $TCID_{50}$). **Group 3-** each pig in this group received an intramuscular injection of 2 ml containing a total of $4x10^4$ $TCID_{50}$ of G16X live PRRS virus vaccine. The fourth group served as a strict (environmental) control and was not vaccinated. Twenty-eight days after vaccination all of the animals in groups 1, 2 and 3 were challenged with $4x10^4$ $TCID_{50}$ of the highly virulent PRRS virus isolate LTX1. This wild-type PRRS virus was isolated in 2012 from a sow farm in Illinois, which was suffering from a severe outbreak of PRRS virus. The syndrome observed was characterized by a conception rate of 60%, late term abortions and stillbirths. In addition, there was a 6 week period with 100% pre-wean mortality, followed by 2 more weeks of 80% mortality of pre-wean pigs. The outbreak was so severe that the owner of the farm and the attending veterinarian decided to depopulate the farm. The RFLP pattern of GP5 gene of LTX1 virus is 1-22-2 and the virus belongs to lineage 1 of type 2 (North American) PRRS virus (Shi et al., 2010). The GP5 of the LTX1 virus has a <88% homology with either of the two vaccines used. Half the dose of the challenge virus was given intranasally using a nasal sprayer and the other half by intramuscular injection. Subsequently the animals were monitored daily for the next 14 days for clinical signs. Blood samples were collected immediately before and at 7, 10 and 14 days after the virus challenge. Body weight was recorded on the day of challenge and at 7, 10 and 14 days after the challenge. At 14 days after the challenge the animals were euthanized and the lungs examined for gross pathology. A bronchoalveolar lavage performed from the right middle lobe using a catheter connected to a syringe containing 10 ml of buffered saline. The catheter was inserted into the bronchi leading to the right middle lobe and the two clamped together with a string to avoid leakage. The fluid was propelled into the lobe and then recovered by retracting the plunger. The amount of infectious virus in the recovered fluid was titrated by standard virological techniques. All other methods used were as previously described (Calzada-Nova et al., 2010; Calzada-Nova et al., 2011 and Calzada-Nova et al., 2012) except that the serum samples and BAL fluids collected were tested for infectious virus load using the porcine alveolar macrophage cell line ZMAC. In a second experiment, the ability of the G16X vaccine to provide protection against another wild type PRRS virus belonging to lineage 1, namely the isolate CCX1, was examined. The CCX1 virus was isolated in 2012 from a sow farm in which the virus was deemed responsible for an outbreak characterized, at the peak of the outbreak, by a 30% abortion rate and 3% sow mortality. The CCX1 virus has a 1-4-4 ORF5 RFLP. The isolate CCX1 was chosen due to the fact that its ORF5 sequence is representative of a number of field virus isolated in 2012 and 2013 that were deemed responsible for several outbreaks in sow farms located in the Midwest. Although also belonging to lineage 1, the CCX1 isolate differs significantly from LTX1 and they only have 86% homology in OR5. The same procedures described above were followed for this second vaccination and challenge experiment. The animals were challenged at twenty-eight days after vaccination with $4x10^4$ $TCID_{50}$ of the PRRS virus isolate CCX1. Half the dose of the challenge virus was given intranasally using a nasal sprayer and the other half by intramuscular injection.

Results

a. Vaccination with the G16X virus stimulates a strong interferon-alpha response at 4 days post-vaccination. Our previous studies with the PRRS virus vaccine candidate G16X, have shown that this virus has some unique biological properties. Unlike all other wild-type PRRS virus isolates that we have evaluated, which we have shown suppress the interferon alpha response of plasmacytoid dendritic cells (Calzada-Nova et al., 2011), G16X strain does not do so. Consistent with the unique biological property of the G16X virus strain, in this study we found that at 4 days after the intramuscular administration of G16X vaccine virus into pigs, a vigorous systemic interferon alpha response was detected in their serum, which was subsiding 4 days later, but was still above background at day 14 post vaccination (Fig. 1). Pigs inoculated with the Ingelvac PRRS MLV vaccine exhibited a much lower (5-fold less) response at the peak of the IFN-α response (day 4 post vaccination), decreasing to background levels by day 7 after inoculation. These results confirm that the G16X virus has a unique biotype regarding the IFN-α response of pigs to their inoculation with this virus.

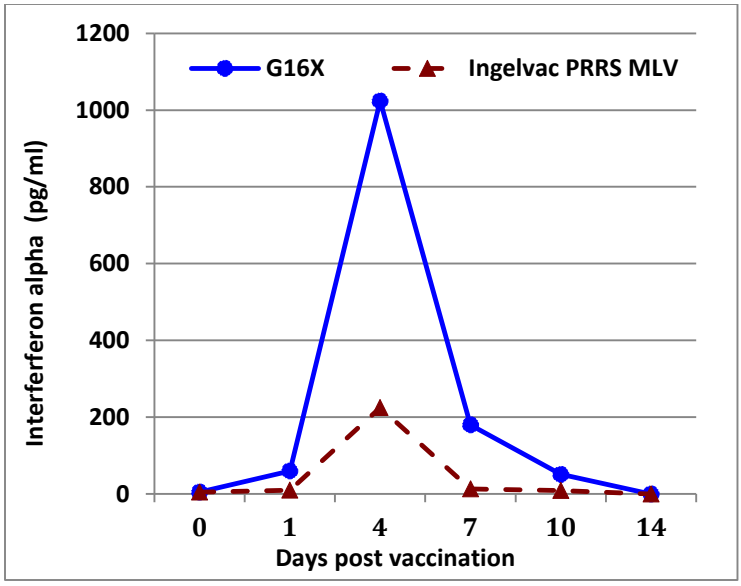

Figure 1. Serum Interferon alpha levels in pigs after their inoculation with either of two different PRRS live virus vaccines. Two groups of pigs (n=6) were inoculated with either Ingelvac PRRS MLV or G16X as described in materials and methods. Serum samples were collected at the indicated time points after vaccination and the level of interferon alpha measured by ELISA. Data represent the mean ± SEM of the 6 samples tested per time point in each treatment group. Mock-vaccinated animals had <2 pg/ml of serum in each time point tested (data not shown).

