

Concomitant Zearalenone Ingestion and Porcine Circovirus-2 Infection

Eduardo Robson Duarte¹, Lincoln Nunes Oliveira², Neide Judith Faria de Oliveira¹,
Flávia Oliveira Abrão³, Rogério Marcos de Souza¹ & Marília Martins Melo⁴

ABSTRACT

Background: Researches have suggested that mycotoxins could corroborate the pathogenesis of porcine viral diseases. Zearalenone (ZEA), one of the most important mycotoxins in pigs result significant reproductive disorders. The circovirus syndromes in pigs have been associated with multifactorial or predisposing conditions. No previous data have correlated ZEA with viral syndromes in *Sus scrofa*. In this report are described anamnesis, clinical and histological findings with zearalenone ingestion and PCV-2 infection in a Brazilian livestock.

Case: Feeding ration was mixed and produced at the farm with low quality corn that was stored in barn with cement floor. During ten months, 28 farrowing occurred. Fifteen of them were normal and produced 145 animals, mean of 9.66 piglets per litter. Thirteen sows presented reproductive disorders, and one died. Eight of them had the delayed farrowing and three aborted. These animals produced 57 mummified fetuses (10-16cm crown-rump length), 23 aborted fetuses (60-72 days of gestation), four stillborn, two teratogenic fetuses and only 16 normal piglets. Skin pustules, hemorrhagic spots and petechiae, as well as coughing were observed in 88% of the animals and four piglets died. Diarrhea outbreaks frequently occurred in this period. Twenty five of 34 gilts (75%) showed longer estrous cycle, estrus returning, vulvae volume and mammary glands enlargement. Necropsies showed enlarged lymphatic nodes and kidneys. Histopathological and immunohistochemical analysis indicated the presence of Porcine Circovirus type 2 (PCV-2) in analyzed tissues. Toxicological tests demonstrated the presence of zearalenone (ZEA) in stomach contents with average of 8.75 µg/g and pesticides and aflatoxins were not detected.

Discussion: The history, the clinical signs, the necropsy and histological findings suggested a mix of zearalenone mycotoxicosis and PCV-2 syndrome that was confirmed by PCV-2 tissue detection in immunohistochemical assay, and by food toxicological analysis. The ZEA average founded in stomach contents (8.75 µg/g could produce the reproductive disorders and could have exacerbated the circovirus. The most frequent reproductive alteration observed in the farrowing was mummified fetuses that corresponded 66.7% of its products. The several reproductive disorders as abortion, stillborn, mummified fetuses, estrus returning, vulvae volume and mammary glands enlargement in gilts are frequently related to zearalenone mycotoxicosis. Appetite loss, apathy, wasting, cough and diarrhea and increased mortality and withdrawal rate in growth and post-weaning phases observed in this study are liked as the several descriptions in the Post-Weaning Multisystemic Wasting Syndrome. The hemorrhagic skin spots and increased kidneys, with multifocal glomerulonephritis, lymphohistiocytic interstitial nephritis to remember the several alterations in Porcine Dermatitis and Nephropathy Syndrome. Both syndromes by PCV-2 could be simultaneity occurring in this herd and could have a co-participation of the ZEA ingestion as aggravated factor. After contaminated corn to sorghum free of ZEA exchange at ration, the reproduction returned to normal level and no PCV-2 lesions were observed, suggesting the ZEA ingestion as one initial or aggravating factor for the PCV-2 syndromes. For the first time, this report describes historical, anamnesis, clinical and histological findings possibly associated with zearalenone contamination and PCV-2 infection in pigs.

Keywords: zearalenone, Porcine Circovirus type 2, high performance liquid chromatography, immunohistochemical method.

INTRODUCTION

The mycotoxin synergisms in the pathogenesis of porcine viral diseases have been previously suggested [5]. The most important mycotoxicosis in pigs is caused by zearalenone (ZEA), produced mainly by species of *Fusarium*. This intoxication can result in hyperestrogenic syndromes produced by competitive binding of ZEA to estrogenic receptors of the uterus, liver, mammary gland and hypothalamus [9]. The circovirus syndromes in pigs have been associated with multifactorial or predisposing conditions. The Post-Weaning Multisystemic Wasting Syndrome (PMWS) and the Porcine Dermatitis and Nephropathy Syndrome (PDNS) have been associated with the *Porcine Circovirus-2* (PCV-2) however other alterations have also been related [10,17].

