

Antibody response between pigs of Piau and a commercial breed naturally infected with Porcine circovirus 2

[Resposta sorológica entre suínos da raça Piau e linhagem comercial em rebanho naturalmente infectado pelo Porcine circovirus 2]

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ABSTRACT

Brazilian pig population is made up of several naturalized breeds; among them the Piau breed is known for its rusticity and large fat stores. The naturalized breeds, in comparison with commercial ones, may have an increased resistance to diseases circulating in their territory. Thus, this study aimed to verify if there are differences between the serologic profile against Porcine circovirus 2 (PCV2) of Piau pigs and that of a commercial breed from a farm naturally infected by PCV2. The serum viral load was measured by qPCR, and levels of anti-PCV2 antibodies were measured by ELISA. The results showed that the serum viral load was similar across all animals. However, Piau piglets showed higher levels of antibodies compared to commercial piglets ($P=0.05$), while sows of the commercial breed showed higher levels than the Piau breed ($P<0.01$). There was not a statistical difference between pigs of different production stages in the seroprevalence of PCV2 or the blood viral load. This work demonstrates that, with regard to a natural PCV2 infection, the Piau breed has a different humoral immune response compared to the response developed by the commercial pigs. The results support the importance of conservation of native breeds.

Keywords: swine, serum viral load, serology, Piau

RESUMO

O rebanho de suínos brasileiro é constituído por diversas raças naturalizadas, entre elas a raça Piau, que é conhecida por sua rusticidade e pela grande deposição de toucinho. As raças naturalizadas, em comparação com as linhagens comerciais, podem ter uma maior resistência a doenças que circulam em seu território. Dessa forma, o presente estudo teve como objetivo verificar se existem diferenças no perfil sorológico contra o Porcine circovirus 2 (PCV2) entre suínos da raça Piau e de uma linhagem comercial de uma granja naturalmente infectada pelo PCV2. Foram realizadas mensurações da carga viral sérica por qPCR e dos níveis de anticorpos anti-PCV2 por meio da técnica de ELISA. Os resultados mostraram que a carga viral sérica se manteve homogênea em todos os animais e que os leitões da raça Piau apresentaram níveis de anticorpos superiores em comparação com os leitões da linhagem comercial ($P=0,05$), enquanto as porcas de linhagem comercial apresentaram níveis superiores aos da raça Piau ($P<0,01$). Este trabalho fornece indícios de que a raça Piau apresenta uma resposta imune humoral distinta diante de uma infecção natural pelo PCV2, quando comparada com a resposta desenvolvida pela linhagem comercial. Os resultados obtidos reforçam a importância da conservação das raças nativas.

Palavras-chave: suíno, viremia, sorologia, Piau

Recebido em 19 de maio de 2016

Aceito em 7 de junho de 2016

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INTRODUCTION

The Brazilian swine population is made up of several naturalized breeds, and among them the Piau breed is considered the most important (Sollero *et al.*, 2006). The main characteristics of this breed are rusticity, low handling requirement and large fat stores, the latter of which is responsible for their classification as a "lard type" pig. Currently all the naturalized breeds are in danger of extinction, possibly due to the emergence of commercial pigs which are more productive (meat type) and better accepted in the market (Mariante *et al.*, 2009).

The domestic-rural pigs had centuries to adapt to the environmental conditions of the country where they were created, and acquired unique characteristics such as rusticity, prolificity, and probably resistance to parasites and/or existing diseases found in different Brazilian regions (Egito *et al.*, 2002). The importance of genetic diversity within the naturalized breeds is reflected in the wide range of types that exist and the variation inside each one. Studies of this diversity can assist in the development of future animal breeding programs (Egito *et al.*, 2002).

Nowadays, *Porcine circovirus 2* (PCV2) is widely distributed in commercial pigs from all over the world, and is responsible for causing a set of multifactorial syndromes collectively named PCV2 associated diseases (PCVDs) (Segalés *et al.*, 2013). The PCVDs represent a health and economic challenge for the pig industry; among them the postweaning multisystemic wasting syndrome (PMWS) is the main syndrome because it is the leading cause of economic losses (López-Soria *et al.*, 2014).

