

Association of porcine circovirus infection with mycotoxicosis: a case report

Infecção por circovírus suíno associado a micotoxicose: relato de caso

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ABSTRACT: Circovirus is widespread in pig farming, and mainly affects piglets increasing morbidity and mortality rates, being vaccination the most effective strategy to control one. However, for an effective vaccine response, certain factors must be considered, such as the adoption of good practices during the conservation and handling of vaccines, environmental challenges, nutritional and health status of the animals, and presence of immunosuppressive agents, such as mycotoxins, in the feed. Here, we describe a circovirus outbreak associated with mycotoxin immunosuppression that occurred in the piglets during the nursery phase at a commercial farm, which initiated with a sudden increase in the mortality of vaccinated piglets. Blood samples were collected and analyzed using RT-PCR, while the feed was subjected to mycotoxicological analysis. RT-PCR analysis revealed the presence of porcine circovirus type 2 (PCV-2) in the blood serum samples, thereby confirming the circovirus outbreak. The feed analysis revealed elevated levels of mycotoxins (deoxynivalenol, aflatoxins, and fumonisins), which were above the levels tolerated by the piglets during the nursery phase. Therefore, the contaminated feed was discarded, and a new ration was made available. Concurrently, the vaccination program was amended to normalize the mortality rate. The presence of mycotoxins in the feed could be the predisposed factor for piglet infection caused by PCV-2 and other diseases. This is an important aspect because the immunosuppressive effect of mycotoxins can alter the vaccine response, thereby making the piglets more susceptible to the diseases even after being vaccinated, although they should be immunologically protected.

KEYWORDS: Circovirus; Piglet; Mycotoxin; Vaccine response; Porcine circovirus type 2; Co-infection.

RESUMO: O circovírus é muito difundido na suinocultura e atinge principalmente leitões, aumentando as taxas de morbimortalidade, sendo a vacinação a estratégia mais eficaz de controle. No entanto, para uma resposta vacinal eficaz, alguns fatores devem ser considerados, como a adoção de boas práticas durante a conservação e manuseio de vacinas, desafios ambientais, estado nutricional e de saúde dos animais e presença de agentes imunossupressores, como micotoxinas, na ração. Descreveu-se um surto de circovírus associado à imunossupressão por micotoxinas que ocorreu em leitões durante a fase de creche em uma granja comercial, que começou com um aumento repentino na mortalidade de leitões vacinados. Amostras de sangue foram coletadas e analisadas por RT-PCR, enquanto a ração foi submetida à análise micotoxicológica. A análise de RT-PCR revelou a presença de circovírus suíno tipo 2 (PCV-2), confirmando assim o surto de circovírus. A análise da ração revelou níveis de micotoxinas (desoxinivalenol, aflatoxinas e fumonisinas) acima dos tolerados pelos leitões nesta fase. Portanto, o alimento contaminado foi descartado e uma nova ração foi disponibilizada. Simultaneamente, o programa de vacinação foi alterado para normalizar a taxa de mortalidade. A presença de micotoxinas na ração pode ser o fator predisponente para infecção de leitões causada por PCV-2 e outras doenças. Esse é um aspecto importante, pois o efeito imunossupressor das micotoxinas pode alterar a resposta vacinal, tornando os leitões mais suscetíveis às doenças mesmo após vacinação.

PALAVRAS-CHAVE: Circovírus; Leitões; Micotoxinas; Resposta vacinal; Coinfecção.

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INTRODUCTION

Swine activity produced approximately 102 million tons of animal protein worldwide in 2019. Brazil remains highlighted for occupying the 4th place in the world ranking among the largest pork producers and exporters (3.9% of pork production; ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL, 2020). This prominent position is attributed to the genetic, nutritional, and management improvement, as well as reproductive biotechniques, in addition to the implementation of biosecurity programs to protect the breeding stock against several diseases and immunosuppressive toxins.

PCV-2 infection is also known as swine circovirus, and it is characterized by a set of syndromes, accompanied with multisystemic swine wasting syndrome being the most frequent and well characterized clinical symptom of this viral infection (ZANELLA, 2012). Swine circovirus is widespread in pig farming and affects different herds, including both the full-cycle herds and piglet production units, daycare centers, and finishing sites. It predominantly affects the piglets between five to 12 weeks of age, with variable morbidity and mortality rates, reaching 70–80% and 4–30%, respectively, which are also related to the management and presence of pathogens in the environment (leading to co-infections). However, it can also occur in the subclinical form in affected herds, thereby resulting in insufficient piglet performance and a higher frequency of occurrence of co-infection (MORÉS; BARCELLOS; ZANELLA, 2012).