b. Inoculation with either the Ingelvac PRRS MLV or G16X vaccines result on a transient viremia of different magnitudes.

To ascertain biological differences between the two PRRS virus vaccines, we determined the extent, frequency and duration of viremia in animals inoculated with either product. To accomplish this task, serum samples were collected from each animal immediately before and at 4, 7, 14 and 28 days after immunization and the infectious virus load determined. In the case of the Ingelvac PRRS MLV vaccine, the simian kidney cell line MARC-145 was used as the cell substrate to measure the titer of infectious virus present in the serum samples. This was done due to the fact most likely due to the fact that the Ingelvac PRRS MLV virus was adapted to grow in monkey cells, it fails to grow in porcine macrophages. On the other hand, the G16X virus readily grows in pig alveolar macrophages. Thus, the virus load in the serum of G16X-vaccinated animals was done using the porcine alveolar macrophage cell line ZMAC. It should be noted that the G16X virus stock used as the vaccine was also produced in the ZMAC cell line. Upon immunization with either vaccine all of the animals developed a viremia that was easily detected in the respective cell line at 4 days after vaccination. In both cases, the viremia persisted for 14 days and was no longer detectable by 21 days. Notably, the extent of the viremia resulting from the administration of the G16X virus was one order of magnitude higher than the level observed following administration of the Inglevac PRRS MLV. The higher level of viremia observed in the G16X-inoculated animals likely reflects the natural ability of the G16X virus to readily grow in porcine macrophages, thus being able to efficiently replicate in the pig's tissues. The ability of a live virus vaccine to elicit a protective immune response is likely to be partly dependent on its ability to replicate in the cells of the inoculated host in order to produce sufficient antigenic mass to stimulate a vigorous protective immune response (Zuckermann et al., 1988). On the other hand, the Ingelvac PRRS MLV virus does not appear to initially readily replicate efficiently in pig's tissues. Rather, it is known to be under selective pressure to adapt back to its natural cell host cell, namely the alveolar macrophage, leading to its reversion to virulence, which has been documented (Nielsen et al 201; Oppriessnig et al., 2002). Regardless, in neither case were clinical symptoms associated with the inoculation into pigs of these two viruses (data not shown). The lack of ill effect of either vaccine is congruent with the equal rates of body weight gain as compared to the mock-vaccinated animals (Fig. 3).

