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Partial Protection in Swine Challenged with Porcine Reproductive and Respiratory Syndrome Virus, Vaccinated Against Classical Swine Fever (PAV-250 Vaccine)

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Abstract: In 2006, a study was carried out to determine whether the attenuated live virus vaccine PAV-250 against Classical Swine Fever (CSF) interacts as a triggering factor in the presentation, pathogenesis and course of the disease caused by the virulent virus of Porcine Reproductive and Respiratory Syndrome (PRRS). Seven Groups (G) were formed and 5 SPF piglets were randomly allocated to each group: G1, negative control; G2, positive control inoculated with PRRSv; G3, vaccinated with PAV-250; G7 and G5, vaccinated with PAV-250 on days-14 and -7, respectively and inoculated with PRRSv on day 0; G8 and G6, inoculated with PRRSv on days -14 and -7, respectively and vaccinated with PAV-250 on day 0. Clinical signs, total white blood cell count by the blood count technique, antibody profile against CSF virus with the ELISA technique and identification of viral nucleic acids by nested RT-PCR were evaluated. Group 6 and 8 showed an increase in clinical signs and groups 5 and 7 did not show apparent clinical signs; body temperature following inoculation increased by 1.5°C on average during 5 days and it subsequently returned to normal ($p>0.005$). Group 5 and 7 showed post-vaccination leukopenia and post-inoculation leukocytosis and post-vaccination and post-inoculation leukopenia was detected in groups 6 and 8 ($p<0.005$). In the antibody profile against CSF in groups 5 and 7, antibody rates of 80 and 86%, respectively, were detected; group 6 and 8 had mean antibody rates of 62 and 60%, respectively ($p<0.001$). When, challenged with CSF virulent Ames strain, these percentages remained constant. Pigs previously vaccinated with the PAV-250 strain did not show clinical and pathological changes when they were infected with the PRRS virus, however, in pigs not vaccinated against CSF and infected with PRRS virus and subsequently with CSF pathogenic virus, the disease worsened, with a 100% mortality rate.

Key words: CSF, vaccination, PAV-250, PRRSv, partial protection, challenged

INTRODUCTION

Swine production is evaluated by its productive efficiency and it may be seriously reduced when health problems occur. Respiratory diseases in swine are of vital importance due to the economic losses they cause. In respiratory problems in swine, bacteria, viruses, parasites and fungi are the main pathogens involved, therefore, they have been designated as porcine respiratory complex. The relationship between different etiologic agents has been demonstrated and virus-virus interactions, virus-bacteria or virus-mycoplasma interactions and bacteria-bacteria interactions are reported (Thacker *et al*., 1999; Iglesias and Trujano, 2000).

The relationship of the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) with other viruses, bacteria and mycoplasmas as part of the Porcine Respiratory Complex (PRC) is currently known. This has originated serious health problems in swine producing regions worldwide (Collins *et al*., 1992) when, PRRSv is associated with another virus, such as the Classical Swine Fever virus (CSFv), it causes severe damages to swine production and to the economy of swine producers, both at national and world level (Kohler, 2002). Classical swine fever is a disease that affects swine and causes severe damages, with clinical presentations that result in production losses (Moennig, 2000; Becher *et al*., 2003; Schirrmeier *et al*., 2004; Heinz *et al*., 2005) and a high

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mortality rate in swine, appearing with necrotic, inflammatory and circulatory lesions (Le Potier *et al*., 2006).

The porcine reproductive and respiratory syndrome virus is characterized by causing an impairment of reproduction in sows, an increased mortality during lactation and pneumonia in fattening swine (Zimmerman *et al*., 2006). It is a highly infectious virus that replicates within the monocyte/macrophage cell line and the lungs are a predominant site of viral multiplication in alveolar macrophages (Albina *et al*., 1998; Thacker *et al*., 1999). The viral replication cycle is fast and the infection causes cell lysis with apoptosis induction in adjacent cells (Benfiel *et al*., 2000).

The PRRS virus has emerged as a serious health problem in many swine producing regions of the world (Shi *et al*., 2007).

In Mexico, PRRS disease appeared in 1994 (Correa *et al*., 1994), probably through swine imports from the United States. Currently, the disease affects most porcine herds. It is a virus that interacts with other etiologic agents, among which mycoplasmas, such as *Mycoplasma hyopneumoniae*, have been reported, as well as bacteria such as *Haemophillus parasuis, Streptococcus suis, Bordetella bronchiseptica* and *Actinobacillus pleuropneumoniae* (Galina *et al*., 1994; Brockmeier *et al*., 2000; Pol *et al*., 1997; Segales *et al*., 1999). It may also, interact with some viruses, such as CSF virus (Dong *et al*., 2006), Aujeszky's disease virus, porcine paramixovirus, porcine parvovirus and porcine circovirus. Upon interacting with these agents, it causes considerable economic losses (Neumann *et al*., 2005), since it increases the morbidity and mortality rate, as well as the presence of severe respiratory signs in growing-fattening swine, with a low food conversion. This leads to a longer time for the affected swine to come out into the market. Also, in breeding sows, various reproductive disorders occur. In order to control this disease, several biosafety mechanisms have been applied in most farms in the country, however, these measures have not yielded the expected results and the control of this virus is still a problem. This causes significant losses in technified swine production and to a lesser degree in courtyard swine production (Gonzalez *et al*., 2001).