The interaction between PCV2 and natural hosts (wild and domestic pigs) is a complex process that begins with viral adhesion and entry into the cell (Nauwynck *et al.*, 2012). This interaction results in the development of an adaptive immune response which tends to end the acute infection, although some animals may develop the persistent infection (Segalés *et al.*, 2012). This persistent infection is expected in animals that will develop subclinical disease but not in animals that will develop the systemic infection (Segalés *et al.*, 2013).

Studies by Opriessnig *et al.* (2006, 2009) showed that purebred Landrace pigs developed more serious lymphoid lesions and clinical disease when compared to purebred Duroc, Large White, and Pietrain pigs. In a field observation study, Walgreen *et al.* (2009) demonstrated that Hampshire pigs developed less serious clinical signs from PCVDs than purebred animals like Landrace and Yorkshire pigs.

In the same context, Souza *et al.* (2011) demonstrated that Piau piglets may have a different immune response than piglets of commercial breeds; however, no other study has evaluated a similar hypothesis comparing naturalized with commercial breeds. Knowledge of the infection rates in these breeds is not clear, and the genetic influence on the pathogenesis of PCVDs is poorly discussed in the literature, although it is widely cited as a predisposing factor (Sliz *et al.*, 2015).

Therefore, the goal of this study was to verify if there are differences between the immune response of pigs from Piau (naturalized) and a commercial breed on the same farm naturally infected with PCV2.

MATERIAL AND METHODS

The experimental procedures of this study are in accordance with the Ethics Committee on Animal Use of the Federal University of Viçosa (process number: 27/2013).

The animals used in this experiment were part of a non-vaccinated herd composed of the naturalized breed (Piau) and a commercial crossbreed (Landrace x Large White x Pietrain). All the animals came from the same swine farm with a farrow-to-finish system.

Calculation of the sample size was conducted using Epi Info software, version 3.5.1 (Statcalc calculator - sample size & Power - population survey), using a prevalence of 95% for PCV2 infection (Barbosa *et al.*, 2008), 5% error and 95% confidence interval. Cross-sectional data for the sample populations by stage of production are shown in (Table 1).

Table 1. Population of animals and number of individuals considered for sampling in each production stage. *The age of each production stage is given in weeks, except for sows which are given in number of parturitions

Production stage*	Herd		Samples	
	Commercial	Piau	Commercial	Piau
Sow (>2)	67	23	9	9
Farrowing (0-3)	135	15	18	6
Nursery (3-8)	153	18	21	7
Finishing (8-22)	106	57	14	22

Blood samples were collected by jugular venipuncture. The serum was separated and used in an *in-house* indirect anti-PCV2 IgG ELISA assay.

The capsid protein of PCV2 was expressed in *E. coli* and used as an antigen as described by Salgado *et al.* (2015). Ninety-six well microplates (Nunc Immuno™ MicroWell™ 96 Plates MaxiSorp™ Sigma-Aldrich, USA) were used and were coated with 5µg/well of antigen diluted in carbonate buffer (0.05M, pH 9.6) for twelve hours. The microplates were washed 4 times with phosphate buffered saline containing 0.05% of Tween 20 (PBS-T), blocked with PBS containing 3% of bovine serum albumin (BSA) and stirred for 90 minutes at room temperature. Then 100µL of the serum samples were diluted in PBS-T and added per well, at a final dilution of 1:400. Each sample was tested in duplicate. After washing again, the microplates were incubated with anti-swine IgG peroxidase conjugate produced in rabbits (Sigma-Aldrich, USA) at a dilution of 1:10,000 and stirred for 90 minutes at room temperature. Finally, 100µL/well of the substrate citrate-phosphate buffer 0.1M (pH5.0) containing 0.02% H₂O₂ and 0.2mg/ml σ-phenylenediamine (OPD) was used and the microplate was incubated at room temperature. After 10 minutes, 50µL/well of H₂SO₄ solution (1.5M) was added, and the presence of antibodies was determined by reading the optical density (OD) at 492nm.