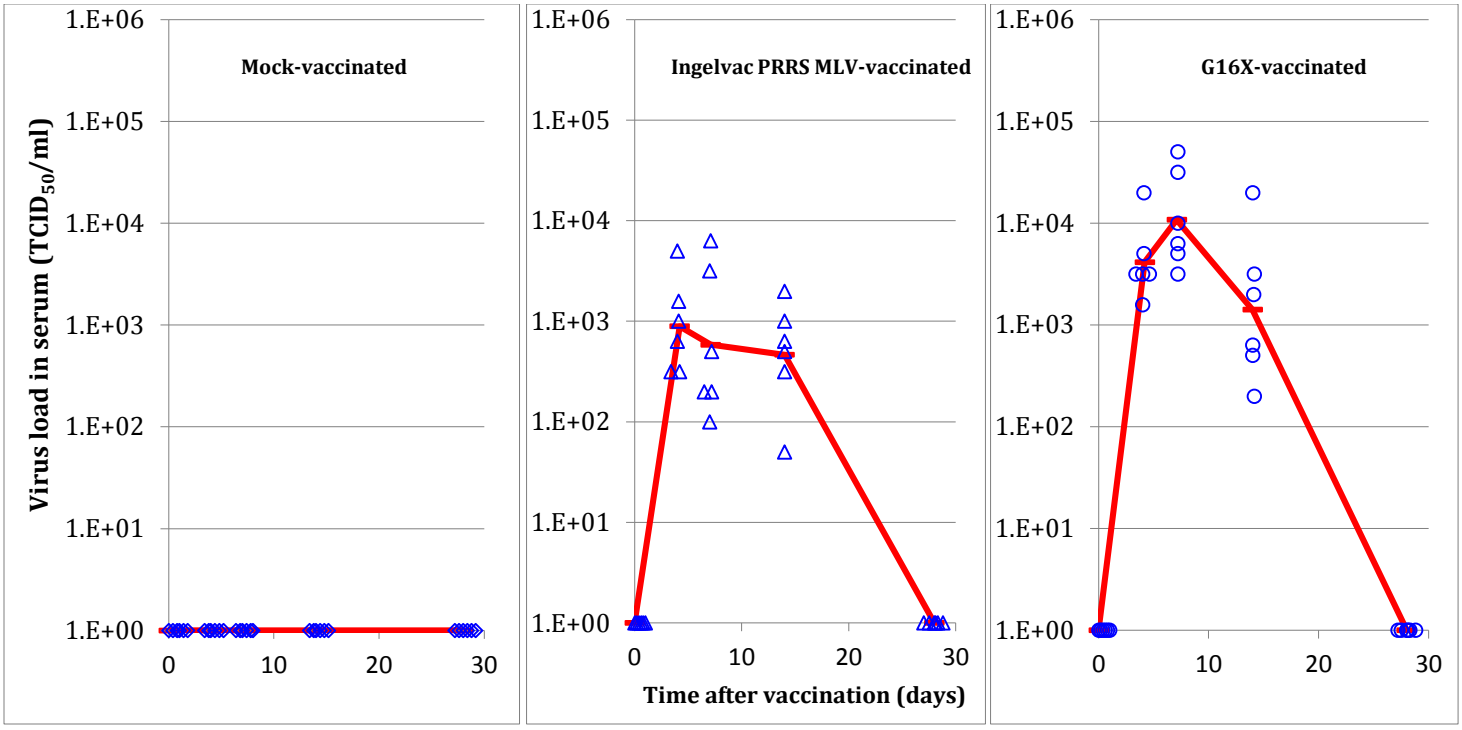

Fig. 2. Extent, frequency and duration of viremia in swine after their vaccination with either of two different PRRS live virus vaccines. Three groups of pigs (n=6) were inoculated with either Ingelvac PRRS MLV, G16X virus or mock vaccinated as described in materials and methods. Serum samples were collected at the indicated time points after vaccination and the virus load in the samples determined by performing infectious virus titrations using either MARC-145 cells (Ingelvac PRRS MLV) or ZMAC cells (G16X). Each data point (blue markers) represents the infectious virus titer for each serum sample of each pig in the group at the corresponding time point. The horizontal red bars represent the geometric mean of the group of the 6 samples tested per time point at the corresponding time point.

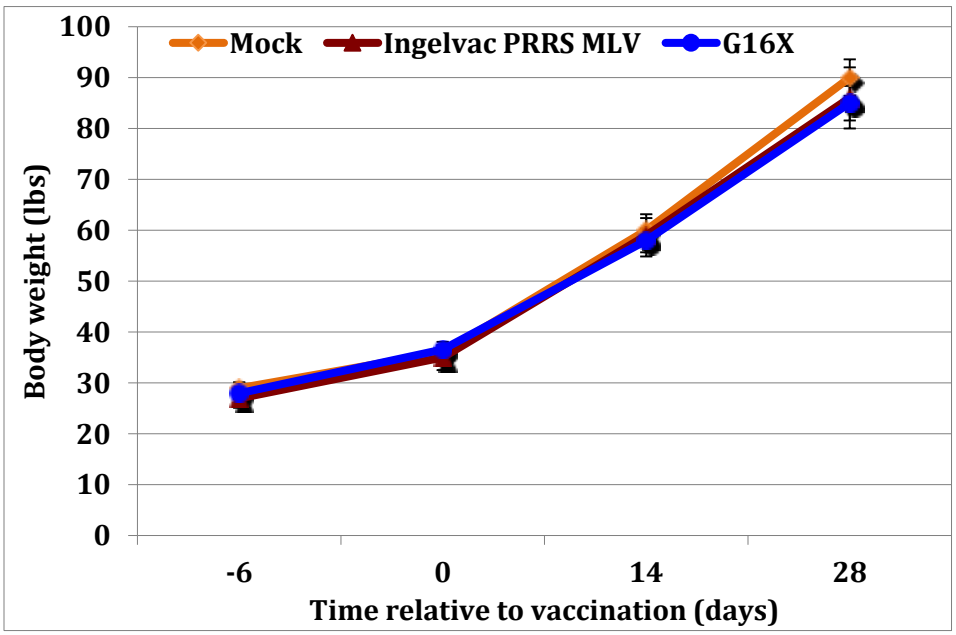

Fig. 3. Body weight gain following vaccination. Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X virus-vaccinated pigs (n=6 for each group) were weighed 6 days before, immediately prior to and at 14, 28 days after vaccination with the indicated virus. Data presented represent the mean BW for each group ± SEM.

b. Efficacy of the G16X vaccine in regards to pig weight gain in pigs challenged with a highly virulent PRRS virus.

At the time of challenge, the average body weight of the 24 pigs in the study was 85±4 lb, and there no differences in the average body weight between the three groups. Thus, exposure of the pigs to either vaccine had no obvious impact on their growth (Fig 3). To measure the protective immunity elicited by the two vaccines being examined with regards to growth, we calculated the % body weight gain for each animal from the day of virus challenge to 7, 10 and 14 days after virus challenge. The pigs in the unchallenged (strict control) group exhibited a steady rate of growth with an average increase of 32% in 14 days (Fig. 4). As compared with the strict control group, infection of the Mock-vaccinated with PRRS virus LTX1 caused a noticeable decrease in their rate of growth, and resulted in a net body weight loss from 7 to 10 days after challenge. Afterwards, the animals began to gain body weight back, ending with a 14% weight gain from the time of challenge (Fig. 4). Prior immunization of the animals with either vaccine counteracted the negative effect of challenge with LTX1 virus in that the groups receiving either vaccine posted similar average BW gains of about 12%, 19% and 29% at 7, 10 and 14 days post challenge, respectively.