A national campaign was carried out in Mexico for the control and eradication of CSF, according to Mexican Official Standard NOM-037-ZOO-1995 (Norma Oficial Mexicana NOM-037-ZOO-1995). This campaign was mainly based on vaccination, using the PAV-250 strain for disease control, followed by a period designated as eradication phase. In this phase, herds were not vaccinated; however, if a focus of infection occurred, quarantine and slaughter of the infected swine were carried out in the outbreaks located in the eradication zone (Gonzalez *et al*., 2001). Finally, the herds from each state of the country enter into a period called CSF-free phase.

The national control and eradication campaign is important, since it specifies the corrective management of the disease. In case this disease occurs by infectious outbreaks or foci in courtyard swine production, all herds located within a 2 km average radius would be seriously affected by the great economic losses it represents due to its high morbidity and mortality and/or association with other infectious agents, with growth delay and poor conversion in growing and developing animals (SAGARPA, 2008). During the vaccination control phase, clinical signs suggesting PRRS disease were reported to have been observed in recent years when the PAV-250 vaccine was used against CSF in herds subjected to disease control process.

In a study performed in 5 farms in La Piedad, Michoacan, Mexico, clinical respiratory manifestations occurred when weaned piglets seropositive to PRRS virus were vaccinated with the PAV-250 strain against CSF and an interaction with this virus was attributed (Pineda *et al*., 2002). Currently, there are publications that describe an increase of respiratory clinical signs and mortality, as well as growth delay in weaned piglets in farms where herds affected by the PRRS virus were vaccinated against CSF Chinese strain (Mogollon *et al*., 2006). This may lead to CSF disease outbreaks, due to an inadequate immunological protection of herds caused by a low antibody concentration in piglets vaccinated under these conditions (Li and Yang, 2003). These clinical presentations observed in swine from the affected herds have a strong impact on production and on producers' economy.

Considering the foregoing, we judged it important to carry out a study to understand the clinical, pathological and molecular changes that occur when swine are vaccinated with CSF vaccinal strain PAV-250, in presence and absence of the porcine reproductive and respiratory syndrome virus and to evaluate whether clinical cases suggesting disease caused by the PRRS virus occur in the following days. It was also, considered important to determine the changes that occur in leukocytic cells, as well as the behavior of antibodies against CSF in presence of the PRRS virus (Thacker *et al*., 1999) to determine if the same happens in presence or absence of the CSFv PAV-250 strain vaccine and to learn, through challenge with virulent Ames strain of CSF virus, whether the humoral immune response (evaluated by ELISA) of vaccination against classical swine fever in presence of the porcine reproductive and respiratory syndrome virus is affected

before and after vaccination against CSF; to verify the presence of CSF virus and PRRS virus nucleic acids during the experiment, by means of nested RT-PCR from blood samples periodically collected from all piglets in the experimental groups, in order to determine whether the attenuated live virus vaccine PAV-250 against Classical Swine Fever (CSF) potentiates or provides protection against the disease caused by the virulent virus of Porcine Reproductive and Respiratory Syndrome (PRRS). This study was carried out to determine whether clinical signs are increased and/or reduced, to determine whether leukocyte counts are altered and whether the levels of antibodies against CSF are influenced by the PRSS virus and if the protection conferred by the PAV-250 vaccine against classical swine fever is affected.

MATERIALS AND METHODS

Pathogenic PRRS virus production: African green monkey kidney cells, known as MA-104, were seeded into 25 cm^2 Falcon dishes, to which 5 mL of growth medium were added (EMEM plus 10% SFB) and they were subsequently incubated at 37°C for 48 h or until an 80% confluence was reached (Kim *et al*., 1993).

Pigs: Thirty five Specific Pathogen Free (SPF) piglets from a crossbreed of Yorkshire, Landrace and Hampshire breeds, of 21 days of age, acquired from a commercial farm, free of viruses and antibodies against CSF and PRRS and born from sows free from these agents were used. This study was carried out at the Virology Laboratory and at the isolation units of the CENID-MA of the National Institute of Forest, Agricultural and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, INIFAP) and at the Virology Laboratory of the Faculty of Higher Studies-Cuautitlan, National Autonomous University of Mexico (Facultad de Estudios Superiores de Cuautitlan, FES-C de la Universidad Nacional Autonoma de Mexico, UNAM).