To determine if the animals were seropositive or seronegative for PCV2 the cut-off for each microplate was calculated according to the following formula: Cut-off = OD average negative control + (2* standard deviation of negative control).

Since all the wells were sensitized with the antigen, a modification was developed to

increase accuracy of the readings. The antibody levels of each sample was calculated using the method of Fort *et al.* (2007): Sample to positive ratio (S/P) = (OD sample)/ (OD positive control - OD negative control).

The percentage of positive and negative animals and the average level of anti-PCV2 antibodies for each production stage among the genotype groups were determined.

To determine the circulating viral load the serum samples were also tested by real-time polymerase chain reaction (qPCR) as described by Olvera *et al.* (2004). The method used was the hydrolyse probe (TaqMan®) system using the TaqMan Master Mix kit on the ABI Prism 7500 system (Applied Biosystems™, USA). Statistical analyses were performed using GraphPad PRISM® 5 (GraphPad Software, Inc., USA). The mean antibody level among the production stages within the same breed was determined using an analysis of variance with the Duncan test. The chi-square test was used to assess the antibody levels of the different production stages among the two genetic groups and to assess the differences in seroprevalence.

RESULTS AND DISCUSSION

Seropositivity of PCV2 in piglets 0-3 weeks of age was 100%, likely due to passive immunity from seropositive sows. Some authors observed that in farms with a farrow-to-finish cycle, the sows and the piglets in the farrowing had high antibody titers, indicating that there is a positive correlation between serum anti-PCV2 antibody titers in sows and the antibody levels in colostrum passively transferred to piglets (Gerber *et al.*, 2012; 2014).

In both, 3-8 week stage and maternity stage (0-3 weeks), there was a lower prevalence of PCV2,

likely due to decreased levels of antibodies from colostrum consumption. In the finishing stage (8-22 weeks of age), the number of seropositive animals increased again as a typical response of pigs to PCV2 in natural conditions. That means that there was a decrease in antibody titers from motherhood until the nursery stage, after which

titers increased gradually until the finishing stage as observed by Fort *et al.* (2007) and Pinto *et al.* (2011) (Figure 1).

The average number of viral copies per milliliter was determined in each production stage of both breeds through qPCR technique (Table 2).