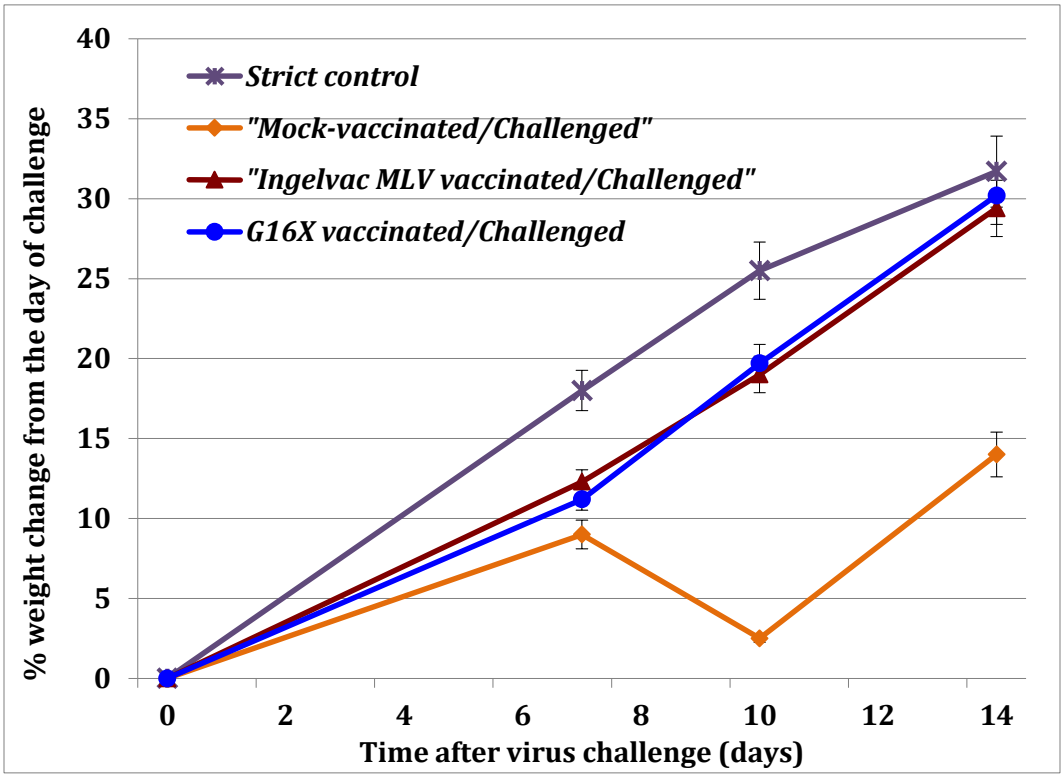

Fig. 4. Weight changes in pigs after exposure to virulent PRRSV. Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X-vaccinated pigs (n=6 for each group) were weighed immediately prior to and at 7, 10 and 14 days after challenge with the wild-type PRRSV isolate LTX1. Unchallenged and unvaccinated animals (strict controls, n=6) were also weighed at these four time points. The changes in BW during the ensuing 7-, 10- and 14-days after challenge were determined on an individual basis and the % weight change relative to its BW at the time of challenge calculated. Results represent the mean % weight change of each group ± SEM. All groups consist of six animals per group except the G16X group. This group had six animals until day 10 when the group was reduced to 5 animals. One animal in this group was eliminated because it developed an intestinal torsion that required that the animal be euthanized at day 10 after virus challenge.

c. Efficacy of the G16X vaccine in regards to the control of viremia in pigs infected with a heterologous highly virulent PRRSV

At the time of challenge (28 days post vaccination) none of the pigs in the trial had a detectable infectious virus in their serum (Fig. 5). All of the mock-vaccinated animals became viremic at 4 days after being challenged with the LTX1 virus. The level of viremia in this group peaked at 7 days post-challenge (pc) and remained at similar levels 3 days later, and although it began to decrease at 14 days pc, the level of viremia was still substantial and present in all animals (Fig. 5). In contrast, while all of the pigs in the two vaccinated groups became viremic at day 4 pc, the level of peak viremia observed in the G16X-vaccinated animals at day 7 pc was over one of order of magnitude lower as compared to the mock-vaccinated animals. The curtailing of the viremia at day 10 pc was significantly better in the G16X-vaccinated animals than in the Ingelvac PRS MLV-vaccinated animal ($p<0.01$). While 5 of the 6 animals in the later group of vaccinated animals were still viremic at 10 days pc, only 1 of the 5 animals in the G16X-vaccinated group were viremic at this time. By 14 days after vaccination, all of the animals in both vaccinated groups no longer had detectable infectious challenge virus in their blood stream.