Piglet inoculation with PRRS virus: Piglets were inoculated with PRRS virus at a $CCID_{50}$ (Cell Culture Infective Dose) 10^4 fluorescent focii of the reference strain (ATCC No. 2332), with 2 mL pig^{-1} by Intramuscular (IM) route. For pig vaccination against CSF, the vaccinal strain PAV-250 at a 2.0 mL dose was intramuscularly administered using one needle per pig. In the negative control group, a placebo consisting of 2 mL of sterile Physiological Saline Solution (PSS) was administered by intramuscular route. Challenge with the vaccine under controlled conditions was carried out in order to determine the efficacy of vaccinal strain PAV-250 against CSF in presence of PRRS virus. On day 30 of this experiment, all piglets from all groups were challenged with 10^2 LD50 mL^{-1} of CSFv virulent Ames strain.

Table 1: Design and composition of the 7 experimental groups

		Vaccination against CSF with PAV-250 strain and inoculation with PRRSv		
Groups*	**Treatments	-14 days	-7 days	0 days
A	Negative control			
B	Control PRRSv			++
C	Control			
	CSF vaccine			+
D	CSF vaccine	++		
	PRRSv			+++
E	CSF vaccine		++	
	PRRSv			+++
F	PRRSv	+++		
	CSF vaccine			++
G	PRRSv		+++	
	CSF vaccine			++

*Each group with 5 SPF piglets of 21 days of age, **10 mL of blood were collected from each pig in all groups on days -14, -9, -7, -2, 0, 5, 7, 14, 21, 28, 35, 37, 44 and 51

Experimental design: Seven groups of 5 SPF piglets each were formed. Piglets were randomly allocated to each group, where treatments with the CSF PAV-250 vaccine strain and PRRS virus were as follows (Table 1):

Group A: Control group, only 2 mL of physiological saline solution were administered on day 0.

Group B: Inoculated with PRRS virus only, on day 0.

Group C: Vaccinated with 2 mL of vaccinal virus against CSF on day 0.

Group D: Vaccinated with 2 mL of vaccinal virus against CSF on day -14 and inoculated with the PRRS virus on day 0.

Group E: Vaccinated with 2 mL of vaccinal virus against CSF on day -7 and inoculated with the PRRS virus on day 0.

Group F: Inoculated with PRRS virus on day -14 and vaccinated with 2 mL of CSF vaccinal virus on day 0.

Group G: Inoculated with PRRS virus on day-7 and vaccinated with 2 mL of CSF vaccinal virus on day 0.

Evaluated parameters

Clinical evaluation: Piglets were clinically observed every day in the morning, during the first hour prior to management. Afterwards, during management, the

ingested amount of food, the increase in respiratory rate, piglet prostration, hair appearance and the presence of circulatory changes on the face (ears, oro-nasal region, etc.) were observed. The observations were recorded and were subsequently described and analyzed.

Serology: One 5 mL blood sample per piglet was collected directly from the jugular vein of all piglets in the experiment on days -14, -9, -7, -2, 0, 5, 7, 14, 21, 28, 35, 37 and 44. Sera were used to measure the antibody profile against CSF vaccinal virus by the commercially available ELISA Herdchek CSF test (IDEXX Laboratories), using the methodology described by the company. A blocking percentage greater than, or equal to, 40% was considered positive and a sample was considered negative (without antibodies) if its blocking percentage was lower than, or equal to, 30% and uncertain if the range was between 30 and 40%.

Total leukocyte count: A 5 mL blood sample was collected directly from the jugular vein in Ethylene Diaminete Traacetic Acid (EDTA) containing tubes on days -14, -9, -7, -2, 0, 5, 7, 14, 21, 28, 35, 37 and 44 from all piglets in the experiment, for the purpose of performing a total leukocyte count by the blood count technique. Total leukocyte count was considered normal when it was within a range between 11,000 and 22,000 white blood cells mm^{-3}, with a mean of 18,000 leukocytes mm^{-3} (Taylor, 1999).

RT-PCR test: A 1 mL blood samples, which were considered as baseline samples, were periodically collected directly from the jugular vein in EDTA-containing tubes starting from day -14 and subsequently on days 0, 7, 14 and 28 in order to process them and to demonstrate, by the nested RT-PCR technique, the presence or absence of classical swine fever virus (PAV-250) and porcine reproductive and respiratory syndrome virus nucleic acids.

Statistical analysis: The data obtained from the different study treatments were evaluated by means of an Analysis of Variance (ANOVA) to determine the differences between group means with a 95% confidence. For the significance analysis, group means were compared by the Least Significant Difference (LSD) method in order to establish significant differences. The SPSS computer program was used for this procedure to determine whether a statistically significant difference existed between the pig groups treated.