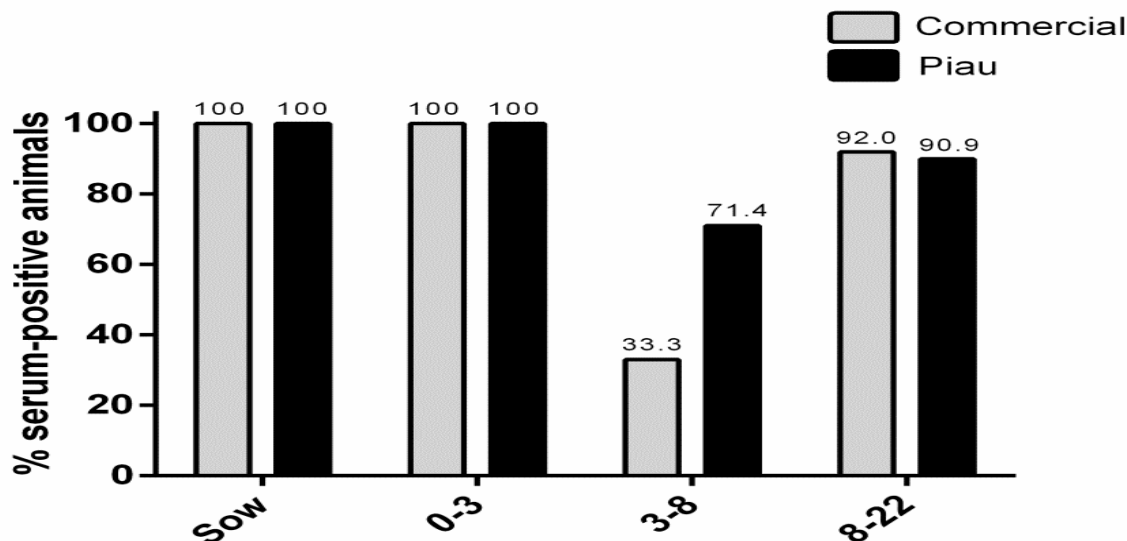


Figure 1. Percentage of animals seropositive for PCV2 in each production stage. Sow: more than two parturitions; maternity: 0-3 weeks of age; nursery: 3-8 weeks of age; finishing: 8-22 weeks of age.

Table 2. Mean and standard deviation of viral load (\log_{10} copies of PCV2/mL) in the different production stages of the Piau and the commercial breed

Production stage*	Serum viral load/Sd**	
	Commercial	Piau
Sows (>2)	3.26 (\pm 0.54)	3.39 (\pm 0.32)
Farrowing (0-3)	3.77 (\pm 1.09)	3.18 (\pm 0.28)
Nursery (3-8)	3.46 (\pm 0.35)	3.18 (\pm 0.59)
Finishing (8-22)	3.49 (\pm 0.38)	3.18 (\pm 0.59)

* The age of each stage is given in weeks, except for the sows which is given as number of parturitions.
 **Sd=Standard deviation

The samples analyzed by qPCR were positive for the virus, and the virus was detected in all production stages as well as in both studied groups. However, there were no statistical differences observed when comparing the Piau with the commercial breed, in all indicating that the pressure of infection remained homogeneous in this farm.

Reiner *et al.* (2010) observed that wild animals showed lower viral loads in tissue samples ($10^{2.8}$ copies PCV2/ μ g DNA sample) compared to animals of commercial breeds ($10^{4.2}$ copies PCV2/ μ g DNA sample), raising the hypothesis that there was a different response among those animals. In another study, Lunney and Chen (2010) suggested that genetic variations of the host regulate the responses of the innate and adaptive immune systems and also the

mechanisms involved in specific disease resistance.

The serologic response of the sows was analyzed, and even though the two groups had the same serum load, the Piau breed showed

lower levels of antibodies compared to the commercial breed ($P < 0.01$) (Figure 2).

In nursery animals, 3-8 weeks of age, there were increased antibody levels in the Piau breed ($P = 0.05$).

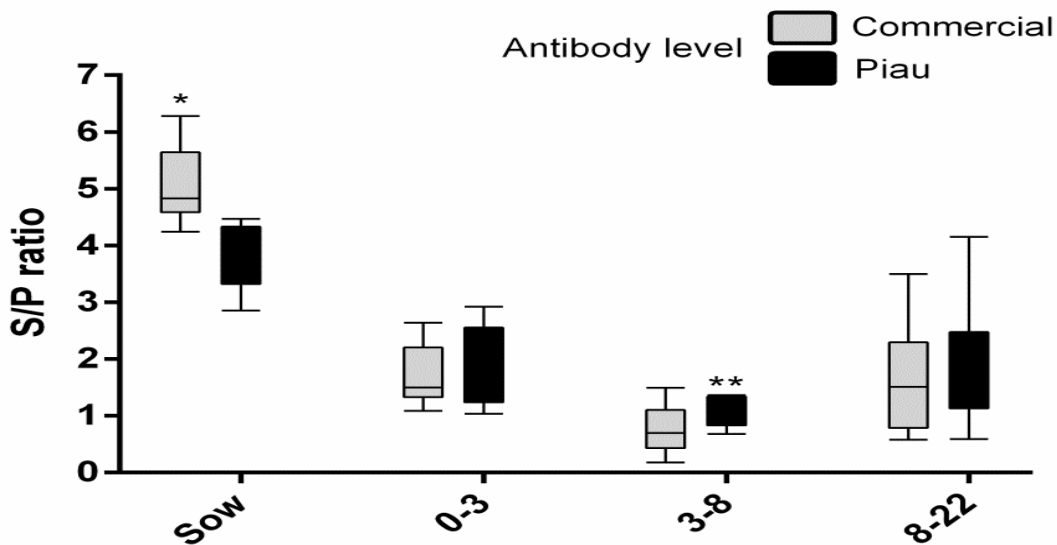


Figure 2. Serologic profile of the Piau and commercial breeds during the production phase. * $p < 0.01$, ** $p = 0.05$. Maternity: 0-3 weeks of age; nursery: 3-8 weeks of age; finishing: 8-22 weeks of age. The age for the sows is given in number of parturitions (>2).