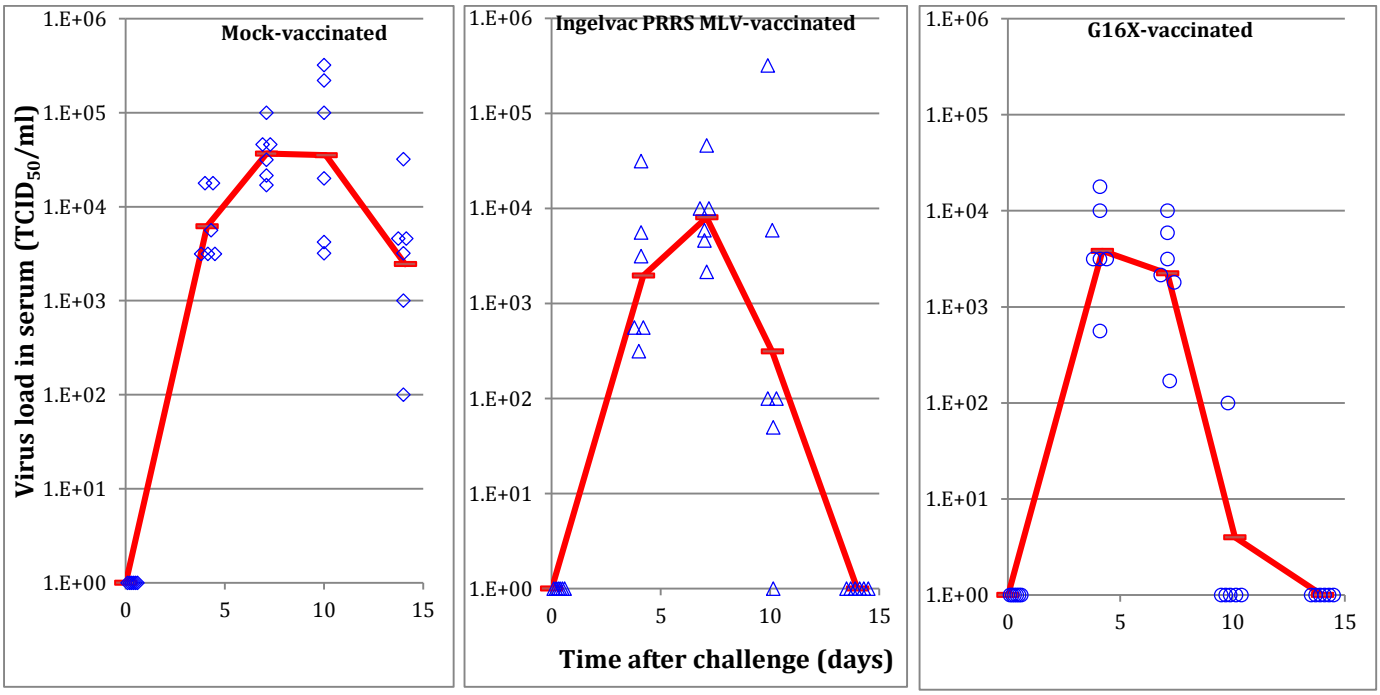

Fig. 5. Extent, frequency and duration of viremia in vaccinated or mock-vaccinated pigs after exposure to the PRRSV isolate LTX1. Serum samples were collected from Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X-vaccinated animals immediately prior to and at the indicated days after challenge with the wild-type PRRSV LTX1. The virus loads in the sera were determined by performing infectious virus titrations in ZMAC cells. Results are presented for each individual pig (blue markers) as well as the geometric mean for all the members of each group (horizontal red bars). One pig in the G16X-vaccinated group was eliminated from the trail at 10 days after challenge (see Fig. 2 legend). Samples were also taken at these time points for the unchallenged and unvaccinated animals (strict controls) (n=6), which remained virus negative at all time points.

d. Efficacy of the G16X vaccine in regards to the control of virus load in the lungs of pigs infected with a highly virulent PRRSV.

At 14 days after challenge with the LTX1 virus, infectious virus was found in the BAL fluid samples collected from all of the members of the mock-vaccinated group, with an average virus load in the pigs of $10^{5.5}$ $TCID_{50}$/ml. At this time, only three of the five animals that had been immunized with G16X virus grown in

ZMAC cells still had detectable amounts of PRRSV in their BAL fluid, while 5 of the 6 animals in the Ingelvac PRRS MLV-vaccinated group had detectable infectious virus. The average virus load in BAL of the animals in the G16X group was $10^{1.7}$ $TCID_{50}$/ml, and $10^{2.7}$ $TCID_{50}$/ml in the Ingelvac PRS MLV group, which represent, respectively, a reduction of almost 4 and 3 orders of magnitude as compared to the amount of virus that was present in the BAL collected from the mock-vaccinated and challenged pigs.