RESULTS

Clinical signs: Piglets in groups A and C did not show apparent clinical signs in the first 30 days. Piglets in Group B, inoculated with PRRSv on day 0, showed moderate clinical signs of anorexia, apathy, prostration, hirsute hair and hyperthermia from 5-12 day; piglets in groups D and E, vaccinated with classical swine fever vaccinal strain PAV-250 on days -14 and -7, respectively, did not show apparent clinical signs when they were exposed to PRRSv on day 0; they showed mild clinical signs of anorexia, apathy, prostration and slight hyperthermia from day 5 to day 12; Groups F and G, challenged with PRRSv on days -14 and -7, respectively and vaccinated with PAV-250 strain on day 0, showed moderate clinical signs of anorexia, apathy, prostration, hirsute hair, sadness, weakness, conjunctivitis, dyspnea, polydipsia, cyanosis of the ears and the oro-nasal region and hyperthermia; these signs persisted for 7 days, after which they disappeared gradually and only growth reduction was observed until the day of slaughter (day 52). Piglets in Group A, negative control, died at 18 days following challenge with CSFv and piglets in Group B, positive control, inoculated with PRRS virus only, died between days 9 and 11 following challenge with CSFv, respectively and it was observed that in Group B, inoculated with the PRRS virus, 80% of pigs died at 9 days and 20% of these died at 11 days following challenge with CSFv and in Group A, negative control without vaccination, 3 pigs died at 16 days and 2 pigs died at 18 days postchallenge. However, groups C-G showed a slight increase in temperature, anorexia and polydipsia within 5 days postchallenge; afterwards they recovered clinically and remained alive until the end of the experiment at 52 days, when they were slaughtered (Table 2).

Body temperature: The following results were obtained for mean body temperature in piglets. Group A, negative control, did not show an increase in body temperature. Group B, inoculated with PRRSv, had a mean temperature of 40°C. Group C, vaccinated with CSF vaccinal strain PAV-250 only, did not show changes in body temperature, with a mean body temperature of 39.2°C. Groups D and E, vaccinated with CSF virus PAV-250 strain on days -14 and -7, respectively and subsequently on day 0, were inoculated with PRRSv and they had a mean temperature of 39.23 and 39.35°C, respectively and Groups F and G, inoculated with PRRSv on day -14 and day -7 and vaccinated with CSF virus PAV-250 strain, had

Table 2: Clinical signs following application of the respective treatments

Groups/pigs	**Treatments	Clinical signs, days after treatment
A/5	Negative control	No apparent clinical signs
B/5	Control PRRSv	Moderate: anorexia, apathy, prostration, hirsute hair, hyperthermia from 5-12 days
C/5	Control	
	CSF vaccine	No apparent clinical signs
D/5	CSF vaccine	
	Day -14	No apparent clinical signs
	PRRSv day 0	Slight: anorexia, apathy, prostration, hirsute hair, from 5-12 days
E/5	CSF vaccine day -7	No apparent clinical signs
	PRRSv day 0	Slight: anorexia, apathy, prostration, hirsute hair, from 5-12 days
F/5	PRRSv day -14	Slight: anorexia, apathy, prostration, hirsute hair, from 5-12 days
	CSF vaccine, day 0	Moderate: anorexia, apathy, prostration, hirsute hair, sadness, weakness, conjunctivitis, dyspnea, polydipsia, cyanosis of the ears and the oro-nasal region, slight hyperthermia from day 5-12 days
G/5	PRRSv day -7	Slight: anorexia, apathy, prostration, hirsute hair.
	CSF vaccine, day 0	Moderate: anorexia, apathy, prostration, hirsute hair, sadness, weakness, conjunctivitis, dyspnea, polydipsia, cyanosis of the ears and the oro-nasal region, slight hyperthermia from 5-12 days

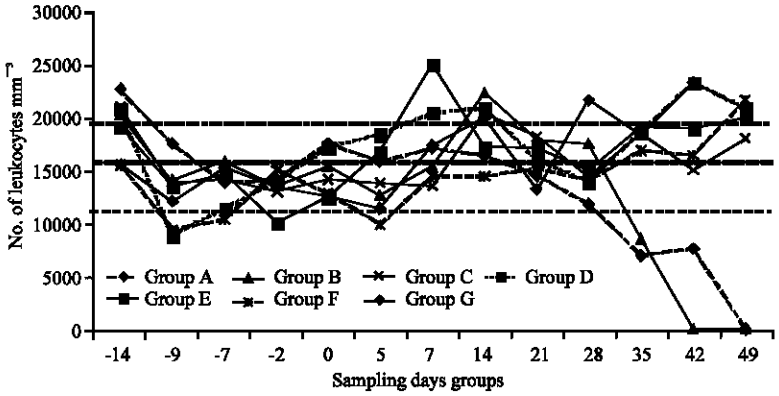

Fig. 1: Mean leukocyte counts found in the 7 groups. The results observed in the present study for total leukocyte count were highly significant ($p<0.001$). Values with different letters (superscript) show significance ($p< 0.001$). Median value of groups 15,332.417, SD ±3909.588, A^{ab}, B^{a}, C^{bcd}, D^{d}, E^{cd}, F^{abc}, G^{cd}. Challenge with Ames strain day 30

mean temperatures of 39.35 and 39.42°C, respectively and in the statistical analysis performed, there were no statistically significant differences ($p>0.05$) in values (Fig. 1).