Circovirus infection may be controlled through implementing biosecurity rules in swine farms, maintaining the immune balance in herds, low microbial (viral) load, and good practices for the conservation and administration of vaccines, to avoid vaccine failures and consequent disease severity (CIACCI-ZANELLA, 2012). The immune system also plays an important role in animal performance, which can interfere with the functioning of other organs and be influenced by several factors, such as the nutritional factors, including the exchange of food and presence of mycotoxins in the feed (DALAGNOL, 2008). The major problem associated with mycotoxins is that the doses below the threshold for exacerbation of known clinical conditions can culminate in the occurrence of immunosuppression in animals, which is characterized by subclinical infections, immunological failures after vaccination, and infectious diseases in animals (DALAGNOL, 2008). In this study, we aimed to describe a case of an outbreak of circovirus associated with mycotoxin immunosuppression observed in the piglets during the nursery phase at a commercial farm.

CASE DESCRIPTION

This case occurred in the nursery of a piglet production unit (PPU), located in the Metropolitan Region of Fortaleza, Ceará, Brazil. The PPU had 1,500 sows, such that the piglets

were vaccinated against PCV-2 at 15 and 35 days of age. The piglets were weaned at 24 days of age and transferred to clean and disinfected nursery warehouses equipped with semi-automatic feeders, 1:10 pacifier-type drinking needles, and side curtains for ventilation and temperature control inside the pen. In the nursery phase, from weaning to 65 days of age, the piglets were fed with a balanced diet (pre-initial 1, pre-initial 2, initial 1, and initial 2), as described by Rostagno et al. (2017).

In December 2017, we observed a sudden increase in the mortality rate in the nursery phase from 1.0% to 5.5% mainly in 50 days old piglets. In addition to an increase in the mortality, some animals exhibited stunted growth accompanied with a sudden increase in joint volume, dyspnea, hypertrichosis, diarrhea, and pallor. Furthermore, a large number of animals experienced ear necrosis in the first 3 days of accommodation, which could be associated with the environmental stress.

Initially, an outbreak of swine circovirus was suspected considering the clinical symptoms. Therefore, the blood samples were collected through jugular puncture from 30 piglets (15 animals per age) that were potentially affected at 45 and 65 days of age to analyze PCV2 viremia. The samples were subjected to quantitative real-time polymerase chain reaction (RT-PCR) analysis to determine the viremia profile, and the viral load (number of PCV2 DNA copies/ μ L of sample) was subjected to logarithmic transformation (\log_{10}) for interpreting the results (Table 1).

Since the clinical symptoms were present in all herds of the nursery piglets, an interference by a common external factor, such as food, was also suspected. Therefore, the feed sampling was conducted, and the samples were subsequently sent for toxicological analysis using ELISA, with the aim to detect mycotoxins, such as deoxynivalenol, aflatoxins, fumonisins, zearalenone, and ochratoxin.

Table 1. Parameters for interpreting the results of quantitative RT-PCR to swine circovirus DNA detection in the blood samples of the piglets.

Values (\log_{10})/ μ L of sample	Interpretation
Negative	Undetectable: circovirus may be present, but not in viremia
Up to 1	Very low viral load: intact immune system
1 to 2	Low viral load: there may be immunosuppression
2 to 3	Average viral load: impairment of immune system with considerable zootechnical losses
3 to 4	High viral load: high immunosuppression and frequent onset of co-infections
Above 4	Severe immunosuppression with the occurrence of co-infections

PCV-2 was detected in the blood samples, and therefore, 45 days old piglets exhibited the high positivity accompanied with high viral load (Tables 2 and 3). Furthermore,

deoxynivalenol (DON), aflatoxins, and fumonisins were detected in all feed samples (pre-initial 1, pre-initial 2, and initial 1) with a prevalence of fumonisin and deoxynivalenol mycotoxins (Table 4).

