Classical Swine Fever: humoral neutralizing antibody induced by a live attenuated vaccine

T.R.P. FREITAS1*, L.A. CALDAS2, E.G. ESTEVES1, A.C.S. DUARTE3, M.A. REBELLO2

1Laboratório Nacional Agropecuário – Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Avenida Rômulo Joviano, s/n Box: 50, Postal Code: 33600-000 – Pedro Leopoldo, Minas Gerais, BRAZIL.
2Laboratory of Molecular Virology II, Instituto de Microbiologia Professor Paulo de Góes – Centro de Ciências da Saúde – Bloco I. Universidade Federal do Rio de Janeiro, UFRJ, 21 941 590 Rio de Janeiro, BRAZIL.

*Corresponding author: taniafrei@hotmail.com

SUMMARY

Classical Swine Fever (CSF) is a highly contagious viral disease of domestic and wild swine caused by a Pestivirus (Flaviviridae) which is controlled in many countries by vaccination with CSF attenuated vaccines. The present study investigated the induction of neutralizing vaccine antibodies against CSF Virus (CSFV) after a virus challenge by the virulent CSFV A-19 strain (2.105 TCID50) on 34 young (2 month old) pigs in the first assay or a vaccine booster on 7 pigs in the second assay at the 22nd day post vaccination whereas unvaccinated pigs served as negative controls. Serum samples were collected weekly from 0 to 42 days post vaccination (dpv) for antibody measurement with the neutralization peroxidase linked assay (NPLA). Although the neutralizing anti CSFV antibody production remained weak before the virulent virus challenge (on days 14 and 21), all vaccinated animals survived and exhibited no clinical sign of the disease. By contrast, the antibody titre increased since the 28th day, reached maximal values at 42 dpv in the 2 assays. Furthermore, the vaccinated pigs presented an antibody titre exceeding the positive threshold fixed to 1:50 at 28 dpv and all animals were sero-positive at 35 and 42 dpv. Consequently, the measurement of serum neutralizing antibodies coupled to the determination of a positive threshold indicating efficient protection may be an alternative way to evaluate the CSF vaccine potency without virus challenge.

Keywords: Classical Swine Fever, neutralizing antibody, vaccine antibody.

RÉSUMÉ

Peste porcine classique : anticorps humoraux neutralisants induits par un vaccin vivant atténué

La peste porcine classique (PPC) est une maladie virale hautement contagieuse des porcs domestiques et sauvages provoquée par un Pestivirus (Flaviviridae) dont le contrôle repose dans beaucoup de pays sur la vaccination par des vaccins atténués. Au cours de cette étude, l’évolution des anticorps neutralisants vaccinaux a été suivie après une épreuve virale par la souche virulente CSFV A-19 (2.105 TCID50) sur 34 jeunes porcs (âgés de 2 mois) lors du 1er essai ou après un rappel vaccinal sur 7 animaux dans le 2ème essai réalisés le 22ème jour après la vaccination ; en parallèle, des animaux non vaccinés ont servi de contrôles négatifs. Les anticorps sériques neutralisants ont été mesurés toutes les semaines, de 0 à 42 jours après la vaccination, par une méthode immunoenzymatique. Bien que la production d’anticorps anti-CSFV soit restée faible avant l’épreuve virale (les 14 et 21ème jours), tous les animaux vaccinés ont survécu et n’ont présenté aucun symptôme. En revanche, le titre en anticorps a augmenté dès le 28ème jour pour atteindre des valeurs maximales le 42ème jour au cours des 2 essais. En outre, les porcs ont présenté un titre en anticorps supérieur au seuil fixé de 1 : 50 à J28 et tous les animaux étaient séropositifs à J35 et J42. Ainsi, la mesure des anticorps neutralisants sériques couplée à la fixation d’une valeur seuil indiquant une réelle protection pourrait constituer une alternative à une épreuve virale dans l’évaluation de l’efficacité d’un vaccin.

Mots clés : Peste porcine Classique, anticorps neutralisants, anticorps vaccinaux.