Leukocyte count evaluation: Group A, noninoculated control, remained within the normal range for mean leukocyte count (11,000-22,000 leukocytes mm^{-3}) until day 30 of the experiment. But in sampling 11, one week after challenge with CSFv Ames strain, leukopenia occurred with a mean of 6,970 leukocytes mm^{-3} and in the sampling performed on day 14 postchallenge, piglets with leukopenia (mean leukocyte count 7,630 leukocytes mm^{-3}) remained alive and they eventually died within 4 days following this last sampling.

Group B, inoculated only with PRRSv on day 0, showed slight leukocytosis at postinoculation day 14, of 22,360 leukocytes mm^{-3} and after challenge with Ames strain of classical swine fever virus, performed on day 30, leukopenia (mean leukocyte count 8,538 leukocytes mm^{-3}) was seen at 5 days postchallenge; piglets in this group eventually died. In Group C, vaccinated with PAV-250 strain against CSF, mean leukocyte counts between 13,930 and 20,340 leukocytes mm^{-3} were always maintained.

In Group D, vaccinated against CSF on day -14 and inoculated with PRRSv on day 0, leukopenia with a mean leukocyte count of 8,870 leukocytes mm^{-3} was observed at 5 days following administration of the vaccine against CSF; normal parameters were subsequently recovered and at day 0, when PRRSv was inoculated, mean levels were 20,510 leukocytes mm^{-3} and an increase in total leukocyte count from 17,220-20,290 was seen in the subsequent samplings. At 7 days after challenge with virulent Ames strain of CSFv, the mean leukocyte count was 18,730-23,360, showing slight leukocytosis.

In Group E, vaccinated against CSF on day -7 and inoculated with PRRSv on day 0, pigs showed slight leukopenia with a mean leukocyte count of 10,075 leukocytes mm^{-3} at 7 days when the vaccine against CSF

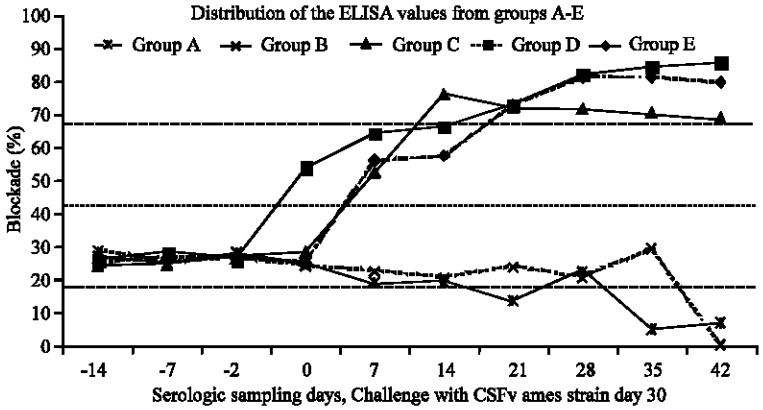

Fig. 2: Level of antibodies against classical swine fever virus determined by blockade ELISA (*). Groups A^a, B^a, C^d, D^e, E^d. Values of this variable with different letters show significance ($p<0.001$). (*) Serum was considered positive with a blockade percentage of 40% or higher. Median value of groups 40.881. SD ±21.486

was administered and when piglets were inoculated with PRRSv on day 0, they had leukocytosis with a mean of 25,100 leukocytes mm^{-2} at 7 days. In subsequent evaluations, it persisted within a range between 15,070 and 17,130 leukocytes mm^{-3}, but starting from seven days after challenge with Ames strain of CSF pathogenic virus, an increase in the normal count from 19,010-20,050 leukocytes mm^{-3} occurred again until the end of the experiment.

In Group F, inoculated with PRRSv on day -14 and vaccinated against CSF on day 0, piglets showed leukopenia with a mean of 9,400 leukocytes mm^{-3} at 5 days after inoculation with PRRSv. Afterwards, when piglets were vaccinated against CSF, they showed leukopenia again with a mean of 9,9000 leukocytes mm^{-3} at 5 days post vaccination and at 7 days, leukocyte levels were recovered with a mean of 14,490 leukocytes mm^{-3}. However, at 7 days following day 30 of the experiment (which corresponded to the day of challenge with Ames strain of CSFv), a progressive increase from 14,120-21,710 leukocytes mm^{-3} was seen until the end of the experiment.