Several studies in humans and mice showed that increased body mass index or obesity can harm the efficiency of the immune system (Milner and Beck, 2012). Nieman *et al.* (1999) observed that T and B lymphocytes of obese humans have reduced proliferation when stimulated to undergo mitosis. O'Shea *et al.* (2013) observed that obese people have a weaker T lymphocyte response and increased production of IL-10 (interleukin suppressor). In another study, obese mice had decreased activation of natural killer cells, dendritic cells and TCD8⁺ lymphocytes (Karlsson *et al.*, 2010).

The Piau breed, like all swine breeds that have not gone through breeding program, had a higher potential to lipid synthesis (Serra *et al.*, 1998). Serão *et al.* (2011) observed that the Piau breed has more intramuscular fat in the *Longissimus dorsi* muscle compared to commercial breeds, especially with regard to sows that are older animals and had excessive fat accumulation over time. Therefore, a possible explanation for the

difference in the antibody response in the Piau breed is the higher percentage of fat stores compared to animals of commercial breed.

In farrowing, 0-3 weeks of age, there was not a statistical difference between the studied groups. We suggest that the Piau breed, although receiving a lower amount of maternal antibodies have a greater recovery of passive antibodies when compared to the commercial breed.

In nursery animals, 3-8 weeks of age, there were increased antibody levels in the Piau breed ($P = 0.05$). In the present work, Piau pigs at the end of the nursery phase (56 days of age) had an average weight of 14.64kg and commercial pigs had an average weight of 20kg, therefore, there was unlikely to be an effect of excess body fat on the immune system of these animals, allowing them to react normally to an infection. This explains the higher antibody levels in nursery phase Piau pigs, which agrees with the theory

that naturalized-rural breeds would be more resistant to pathogens (Amarante *et al.*, 2009).

In the finishing stage, 8-22 weeks of age, there was no statistical difference between the studied groups. At this stage, there was an increase in the serologic response in both groups, as a typical response of positive pigs to PCV2, as observed by Fort *et al.* (2007) and Pinto *et al.* (2011).

The methodologies used in other studies to verify the difference in susceptibility to PCV2 infection would not apply to this study, because they were based on differences in the occurrence of clinical disease. The traditional way of evaluating the susceptibility difference to PCV2 subclinical infections is based on production parameters (Young *et al.*, 2011), however the different productivity between the Piau and the commercial breed would make this methodology invalid (Veroneze *et al.*, 2008). Therefore, this study showed that serologic analysis was the most appropriate methodology to evaluate the response of the two different genetic groups exposed to PCV2 infection.

This study demonstrated that the Piau breed has a humoral immune response different from that of the commercial breed regarding PCV2 infection, as the Piau sows had lower antibody levels, and the nursery piglets had higher antibody levels, compared to the commercial pigs. Importantly, infection prevalence was similar between the two groups, and the viral load found in both breeds did not differ statistically. These results reinforced the hypothesis that there were differences between the immune response of pigs from Piau and a commercial breed even when the pressure of infection was homogeneous in this farm.

CONCLUSION

The information obtained in this study reinforces the hypothesis that the Piau breed is an interesting model with which to study the immune response, because the Piau showed serologic differences compared to commercial pigs when undergoing similar pressure by PCV2 infection. Overall, more studies are needed to elucidate details about breed-dependent differences and the type and efficacy of immune response in the face of an infection.

ACKNOWLEDGMENTS

This study was supported by grants from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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