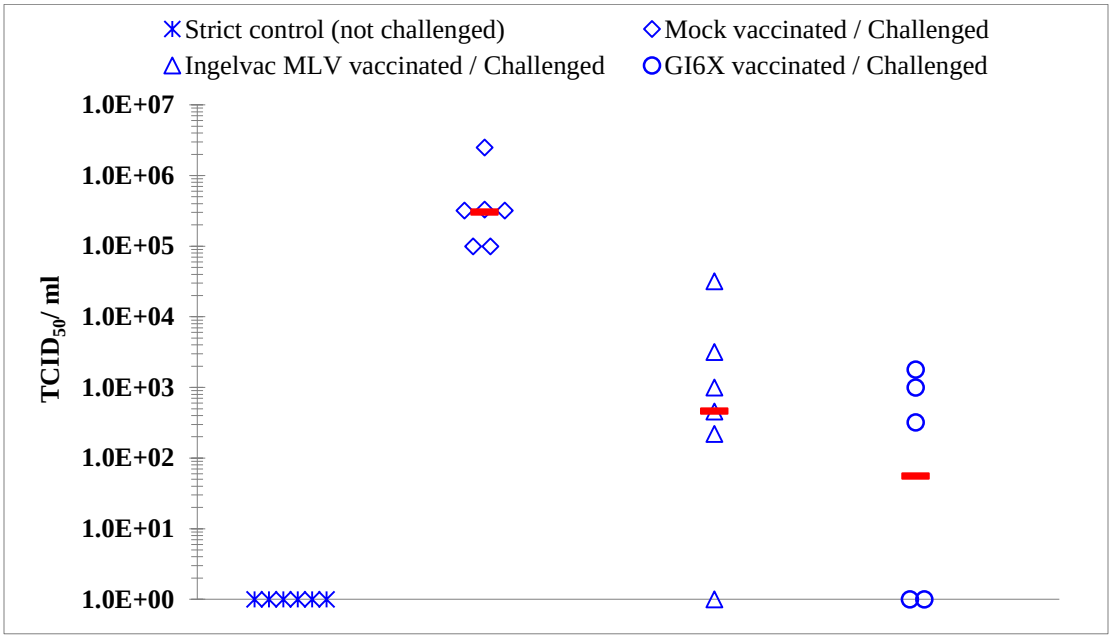

Fig. 6. Virus load in the BAL fluid of pigs 14 days after challenge with virulent PRRS virus. BAL fluid was collected from the lungs of Mock-vaccinated, Ingelvac PRRS MLV-vaccinated or G16X-vaccinated animals at 14 days after challenge with the wild-type PRRSV LTX1. Samples were also obtained at this time from unchallenged and unvaccinated animals (strict controls) (n=6). The virus load in the BAL fluid of each animal was determined by performing infectious virus titrations in ZMAC cells. Results are presented for each individual pig and then the geometric mean for all the members of each group (horizontal red bars).

e. Efficacy of the G16X vaccine in conferring protective immunity to the isolate CCX1, another wild-type PRRS virus of a lineage different (heterologous) from that of the vaccines

A second experiment was conducted to evaluate the cross-protective protective immunity elicited by the G16X vaccine against another lineage 1 virus. The isolate CCX1 was chosen due to the fact that its ORF5 sequence is representative of a number of field virus isolated in 2012 and 2013 that were deemed responsible for several outbreaks in sow farms located in the Midwest. As in the previous study at the time of challenge, there were no differences in the average body weight between the mock-vaccinated and the G16X-vaccinated animals. Thus, exposure of the pigs to the vaccine had no obvious impact on the growth of the animals and had no ill effect on their health as evidenced by the lack of respiratory signs or changes in their attitude after vaccination (data not shown). To measure the level of protective immunity elicited by the vaccine with regards to pig growth, we calculated the % body weight gain for each animal from the day of virulent virus inoculation to 7, 10 and 14 days after the challenge. The pigs in the unchallenged (strict control) group exhibited a steady rate of growth with an average increase of 33% in 14 days (Fig. 7). As compared to the strict control group, the mock-vaccinated and CCX1-challenged pigs exhibited a noticeable decrease in their rate of weight gain. The mean of the weight gain observed in this group of animals (27%) at 14 days after the challenge was significantly lower

($p<0.02$) than the group mean of the strict control (unchallenged) group (Fig. 7). It is worth noting that the impact of the challenge with the CCX1 virus in the rate of weight gain of the animals was not as severe as the negative effect that was observed in the previous experiment upon challenge with the LTX1 virus, in which case resulted in a net body weight loss from 7 to 10 days after challenge (Fig. 4). The effect of the prior immunization of the animals with the G16X vaccine in counteracting the negative effect of CCX1 virus challenge on rate of weight gain became evident during the second week post challenge. At this time the rate of growth of the G16X-vaccinated animals appeared to have a compensatory rate of growth as compared to the mock-vaccinated animals. Consequently, by day 14 pc the calculated rate of weight gain of the G16X-vaccinated pigs was not statistically different ($p>0.05$) from the unchallenged strict controls pigs.