Group G, inoculated with PRRSv on day -7, showed a reduction in leukocyte count from 15,300-12,600 leukocytes mm^{-3} and after administration of the vaccine against CSF on day 0, the leukocyte count decreased again to 11,460 leukocytes mm^{-3} and afterwards it remained between 13,000 and 20,000 leukocytes mm^{-3}. Upon challenge with virulent Ames strain, there was an average increase of 23,360 leukocytes mm^{-3} at 7 days postchallenge (Fig. 2).

Level of antibodies against classical swine fever: Antibodies were detected starting from day 14 post vaccination against CSF in the serologic evaluation of all piglets from the groups vaccinated with classical swine fever PAV-250 strain.

Groups A and B, negative control group and group infected with the PRRS virus, respectively, had a negative response. Group C had a 52.4% positive response starting from day 7 post vaccination and by day 14 it had a 76% blocking percentage and remained constant beyond challenge with Ames strain of CSF virus 70%. Groups D and E, vaccinated against classical swine fever at days -14 and -7, respectively, had a positive response at 7 days post vaccination, with 64 and 56%, respectively, when they were inoculated with the PRRS virus and it did not cause any reduction in the immune response. However, in the following 14 days, both groups remained within a range between 85 and 80% and when they were challenged with virulent Ames strain of classical swine fever, which did not affect them, they remained constant within that percentage. Groups F and G, inoculated with PRRS virus at day -14 and day -7, respectively, which were vaccinated with classical swine fever vaccinal strain PAV-250 at day 0, had a negative result at 7 days post vaccination, of 27.8 and 23.5%, respectively and a positive serologic response of 60 and 62%, respectively, was only detected at 21 days post vaccination.

Following challenge with virulent Ames strain, they had a 79 and 77% increase, respectively. The results observed in the antibody level for the 7 groups treated showed highly significant statistical differences ($p<0.001$) (Fig. 3).

Viral identification by RT-PCR: The presence of classical swine fever virus and porcine reproductive and respiratory syndrome virus was determined by the nested RT-PCR molecular technique, with the following results.

The results obtained to demonstrate the presence of nucleic acids of classical swine fever vaccinal strain PAV-250 were negative in all groups tested, from Group A

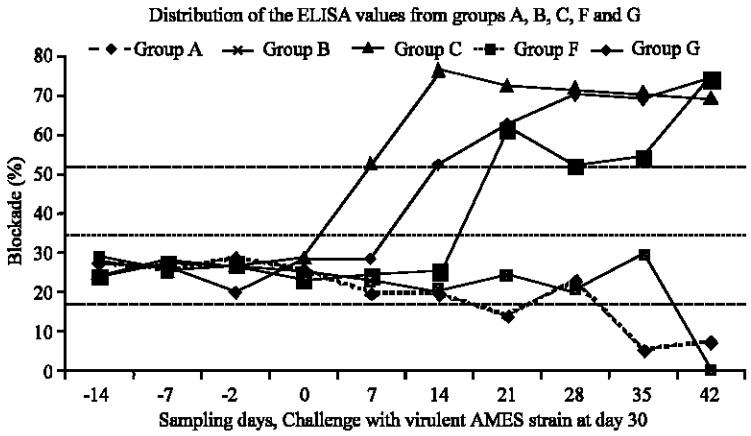

Fig. 3: Level of antibodies against classical swine fever virus determined by blockade ELISA (*). Groups A^a, B^a, C^d, F^b, G^c. Values of this variable with different letters show significance ($p<0.001$). (*) Serum was considered positive with a blockade percentage of 40% or higher. Median value of groups 35.321. SD ±16.425

to Group G. The following results confirmed the presence of PRRS by nested RT-PCR: negative results were obtained for Group A and C, which were the control group and the group vaccinated with the CSF vaccine, respectively. Groups B, D, E, F and G, which were all inoculated with the PRRS virus according to the experimental design, had positive results.

DISCUSSION

Piglets infected with the PRRS virus show pathological changes in their organs, tissues and cells. This is reflected by the presence of clinical signs that modify their behavior for an optimal production, manifesting a chronic disease, a situation that worsens when this virus interacts with the classical swine fever virus, with a greater intensity of clinical manifestations being observed as a consequence of the damage caused by these viruses to the cell immune system. They both replicate in alveolar and intravascular macrophages, which compromises the respiratory system's defense (Fraile *et al*., 2008; Suradhat *et al*., 2006).

In the present study, mild to moderate clinical signs were observed, such as anorexia, apathy, prostration, hirsute hair, sadness, weakness, conjunctivitis, dyspnea, slight polydipsia, cyanosis of the ears and the oro-nasal region, which are similar to those reported (Thacker *et al*., 1999; Pineda *et al*., 2002) for mixed infections by PRRS and its interactions. Groups A and C, which were the control group and the group vaccinated only with CSF vaccinal strain PAV-250, respectively, did not show apparent clinical signs. Groups D and E, vaccinated with PAV-250 strain on days -14 and -7, respectively, did not show clinical signs after vaccination.