Introduction

Classical swine fever (CSF) or hog cholera is a highly contagious viral disease of domestic and wild swine caused by a Pestivirus (Flaviviridae). This disease is considered as the major factor of economic losses to the swine industries and pig farmers. Classical Swine Fever Virus (CSFV) is enveloped, single-strand with positive polarity RNA genome [4]. Pigs can be protected against CSF by vaccination with attenuated CSFV [7-8, 13]. The CSF live attenuated vaccine, a Chinese strain (C), is preferred over inactivated or subunit ones because is able to induce a long lasting humoral response as well as cellular immune response [6, 9]. In order to avoid trade restrictions, eradicating programs established the destruction of infected and serologically positive animals without vaccination recovering [3]. Nevertheless, due to the efficacy of attenuated live vaccine to control or to eliminate the disease, vaccination is still used in many countries [11]. In Brazil, the production of CSF-vaccine is directed to both, the stock for use in emergencies, and for exportation. From 2003 to 2007 the yield of CSF vaccine exported to seven and five countries in Asia and South America, respectively, reached 88.035.450 doses (Pest Vac® - Fort Dodge). CSF-vaccine is produced by passage either in cell culture or in suitable host species. The vaccine must be validated to identity, sterility, purity, safety, non-transmissibility, stability and immunization. Conventionally, the vaccine efficacy is directly validated by cellular immune response, i.e., vaccination of pigs following virus challenge. In areas where eradication campaigns are in progress, all security conditions must be
respected in order to avoid virus dissemination. Consequently, the use of CSFV challenge is restricted for safe areas. The evaluation of neutralizing antibodies induction in vaccinated swine could be a resource to minimize the virus dissemination risk and to decrease the vaccine test costs.

The present study was performed in order to investigate the starting of serological protective value of cell culture C strain vaccine against CSF. To describe the patterns of seroconversion we analyzed neutralizing antibodies induced by vaccination with and without viral challenge from 0 until 42 days post-vaccination (dpv). The frequency distribution analysis (Chi square) was applied to compare between antibodies induction before and after the virus challenge and central tendency measures, to plot a profile of vaccine induced antibodies.

Materials and Methods

ANIMALS AND EXPERIMENTAL DESIGN

Two experimental vaccination assays I and II were performed at Laboratory of Animal Support (Laboratório de Apoio Animal - LAPA/PR). In both assays, pigs, approximately 2 month old, devoid of neutralizing antibodies against CSFV and BVDV were obtained from a closed herd and were weighted and housed in isolation units. The healthy state of animals was considered. During the assay, the rectal temperature of each swine was recorded twice daily. All experimental vaccination was performed at a highly safety area. One vaccine dose (VD) was applied in each pig by intramuscular route according to producer recommendations (Pestiffa - Fort Dodge Saúde Animal Ltda).

In assay I, 34 pigs divided into 7 groups were inoculated with the commercial C strain vaccine produced in tissue culture. The pigs were divided in seven groups to better observe the possibility of a horizontal transmission of vaccine virus to contact control pigs. Those pigs were challenged with the virulent CSFV strain (CSFV A (AIfort)-19, supplied by Laboratório Regional de Apoio Animal/LAPA/PR) by intramuscular inoculation (2 mL of 10^5 TCID50/mL inoculum) (TCID: Tissue Culture Infectious Dose) at 22 days post-vaccination (dpv). Seven pigs (one pig of each group) were not vaccinated and served as negative control and contact control. In this assay, no vaccination booster was applied.

In assay II, one group with eight pigs was used: 7 pigs were vaccinated in the same way as for assay I (one VD was applied by intramuscular route according to producer recommendations) and 1 pig was kept as no vaccinated control. In this assay, no viral challenge was applied but pigs received a vaccine booster at 22 dpv according the same modalities as previously described.

In both assays, blood samples were collected from the vein cava at the pre vaccination day (D0) and on the 7th, 14th, 21st, 28th, 35th and 42nd days after vaccination (dpv). The blood samples were allowed to clot at ambient temperature for 1-2 hours before centrifugation at about 1 000g for 10-15 minutes at room temperature for serum achievement.