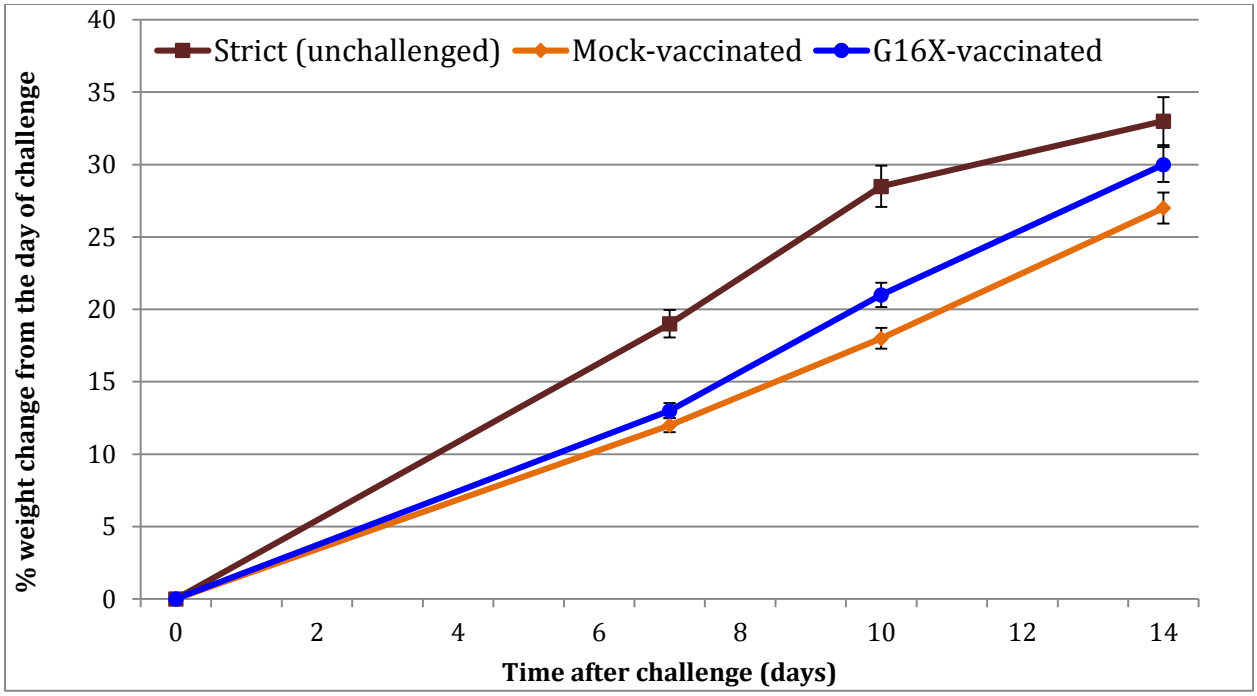

Fig. 7. Weight changes in G16X vaccinated and mock-vaccinated pigs after exposure to the virulent PRRSV isolate CCX1. Mock-vaccinated or G16X-vaccinated pigs (n=6 for each group) were weighed immediately prior to and at 7, 10 and 14 days after challenge with the wild-type PRRS virus isolate CCX1. Unchallenged and unvaccinated animals (strict controls, n=3) were also weighed at these four time points. The changes in BW during the ensuing 7-, 10- and 14-days after challenge were determined on an individual basis and the % weight change relative to its BW at the time of challenge calculated. Results represent the mean % weight change of each group ± SEM.

At the time of challenge (28 days post vaccination) none of the pigs in the trial had a detectable infectious PRRS virus in their serum (Fig. 8). Four days after being challenged with the CCX1 virus, all of the mock-vaccinated animals (6/6) became viremic and remained so until day 10 post-challenge. By 14 days pc, 2 of the 6 (33%) mock-vaccinated pigs were still viremic (Fig. 8). In the G16X-vaccinated pigs a decrease on frequency of the viremia was evident by 7 days post-challenge (Fig. 8). At this time, 2 of the 6 pigs (33%) in the group were no longer viremic, and by 10 days post challenge 4 of 6 pigs (66%) had cleared the virus from their blood stream. By 14 days post-challenge none of the G16X-vaccinated animals were viremic.

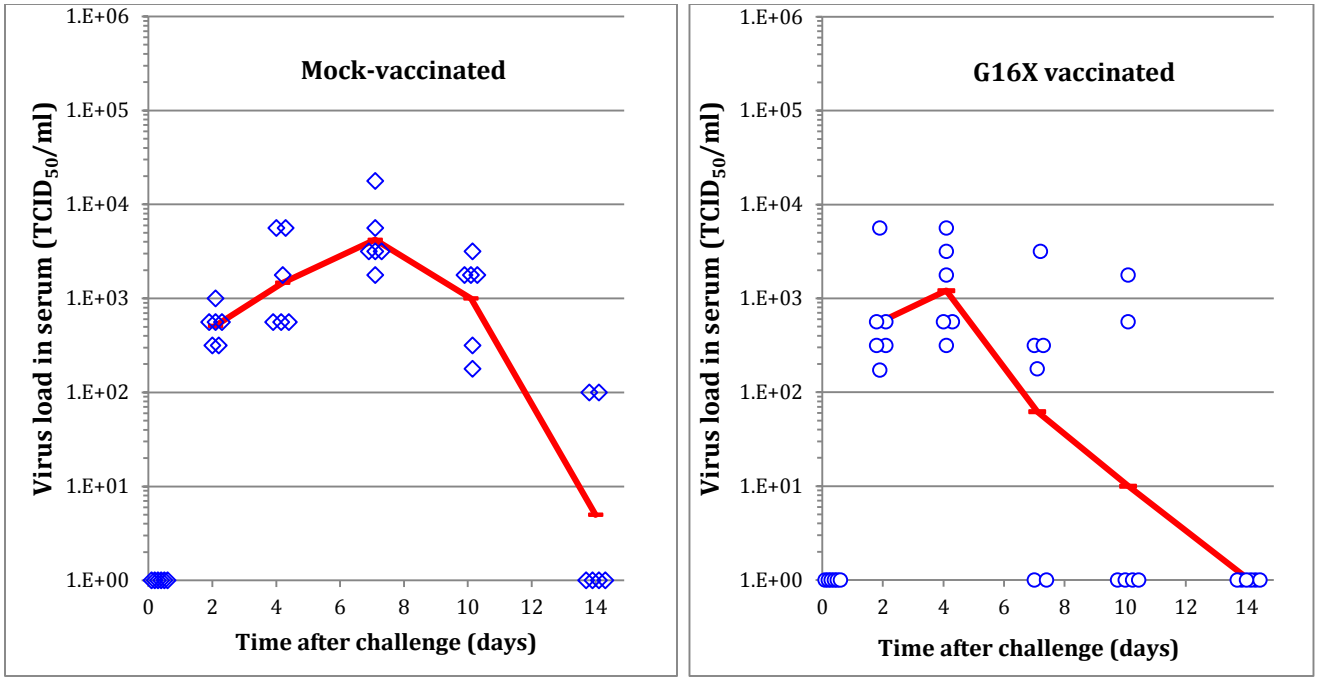

Fig. 8. Extent, frequency and duration of viremia in G16X-vaccinated or mock-vaccinated pigs after inoculation with the PRRS virus isolate CCX1. Serum samples were collected from Mock-vaccinated or G16X-vaccinated animals immediately prior to and at the indicated days after challenge with the wild-type PRRSV CCX1. The virus loads in the sera were determined by performing infectious virus titrations in ZMAC cells. Results are presented for each individual pig (blue markers) as well as the geometric mean for all the members of each group (horizontal red bars). Samples collected from the strict control pigs (n=3) were virus negative at all time points (not shown).

