DETECÇAO DE CIRCOVIRUS SUINO 2 (PCV2) POR REAÇÃO EM CADEIA PELA POLIMERASE (PCR) NAS FEZES E BAIA DE SUÍNOS EM GRANJA SEM VACINAÇÃO

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RESUMO - A circovirose causada pelo circovírus suíno 2 (PCV2) é responsável por gerar prejuízos econômicos na atividade suinícola no Brasil. Os animais infectados podem desenvolver a síndrome multissistêmica do definhamento, distúrbios reprodutivos e doenças do complexo respiratório. Há uma manifestação subclínica importante onde observa-se a queda no desempenho. A disseminação ocorre primariamente pelas vezes. A vacinação é a forma mais eficaz para a diminuição da circulação viral, pois minimiza a eliminação viral pelas fezes. Infelizmente, o alto custo inviabiliza a utilização da vacina por muitos produtores. O objetivo deste trabalho foi detectar o PCV2 em amostras de fezes coletadas de leitões e da baia de uma granja de suínos não vacinados para o agente. Foram coletadas 16 amostras usando suabes, sendo seis delas da ampola retal de animais e dez da baia. A extração do DNA foi feita com o kit QIAamp da Qiagen, seguida de amplificação por PCR. Os amplicons foram verificados em eletroforese em gel de agarose. 87,5% das amostras foram positivas para o PCV2 (14/16), sendo que 83,3% dos animais testados estavam infectados, (5/6) bem como 90% das amostras da baia (9/10), indicando uma alta disseminação do vírus no ambiente. Esses resultados foram semelhantes aos descritos em literatura para granjas que, também, não utilizavam a vacinação para o controle da infecção por PCV2.

Palavras-chave: circovirus; PCV2; PCR; suínos.

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Porcine circovirus 2 detection in feces and sty samples from an unvaccinated farm using PCR

ABSTRACT- The circovirosis caused by porcine circovirus 2 (PCV2) is responsible for generating economic losses in swine production in Brazil. Infected animals can develop post weaning multisystemic wasting syndrome (PMWS), reproductive disorders and respiratory diseases. An important subclinical manifestation leads to arrested development. Feces are the main form of contamination. Vaccination is the most effective way to decrease the viral circulation, because it minimizes viral shedding in feces. Unfortunately, the high cost prevents the use of the vaccine for many producers. The objective of this study was to detect PCV2 in stool samples collected from piglets and from the sty of a pig farm, unvaccinated for the agent. A total of 16 samples were collected using swabs, six from the rectum of animals and 10 from the sty. DNA extraction was performed using the Qiagen QIAamp kit, followed by PCR amplification. The amplicons were checked on agarose gel electrophoresis. Our results showed that 87.5% of the samples were positive for PCV2 (14/16), 83.3% of tested animals were infected (5/6) as well as 90% of the sty samples (9/10), indicating a high spread of the virus in the environment. These results were similar to those described in the literature for farms that also did not use vaccination to control PCV2 infection.
Keywords: circovirus; PCV2; PCR; porcine.

INTRODUCTION

In Brazil, the porcine circovirus 2 (Porcine circovirus 2 - PCV2) is the primary causative agent of various syndromes which are collectively designated circovirosis. Worldwide, the virus is responsible for huge economic losses in swine production, and it is assumed that prevention is a key point in the control of PCV2 (Opriessnig et al. 2007, Segalés 2012, Segalés et al. 2013).

The clinical and etiological scope of PCV2 infection has expanded over the past 20 years, giving the virus a causative role in a large number of clinical syndromes. The most described is the postweaning multisystemic wasting syndrome (PMWS). PCV2 also plays a role in the occurrence of reproductive disorders, respiratory diseases and proliferative necrotizing pneumonia. A more subtle manifestation, but not less

important, is subclinical, where apparently "healthy" animals decline in performance (Opriessnig et al. 2007, Segalés 2012).

PCV2 has two major genotypes, PCV2a and PCV2b, which have been associated with circovirosis on five continents, with a prevalence ranging from 50 to 100%. In terms of continual evolution of PCV2, genome analysis studies revealed a mutation rate of 3.3×10^{-3} a 1.2×10^{-3} substitutions/ locus/ year, demonstrating that PCV2 has the highest mutation rate between DNA viruses and is very close to that observed in RNA viruses (Dupont et al. 2008).

The spread of PCV2 by pig faeces is already well documented in the literature, and described both in animals infected naturally and experimentally. The quantification of viral DNA in stool samples showed larger amounts of viral DNA in clinically ill animals when compared to subclinical individuals (McIntosh et al. 2009). Vaccination against PCV2 introduced in Brazil in 2008 has, among other impacts, minimized viral shedding in the faeces, reducing the spread and maintenance of viral circulation. However, although well-publicized, there are still producers who do not use the vaccine due to its high cost.

The present study aimed to detect PCV2 in stool samples collected from piglets and the sty of a pig farm unvaccinated for the agent.

MATERIAL AND METHODS

The collection of biological material was carried out in a pig farm that does not use vaccination for PCV2. A total of 16 samples were collected, six from feces and 10 from the sty. The animals were contained and feces were collected from the rectum using sterile nylon tip swabs. The sty samples were collected randomly with the aid of a 40 cm^2 area delimiter and swabs. After collection, samples were placed in micro eppendorfs containing sterile saline solution 0.9%.

The samples were kept refrigerated during their transport to the laboratory, where they were stored at -20°C until the start of the extraction of nucleic acids.