Groups B, D, E, F and G, in which the PRRS virus was inoculated, showed mild to moderate anorexia, apathy, prostration and hirsute hair; only the piglets in Groups F and G showed mild signs of anorexia, apathy, prostration and hirsute hair. When the CSF PAV-250 strain vaccine was administered to these piglets on day 0, they showed more marked signs of anorexia, apathy, prostration and hirsute hair and they also manifested clinical respiratory signs, such as dyspnea, polydipsia and cough, accompanied by sadness, weakness, conjunctivitis and cyanosis of the ears and the oro-nasal region; these clinical signs persisted for 5 days and then gradually disappeared. In the more affected piglets (60%), weight loss and growth reduction were observed until the time of slaughter. This is probably due to the fact that the PRRS virus initially infects cells in the intravascular and alveolar monocyte/macrophage cell line, where the virus multiplies itself and causes damage (Molitor *et al*., 1997; Thanawongnuwech *et al*., 1997, 1998). The vaccine against classical swine fever is subsequently administered. This virus will use the remaining macrophages for replication (Mogollon *et al*., 2006). This leads to a marked increase in clinical signs and to pathological changes in these piglets.

The results showed mean total leukocyte counts in this experiment and a slight mean leukopenia was found at 5 days in the groups to which the classical swine fever vaccine was administered and also, in the pigs from the groups inoculated with the porcine reproductive and respiratory syndrome virus. Similar results were reported (Nielsen and Botner, 1997; Feng *et al*., 2001; Ramirez *et al*., 2008), who mention leukopenia from 3-7 days post inoculation. It was observed that groups D and E, vaccinated with the CSF strain on days -14 and -7 and

inoculated with PRRSv on day 0, showed a mean leukopenia of 8,870 and 10,075 leukocytes mm^{-3}, respectively, at 5 days following vaccination with CSF, but when they were inoculated with the PRRS virus on day 0, they showed a mean leukocytosis of 12,490-25,100 leukocytes mm^{-3} in Group E and an increase from 17,220 (day 0)-20,920 (day 21) leukocytes/mm^3 in Group D at 7 days.

In groups F and G, inoculated with PRRSv on days -14 and -7, respectively and vaccinated with CSF vaccinal strain PAV-250 on day 0, 4 pigs in Group 8 showed leukopenia at 5 days following inoculation with PRRSv, with a mean leukocyte count of 9,400 leukocytes mm^3 and one pig in group 6 had slight leukopenia at 5 days following inoculation with PRRSv and when they were vaccinated with CSF vaccinal strain PAV-250, they again showed a slight reduction: from 12,600-11,400 leukocytes mm^{-3} in Group G and a reduction in leukopenia in Group F, from 12,845-9,900 leukocytes mm^{-3} on average. Afterwards, following challenge of piglets with Ames strain of CSF pathogenic virus, piglets in Group F showed an increase in the number of leukocytes, which was somewhat higher than the normal range, since it increased from 14490-21710 leukocytes mm^{-3} on average and in Group G it remained normal. The increase in the number of leukocytes after leukopenia and following challenge with CSFv Ames strain promotes piglet recovery due to the increase in leukocytes (Diosdado *et al*., 2000). In this research, it is demonstrated that inoculation with the PRRS virus and subsequent vaccination with CSFv PAV-250 strain induce white blood cell damage in pigs, which probably causes a significant reduction in the phagocytic effect, as well as a negative effect on the immune response. This demonstrates the potential clinical and pathological changes that cause losses in swine production.

The presence of nucleic acids of classical swine fever virus and of porcine reproductive and respiratory syndrome virus was determined by the nested RT-PCR molecular technique. In the evaluation by this molecular technique in this study, all groups tested had a negative result for the presence of CSF virus nucleic acids. The results which demonstrated the presence of PRRS by nested RT-PCR were: Groups A and C, which were the negative Control Group and the Group vaccinated with the CSF vaccine, respectively, had negative results; and groups B, D, E, F and G, which were all inoculated with the PRRS virus according to the experimental design, had a positive result.

Control Group A and B, infected with the PRRS virus, had negative results. In the first analysis, they showed a 19 and 22% blockade, respectively, which persisted throughout the experiment in the 10 samplings analyzed by blockade ELISA. These piglets died after challenge with virulent Ames strain between 7 and 20 days postchallenge and 80% of piglets in Group B, inoculated only with the PRRS virus, died on day 6 and the other 20% died on day 7 post inoculation, but piglets in Control Group A died at 14 and 20 days postchallenge with virulent Ames strain.

Group C, vaccinated with CSF vaccinal strain PAV-250, had a positive serologic response starting from day 7 post vaccination, with a 52.4% blockade and remained constant with a 76% blockade, approximately, since day 21 until after challenge with CSF virulent AMES strain.