Discussion

The results of this study indicate that different levels of cross-protective immunity were provided by two different type 2 PRRS live virus vaccines of the same lineage (lineage 5), namely the G16X and Ingelvac PRRS MLV. These differences were evidenced by a reduction in the deleterious effect of the infection with a genetically divergent wild-type PRRS virus belonging to a distant lineage (lineage 1) than the vaccines. Evidence of different levels of cross-protection is based on an improvement on the pig's rate of growth, a reduction on the magnitude of the post-challenge viremia, as well as a faster elimination of the challenge virus from the circulation and lung tissue. The fact that both vaccines belong to the same lineage but differ in their ability to provide cross-protective immunity, suggests that differences in the biological properties of these two vaccine viruses are responsible for their disparate abilities to elicit protective immunity. Interferon-α has been shown to play a role in promoting the development of protective anti-viral immunity (Cousens et al., 1997; Cousens et al., 1999; Levy et al., 2003). The fact that the G16X vaccine stimulated a much higher systemic IFN-α response as compared to the response elicited by the Ingelvac PRRS MLV, suggests that this or other related biological properties of the G16X virus, might be contributing to its superior efficacy. In addition, since the G16X virus readily replicates in porcine alveolar macrophages. The ability of the G16X virus to readily replicate in its naturally host was clearly illustrated by the higher level of viremia observed in the G16X vaccinated pigs. The aptitude of the G16X virus to replicate in its natural host without producing disease might very well give this vaccine virus an edge in eliciting protective immunity. Previously, we have demonstrated that the efficacy of a different PRRS live virus vaccine, namely the PrimePac vaccine strain, can be improved by simply allowing this attenuated virus to gain back its ability to replicate in porcine alveolar macrophages.

Notably, the PrimePac srain gained back its ability to efficiently replicate in porcine alveolar macrophages after only three serial passages in ZMAC cells. We observed that the PrimePac vaccine grown in ZMAC cells provided better level of protective immunity as compared to the protection provided by the original PrimePac virus that was instead grown in simian cells (Calzada-Nova et al., 2011). Thus, it seems likely that a combination of the features of the G16X biologic contributed to its superior efficacy. Another major advantage of G16X virus is the likely lack of selection pressure to become fit to grow in its host. The Ingelvac PRRS MLV has been shown to adapt back to grow efficiently in its natutral host, become pneumotropic and revert to virulence (Opriessnig et al., 2001).

The results of this study can be summarized as follows:

1. The inoculation of pigs with wither the G16X or the Ingelvac PRRS MLV vaccines resulted in a viremia that lasted less than 4 weeks. In neither case as the viremia associate with ill-effects. The viremia resulting from the G16X virus was 10-fold higher magnitude than the one resulting from the other vaccine. However, in the case of the G16X virus, the viremia was associated with substantial but transient systemic IFN-α response.
2. Immunization of the animals with either vaccine (G16X or Ingelvac PRRS MLV) 4 weeks before challenge with a genetically divergent wild-type PRRS virus, similarly counteracted the negative effect in pig's growth.
3. Vaccination with G16X resulted on a lower peak viremia at 7 days after challenge and also promoted the faster elimination of from the serum by 10 days post challenge in 80% (4 of 5) of the pigs vaccinated with G16X as compared to only 16% (1 of 6) in the group vaccinated with the Ingelvac PRRS MLV.
4. Vaccination with the G16X resulted in either a complete elimination (40% of cases) or a major (2 orders of magnitude) reduction in the amount of virus present in the lungs of pigs at 14 days after challenge with a genetically divergent (heterologous) wild-type PRRS virus. The Ingelvac PRRS MLV had similar effects but with lesser efficacy.
5. The G16X was able to confer protective immunity against a second representative of type 2 PRRS virus belonging to lineage 1.

It is worth noting that, using viremia and weight gain as the parameters to assess virulence, the CCX1 virus appears to be much less virulent than the LTX1 virus. While the LTX1 virus triggered a major decrease in the rate of the pig's growth, the CCX1 virus had a much lesser impact in this regard. In addition, the LTX1 virus produced a much more intense viremia as compared to the CCX1 virus isolate. While all of the LTX1 animals were viremic at 14 post-challenge (Fig. 5), only 2 of 6 pigs challenged with the CCX1 virus were viremic at this time point (Fig. 8). Thus, the assessment of protective efficacy of a vaccine can be influenced by differences in the clinical outcomes resulting from challenge with different PRRS viruses.

The results of this study indicate that the level of IFN-α response to immunization with a PRRS MLV vaccine can be used as a predictive parameter of the potential effectiveness (potency) of PRRS virus vaccine and that the use of this biological property of this virus as selection criteria for vaccine strain selection will aid in the development of a more effective PRRS virus vaccine. The results of this study support the notion that the efficacy of a vaccine is influenced its biological properties.

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