DNA extraction was performed using 100 μL of fecal solution and the extraction kit QIAamp DNA Mini Kit (Qiagen) under manufacturer's recommendations. PCV2 PCR was conducted in a final volume of 25 μL containing 2.5 μL DNA sample, 12.5 μL of DreamTaq™ Green PCR Master Mix (2X) (Fermentas, USA), 10 pmol of each primer (SybPCV2F: 5´ATA ACC TCC TTC CAG CCC TAC C 3´/ SybPCV2R: 5´ CGT CTA

GGC CTA GGT CAT TTC C3´), which amplifies a fragment of 145 pb (Yang et al., 2007). Positive and negative controls were used in the reaction. Amplification was carried out in PTC-100 thermal cycler (Peltier Thermal Cycler MJ Research, Bio-Rad, USA) under the following conditions: 95°C/ 5 min followed by 29 cycles (94°C/ 30 seconds, 55°C/ 30 seconds and 72°C/ 30 seconds) and a final extension at 72°C/ 5 minutes. Amplicons were visualized by ultraviolet light transillumination of 2% agarose gel after staining with GelRed (Uniscience), according to the manufacturer's instructions. The size of the amplified fragment was compared to a standard of 100 pb (Invitrogen).

RESULTS AND DISCUSSION

The growth of Brazilian pig farming productivity is the result of recent modern techniques that have transformed this activity into an industry that seeks maximum productivity and profitability.

In this context, health has a great impact on swine productivity, since the outbreaks of diseases cause large losses, either through death or low animal performance. In recent decades, viruses have emerged and/or resurfaced in the global swine population, some with great economic impact, such as PRRVS and PEDV.

Experimental and field studies have been performed to demonstrate the effectiveness of commercial vaccines available for the control of circovirosis. These also contribute to load reduction and duration of viremia, viral load in faeces, lesions and increased animal performance (Segalés 2012).

Studies on the maintenance of PCV2 in farms that do not use vaccines are scarce, and the importance of this study was to precisely demonstrate the spread of the virus in these conditions. Of the total number of samples tested, 87.5% (14/16) were positive, 83.3% (5/6) of the animals were infected, as well as 90% (9/10) of the sty samples, demonstrating a great spread of the virus in the environment (Figure 1). These findings are similar to those observed by other authors who conducted studies on farms that also did not use vaccines for the control of PCV2 infection (Gillespie et al. 2009, Rose et al. 2012, Patterson; Opriessnig 2010).

Although several studies are available about PCV2 detection in different types of samples, the high frequency of positive samples (90%) in this study shows the significance of the impact of interventions which minimize maintenance of viable viruses in the environment. It should be emphasized, however, that we did not

aggregate another technique that allows the determination of viral viability (Opriessnig et al. 2007).

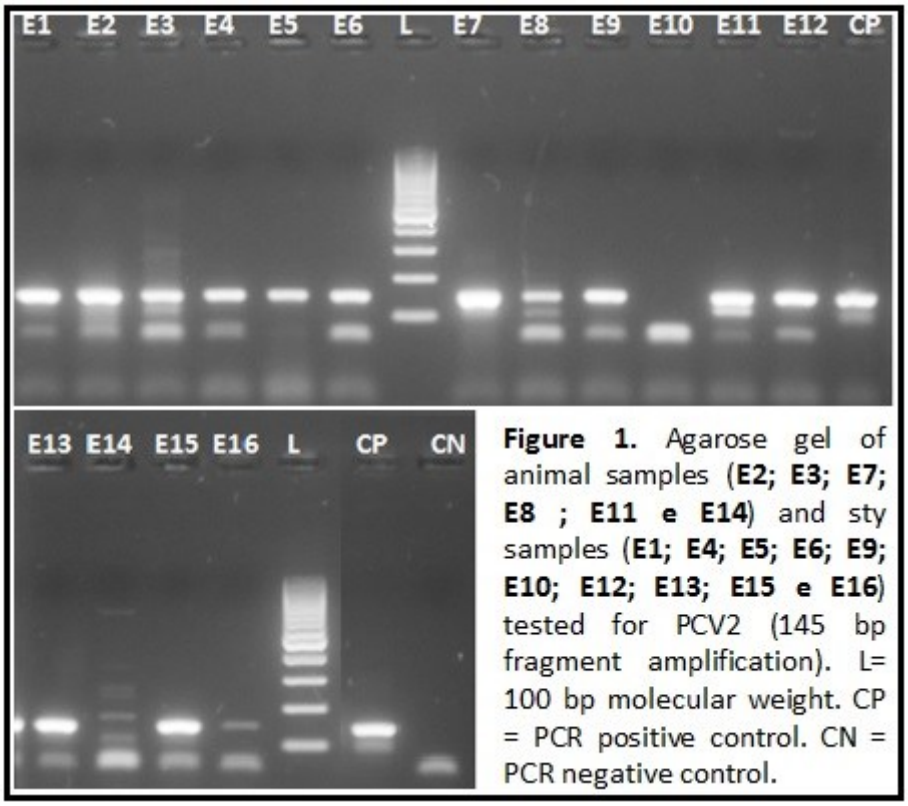

Figure 1. Agarose gel of animal samples (**E2; E3; E7; E8 ; E11 e E14**) and sty samples (**E1; E4; E5; E6; E9; E10; E12; E13; E15 e E16**) tested for PCV2 (145 bp fragment amplification). L= 100 bp molecular weight. CP = PCR positive control. CN = PCR negative control.

CONCLUSION

This study demonstrated a high frequency of positive samples for DNA circovirus collected at a farm where there is no vaccination scheme for the control of circovirosis. Viral DNA was detected in 90% of sty samples, demonstrating a high spread of the virus and its maintenance in that environment. The results show the importance of introducing measures to minimize the disposal of the virus in faeces of animals, since this is an important environmental contamination pathway.

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