SEROLOGY ANALYSIS

Serum samples of experimental vaccinated swine were sent to the Molecular Virology Laboratory at Universidade Federal do Rio de Janeiro where they were tested by neutralization peroxidase linked assay (NPLA) [2] to detect the protective humoral antibody titre. Before the test, all serum samples were identified and inactivated by heat. They were then analyzed by serial dilutions for determining the maximal titre obtained; the titre was given by the end dilution sufficient to neutralize 100 TCID50 of the CSFV A-19. Briefly, 50 µL of diluted serum (1:25) were incubated with 50 µL of 100 TCID50 of the virulent virus suspension in 96 well microplates for one hour at 37°C in an atmosphere of 5% CO2. After this period, 200 µL of PK15 cells suspension containing around 2 x 10^5 cells per mL were added to each well and incubated for 48h at 37°C. After confluence, cells were gently rinsed and fixed with acetone 20%. For viral neutralization detection, 50 µL of CSFV-hyper immune swine serum (1:80) or CSFV specific monoclonal serum (1:100) were applied. The plates were incubated for 45 minutes at 37°C in an atmosphere of 5% CO2. Thereafter, the anti-pig or anti-mouse peroxidase conjugate (Sigma, USA) were used at 1:2500 as described in [2]. The results were read at optical microscopy using 9-ethyl 3 amino Carbazol (Sigma Chemicals, USA).

Concerned to pigs’ immune response to CSFV attenuated-vaccine the sera were considered positive or reactive when the neutralizing antibody titre reached values equal or superior to 1:25 preventing cross-reactions with pestivirus, especially Bovine Viral Diarrhoea Virus (BVDV). When the antibody titre is equal to 1:32, the pig is considered individually protected against the virus challenge. But when the antibody titre is equal or over 1:50 no virus is excreted to environmental so, the herd is protected too [13].

STATISTICAL ANALYSIS

The data of each period (Day 1 – Day 22 and Day 22 – day 42) was analyzed by Qui Square frequency of distribution. Differences were considered as significant when p values were less than 0.05. The arithmetical mean to design the neutralizing antibodies profile induced at each experimental week was also applied. The profile of neutralizing antibody mean titre against CSFV was plotted in diagram.

Results

In assay I, all vaccinated pigs survived to virus challenge without presented CSF symptoms and rectal temperatures remained below 40°C whereas negative control controls (unvaccinated pigs) exhibited strong typical clinical signs of CSF as prostration, depression, weakness, anorexia, diarrhoea or vomiting incoordination and tremors. The haemorrhagic lesions (petechial and cyanoses) of tips extremities (ears, tail, vulva) associated with hyperthermia (40.5 - 41.5°C) took place approximately 2 to 7 days after the virulent viral challenge.
As shown in Table I, no neutralizing antibodies against CSFV could be detected by NPLA in vaccinated pigs until the 14th day. At this date, neutralizing antibodies (1:25) occurred in 5 pigs (14.7%). On the 21st dpv, 13 pigs (38.2%) pigs presented increased antibodies titres but no pig exhibited an antibody titre above 1:50 (threshold value for a herd). The mean antibody titre was over 1:27 ranging from 1:25 to 1:40. One week after the virus challenge, all serum tested showed protective neutralizing antibodies that increased until 30% for 24 pigs (70.6%) and above 30% for 9 pigs (26.5%). The antibody mean titre was over 1:31 ranging from 1:25 to 1:64 reaching values equal or over 1:50 in 5 animals (14.7%) at 28th day post vaccination. Two weeks after challenge, neutralizing antibodies titre strongly rose for all pigs. The mean titre of NPLA antibodies was 1:132.5 ranging from 70.4 to 224 and despite the heterogeneity of the individual humoral response, 100% of vaccinated pigs presented protective antibody titre. Furthermore, on day 42, all animals presented an antibody titre over 1:100.

The frequency distribution analysis significantly evidenced the increase of the proportion of seropositive vaccinated animals between days before virus challenge (days 7, 14 and 21) and after the virus challenge (Days 28, 35 and 42) (p < 0.001) (figure 1A). Moreover, the genesis of serum neutralizing anti-CSFV antibodies appeared stable since the 35th dpv (day 35 vs. day 42: not significant).

By contrast, no neutralizing anti-CSFV antibody was detected in negative contact controls (unvaccinated pigs) and after the challenge all were died until seven days.