Table 2. Individual results of RT-PCR analysis for Porcine Circovirus type 2 (PCV2; the number of viral DNA copies/ μ L of samples; \log_{10}) in the blood samples of piglets in the nursery phase at 45 and 65 days exhibiting clinical symptoms of circovirus.

n°	Age (days)	Qty.	Log
1	45	138.02	2.14
2	45	NEG.	-
3	45	206.2	2.31
4	45	55.6	1.75
5	45	65.12	1.81
6	45	28	1.45
7	45	54.91	1.74
8	45	98.5	1.99
9	45	NEG.	-
10	45	NEG.	-
11	45	57.86	1.76
12	45	121.68	2.09
13	45	195.88	2.29
14	45	83.18	1.92
15	45	108.14	2.03
16	65	100.05	2.00
17	65	174.14	2.24
18	65	173.73	2.24
19	65	211.82	2.33
20	65	204.52	2.31
21	65	90.98	1.96
22	65	NEG.	-
23	65	NEG.	-
24	65	NEG.	-
25	65	NEG.	-
26	65	NEG.	-
27	65	61.29	1.79
28	65	NEG.	-
29	65	NEG.	-
30	65	101.37	2.01

DISCUSSION

The occurrence of symptoms of refuge, including growth retardation and characteristic hypertrichosis, accompanied with pale skin, dyspnea, arthritis, diarrhea, and a sudden increase in mortality in piglets in the nursery phase suggested the incidence of multisystemic swine wasting syndrome (circovirus). Previously, Morés; Barcellos; Zanella (2012) described rapid progressive weight loss accompanied with symptoms similar to those described in this study, and were also observed: apathy, due to opaque, anorexia, conjunctivitis, enlarged lymph nodes, jaundice, signs of pneumonia, and cachexia.

Withering is a common sign of other diseases, such as diarrhea caused by *Lawsonia intracelullaris*, *Brachyspira* sp., and Glasser's disease, for which a differential diagnosis must be conducted. However, in this study, the age of occurrence of the symptoms and the broad symptomatology involving the respiratory and enteric systems was consistent with that of the multisystemic disease. The clinical presentation of can vary greatly between herds due to the occurrence of co-infections (MORÉS; BARCELLOS; ZANELLA, 2012), such as colibacillosis, salmonellosis, enzootic pneumonia, Glasser's disease, and skin infections caused by *Staphylococcus* sp. (ZANELLA; MORÉS, 2016). In addition to the clinical signs associated with the disease, the quantification of viral DNA using RT-PCR analysis was sufficient to diagnose the incidence of PCV-2 in an immunized herd.

Due to the age at which the worsening of clinical symptoms and higher mortality were observed (from 50 days onward), which increased after the change of feed, the possibility of the existence of a factor related to food was raised. During the transition of the rations, the piglets were left with a ration with high inclusion of mineral and vitamin nucleus and less of other ingredients (pre-starter ration 2), and then, they were fed the one with less inclusion of mineral and vitamin nucleus and high of corn and bran soy (initial ration 1). Therefore, in the presence of corn contaminated by mycotoxins, these would exert a more pronounced effect on diets with higher amount of corn, which

Table 3. Prevalence of porcine circovirus type 2 (PCV2) assessed using RT-PCR in the blood samples of piglets in the nursery phase at 45 and 65 days exhibiting clinical symptoms of circovirus.

Age	Sampling			Number of viral copies of positive samples	Average \log_{10} of positive samples	Prevalence (%) of positive samples
	N° samples	+	-			
45	15	12	3	101.09	2.00	80.0
65	15	8	7	139.74	2.15	53.3

Table 4. The concentration of mycotoxins in the feed of piglets during the outbreak of porcine circovirus type 2 (PCV2) in nursery phase in 45 and 65 days old piglets exhibiting clinical symptoms of circovirus.

Feeds	DON (ppb)	Aflatoxin (ppb)	Fumonisin (ppb)	Zearalenone (ppb)	Ochratoxin (ppb)
Pre-initial 1	630	8	2170	<BQL	<BQL
Pre-initial 2	260	8	2140	<BQL	<BQL
Initial 1	900	<BQL	3450	<BQL	<BQL

DON: deoxynivalenol; <BQL: below quantification levels; ppb: parts per billion.

would coincide with an increase in the animal's ingestion capacity. This justifies the feed mycotoxicological analysis conducted in this study.