Groups D and E, vaccinated against classical swine fever on days -14 and -7, respectively, had a similar behavior, with a positive antibody response at 7 days post vaccination and a 56 and 53% blockade, respectively and inoculation with PRRS virus did not cause antibody reductions, but in the following 14 days, both groups remained within an 82-85% blockade range and were not affected when challenged with classical swine fever virulent Ames strain and remained constant within this percentage.

Groups F and G, inoculated with PRRS virus on days -14 and -7, respectively and subsequently vaccinated with CSF vaccinal strain PAV-250 on day 0, had a positive result at 21 days post vaccination, with a 62 and 60% blockade, respectively. Following challenge with virulent Ames strain, they had an increase, reaching an 84 and 79% blockade, respectively.

The present study demonstrates that piglets with primary infection with porcine reproductive and respiratory syndrome virus, before and after vaccination with vaccinal strain PAV-250 against classical swine fever, did not have a significant decrease in the serologic response against this vaccinal strain. The results of the present study also demonstrated that piglets vaccinated with PAV-250 and challenged with virulent Ames strain did not have clinical signs or deaths within 22 days following challenge, contrary to the observations made by other investigators, who mention in their studies that PRRS virus infection causes a supression of antibody response upon vaccination against classical swine fever virus with the Chinese strain or with the modified live virus vaccine, produced in cell culture or lapinized (Pineda *et al*., 2002; Li and Yang, 2003; Mogollon *et al*., 2006).

Piglets from groups D and E, which were initially vaccinated with CSF vaccinal strain: PAV-250 and were subsequently challenged with PRRSv, showed a greater antibody percentage compared with Groups F and G,

which were first infected with PRRSv and were subsequently vaccinated with CSF vaccinal strain PAV-250, due to the possibility that they may contain epitopes in common.

A potentiation of CSF virus infection was observed in piglets in Group B, which were inoculated with the PRRS virus and were subsequently challenged with virulent Ames stratin at study day 30; a similar investigation was reported by Depner *et al*. (1999).

A document published by Suradhat *et al*. (2006) mentions the immunomodulating properties of PRRSv when it interacts with other viruses and inhibits the immune response in pigs when the vaccine against CSF was administered. Piglets already infected with PRRSv showed failure in protective antibody production (Li and Yang, 2003), contrary to the observations reported in this study, in which no failure in antibody production occurred. This is probably due to vaccination with a strain different from that used in this study, as well as to the characteristics of classical swine fever vaccinal strain PAV-250.

CONCLUSION

Piglets infected with PRRS virus and subsequently vaccinated with vaccinal strain PAV-250 against CSF, show clinical and respiratory changes, as well as slight leukopenia in total leukocyte count. The protective antibody response was not affected and piglets showed a minimum percentage of macroscopic lung lesions, on the other hand, the lung lesion percentage was high, when piglets were not vaccinated with this vaccinal strain and were challenged with the pathogenic CSF virus.

Eighty percent of piglets vaccinated with the PAV-250 strain had post vaccination leukopenia and those that were subsequently inoculated with the PRRS virus had slight leukocytosis post inoculation and afterwards, when challenged with virulent Ames strain, they had another increase in leukocyte count.

Piglets inoculated with PRRSv on day -7, that were vaccinated with PAV-250 strain on day 0, had only slight leukopenia, with 9050 and 7950 white blood cells per mm^3 post vaccination and there were no marked changes following challenge with CSFv.

Piglets that were first inoculated with the PRRS virus (on days -14 and -7) showed leukopenia and when they were vaccinated with the PAV-250 strain, they showed slight leukopenia again. Likewise, leukocyte count was not reduced following challenge with virulent Ames strain.

Piglets vaccinated with the PAV-250 strain before or after inoculation with PRRS virus and challenged with virulent Ames strain, had minimum percentages (3%) of lung lesions, in contrast with vaccinated controls, which reached up to 18%. The piglets that were not vaccinated against CSF, which were subsequently infected with the PRRS virus and were then exposed to virulent Ames strain showed a high percentage of lung lesions (60%).

Based on the data obtained in this study, it was demonstrated that infection with the PRRS virus in SPF piglets before or after vaccination with PAV-250 strain against CSF did not affect the antibody response stimulated by the vaccinal strain and when challenged with virulent Ames strain, they had a 0% morbidity and mortality.

In control pigs without antibodies against classical swine fever that were infected with PRRS virus, CSF clinical and pathological changes were potentiated upon infection with virulent CSF strain and they died within an average of 6-7 days postinfection.

Piglets not vaccinated with the PAV-250 strain and infected with 10^2 of CSF Ames strain could be kept alive for up to 22 days.

In SPF pigs, it was demonstrated that the vaccine against classical swine fever, PAV-250 strain, is an adequate, good quality and reliable vaccine to continue to be used in the control and eradication of CSF in enzootic zones, even in presence of porcine reproductive and respiratory syndrome virus.

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