In assay II, NPLA antibodies were not detected in vaccinated pigs before the vaccine booster (on day 22) (Table II, figure 1B). Thereafter, the mean titre of neutralizing antibodies markedly enhanced on day 28 and gradually increased until the end of the experimental period (day 42) (Table II). In parallel, 6 / 7 pigs exhibited titres above 1:50 since the 28th day post vaccination and all pigs were seropositive at 35 and 42 dpv (antibody titres were ranged from 1:60 to 1:190 and from 1:100 to 1:190 respectively) (figure 1B). Again, the contact controls (non vaccinated pigs) did not develop neutralizing antibodies against CSFV. Like in the assay I, the serum anti-CSFV antibody profiles significantly differed (p < 0.001) between the period before vaccine booster and the period after (figure 1B).

### Discussion

In the present study, a commercial C strain vaccine produced and validated by international rules was used. DAHLE and LIESS [1] observed that some weaner pigs vaccinated with a commercial C vaccine started the seroconversion as early as...
one week post vaccination, while others presented neutralizing antibodies in two weeks post vaccination. In ours experiments, the raising of humoral neutralizing antibodies was measured every week from pre vaccination (day 0) to 42 days post vaccination, and until the 14th day, no serum NPLA antibodies could be detected with a titre equal or superior to 1:25. Below this limit, cross-reactions with other Pestivirus \[12\] would interfere in the dosage \[12, 13\]. Since cell-mediated immunity may be either absent \[15\] or transient \[10\], humoral antibodies can be assumed to play a major role in the immunity of CSF. Based on that information, serum NPLA antibody titres were compared from vaccinated swine before and after the viral challenge. Whereas antibody titres slowly increased after vaccination, a strong humoral immune response was obtained in all vaccinated pigs at least 2 weeks after the viral challenge. This humoral response kinetic could firstly suggest a possibility of virus replication, but in agreement with DAHLE and LIESS \[1\], none of the vaccinated pigs died or presented clinical signal of CSF and rectal temperature remained below 40°C. Furthermore, unvaccinated control pigs housed together with vaccinated swine have not developed antibodies to CSFV for the whole experimental period, suggesting that no direct viral transmission occurred between vaccinated animals and exposed unvaccinated controls. TERPSTRA and WENSOORT \[13\] affirmed that vaccination protected and avoided virus dissemination when the humoral antibodies reached over 1:32 on the 42th and 56th dpv. In the present study, during the first assay, all swine vaccinated and challenged with virulent CSF virus reached antibody titre greatly over 1:32 since the 35th dpv. Besides, the proportion of animals presenting an antibody titre superior to the value of 1:50 was significantly increased at this date compared to the proportion of positive animals before the viral challenge. In the same way, all vaccinated pigs except one of the assay II (submitted to a vaccination rappel) exhibited a protective humoral response with antibody titres exceeding 1:50 since the 28th day and all these animals reached neutralizing antibody titre > 1:50 on the 35th day. Again, the challenge on day 22 (here, vaccination rappel) has significantly exacerbated the humoral response of pigs (p < 0.001). Nevertheless, the serum neutralizing antibody production appeared to reach a plateau when all animals were protected since no significant difference in the repartition of the anti-CSFV antibody titres was observed between the 35th and the 42th days in the 2 assays. KADEN et al. \[5\] reported that a swineherd was totally protected with antibody mean titre over 1:79. In agreement with that, all pigs analyzed during the 2 assays presented antibodies titre over 1:100 on the 42th day. So, despite the experimental design included the immunogenic stimulation with a viral challenge or vaccine booster the results suggest that all vaccinated pigs were protected against CSFV at 35 dpv whereas TORLONE et al. \[14\] considered that neutralizing antibodies would be established only after 56 dpv. Consequently, the detection of humoral neutralizing antibodies, considered to play essential roles in immunity against CSF, allows the investigation of vaccine protection without necessary recurring to a virulent viral challenge. The comparison of vaccine induced antibodies profiles between pigs submitted or not to a virulent virus challenge could contribute to determine the vaccine antibodies titre necessary for pig protection.

As a conclusion, neutralizing antibodies are undoubtedly considered as the most important specific defense against CSF. But, experimental design and the historical of healthy animals are very important to establish a protective antibody profile.

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