The levels obtained in the feed analysis of piglets exceeded the maximum tolerated limits for pigs in the initial phase (deoxynivalenol 0 ppb, aflatoxin 0 ppb, and fumonisin 100 ppb) that were defined by the Laboratory of Mycotoxicological Analysis (LAMIC, 2005) and are sufficient to trigger harmful effects in animals (LIU et al, 2020). The mycotoxins present in the feed can cause several metabolic changes, which cause the occurrence of diseases named mycotoxicosis. Deoxynivalenol, fumonisin B1 (FB1), and zearalenone are the most frequently detected mycotoxins produced by *Fusarium* fungi (STREIT et al., 2013; DE BOEVRE et al., 2013). Deoxynivalenol is the most prevalent mycotoxin worldwide, contaminating 46–64% of the analyzed samples, followed by fumonisin (50–63%; STREIT et al., 2013). The toxic effects of aflatoxins, deoxynivalenol, and fumonisins include immune modulation, the disruption of intestinal barrier function, and cytotoxicity that lead to cell death, and results in impaired performance of pigs and poultry (CHEN; LI; LIN, 2017; HOLANDA; KIM, 2021). Therefore, mycotoxicosis can cause dysphagia or anorexia, irritations and hemorrhages in the digestive tract, changes in the reproductive organs, and failures in the bone marrow and spleen regenerative processes (PRESTES et al., 2019).

Most mycotoxins, such as deoxynivalenol, aflatoxin, and ochratoxin, potentially inhibit protein synthesis. Therefore, mycotoxins act via targeting the protein synthesis cascade, which is necessary for the defense mechanisms of the organism (from mitosis to the production of proteins, antibodies, and lymphokines), thereby causing immunosuppression in animals exposed to them (DALAGNOL, 2008). The immunosuppressive action of mycotoxins involves alteration of host resistance to the infectious diseases and leads to vaccine failure. Fumonisin, when present at high levels in food, increases the susceptibility of animals to lung infections and potentiates the pathogenicity of viral and bacterial agents (PIERRON; ALASSANE-KPEMBI; OSWALD, 2016). The effect of mycotoxins on the immune response to vaccinations has already been described by Cysewski et al. (1978), demonstrating that the diets contaminated with aflatoxin decreased the pigs response to vaccine against

Erysipela sp. Likewise, in this study, it is believed that the response to vaccination against circovirus was impaired due to the presence of mycotoxins in the feed, thereby triggering the disease outbreak.

The major problems related to mycotoxins are at the dietary levels that do not determine the appearance of clinical symptoms, but can be harmful, both at high and low doses (LIU et al, 2020) and cause innate immunosuppression in animals (KHATOON et al., 2018), and inflammation in many cell lines (ALASSANE-KPEMBI et al., 2017), culminating in the reduction of animal productivity (KHATOON et al., 2018). Altogether, the results indicate towards an outbreak of circovirus associated with immunosuppression caused by mycotoxins. Maternal antibodies against PCV disappear between 8 and 9 weeks after birth, and thus, the vaccination programs adopt a period prior to the natural disappearance of maternal immunity, so that the piglets are immunized before a possible viral infection (ALLAN et al., 1994). In this study, the piglets vaccinated at 15 and 35 days immunosuppressed due to mycotoxicosis were unable to develop sufficient immunity against circovirus (PCV-2) clinically presenting the disease.

Upon diagnosing the cause of this problem, the feed contaminated with mycotoxins was discarded and the vaccine program against circovirus was brought forward by 1 week, so that the piglets could attain a better immune response in the period of greatest health challenge, around 50 days old. Additionally, there was a mass vaccination against circovirus in all breeding sows up to 70 days of gestation to increase the herd immunological load. After carrying out these management changes, mortality was controlled, falling to 1.42%, indicating the relevance of this revised management plan.

CONCLUSION

The presence of mycotoxins in the feed can be considered as a crucial factor affecting the infection of PCV-2 in piglets. Moreover, owing to the associated immunosuppressive effect, mycotoxins change the vaccine response, making the animals more susceptible to other diseases. Therefore, a strict control over raw materials for the preventive use of adsorbents is essential to avoid this issue. Additionally, it is important to create a standard operating procedure for the incidence of mycotoxicosis.

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