No previous data have correlated ZEA with viral syndromes in *Sus scrofa*. This report describes historical, anamnesis, clinical and histological findings possibly with zearalenone ingestion and PCV-2 infection in a Brazilian livestock.

CASE

In Montes Claros City, 16°51'38" S latitude and 44°55'00" W longitude, north region of Minas Gerais State, Brazil, porcine reproductive disorders and sudden mortality outbreaks occurred during nine months in a 21-sow herd with a total of 269 animals.

The alterations occurred between July 2007 and April 2008, within the critical raining season, from December to January. Feeding ration was mixed and produced at the farm with low quality corn that was stored in barn with cement floor. During these ten months 28 farrowing occurred. Fifteen of them were normal and produced 145 animals, mean of 9.66 piglets per litter. Thirteen sows presented reproductive disorders, and one died. Eight of them had the delayed farrowing and three aborted. These animals produced 57 mummified fetuses (10-16cm crown-rump length, Figure 1), 23 aborted fetuses (60-72 days of gestation), four stillborn, two teratogenic fetuses and only 16 normal piglets.

Withdrawal rate increased to 20% from October 2006 to April 2007 and alimentary conversion changed from 2.8:1 to 3.1:1 on the growing phase. Skin pustules, hemorrhagic spots and petechiae, as well as coughing were observed in 88% of the animals and four piglets died. Diarrhea outbreaks frequently

occurred in this period. Twenty five of 34 gilts (75%) showed longer estrous cycle, estrus returning, vulvae volume and mammary glands enlargement.

Skin lesions with round to irregular, red to purple macules was observed in 15 animals of 22 finishing pigs (Figure 2). After the slaughter inspection, all carcasses there were enlarged inguinal and mesenteric lymph nodes, 35% showed intestinal adhesions and the kidneys were two times increased to five animals. After this inspection, fifteen carcasses were entirely condemned.

Pathological lesions

In this period three diseased animals were necropsied. After slaughter inspection, the first, a gilt with 140 days old, 112 kg body weight (bw), 3.5 corporal score (one to five) that showed infertility during four consecutive times. Petechiae and suffusions at tongue were found. Accentuated liver lobulation, with diffuse suffusions and petechiae at surface and under cutting and larynx, trachea and lungs with petechiae and suffusions were also observed. Enlarged mesenteric lymph nodes were registered. Petechial hemorrhages were founded in the bladder, epicardium, endocardium and aorta. Kidneys with petechiae and suffusions at surface and under cut, and a two mm abscess. The reproductive system was increased with vulvae and uteri volume three times enlargement and uncountable cysts 0.5-1.5 cm in uterine tubes. Hemorrhagic spots and suffusions were noted in skin and muscle of the forelegs.

The second was a 90-day-old male piglet with petechiae and suffusions on the skin. This animal was 72 kg bw, three corporal score and was also euthanized. Liver adhered to parietal peritoneum and with a 2x5 cm abscess, dense bilis and biliary vesicle mucosa with diffuse petechiae and congestion were founded. Spleen was reduced, firm under cut, with 2x4 cm abscess and greater omentum adherence. Petechiae and suffusions at larynx, glote and trachea were observed. Lung with diffuse petechiae and a 2x3 cm hematoma in the cranial lobe were observed. Epicardium, endocardium and ventricular myocardium with diffuse petechial hemorrhages; congested, diffused petechiae kidneys, bladder adhered to medium abdominal line and with 5x4 cm intramural abscess and focal petechiae were found. Reduced adrenal, 1x0.7 cm in length, with narrow cortical region (0.3

cm) were observed. Muscle hemorrhagic spots and suffusions were noted in forelegs.

The third necropsied pig was a sow with 2.5 years, 250 kg bw and 3.5 corporal score that died six days after the parturition. In the farrowing, it produced 11 mummified fetuses and showed continuous and intense abdominal contractions, muscular tremors, hypothermia, weakness, locomotive difficulty, agalactia and ocular congestion. Intramuscular antimicrobial therapy with oxitetracycline1 (20 mg kg⁻¹) was instituted, however the death occurred two days after. The oral, abdominal and intestinal mucosa presented diffuse petechiae and suffusions. Intestines adherences to liver, and peritoneum with increased and congested mesenteric lymph nodes were found. Ileum and cecum ruptures were identified together enlarged uterus, thinner adhered serous to intestines with purulent exudates inside

Toxicological analyses

After necropsy, samples of kidneys, liver, spleen, stomach contents of the three necropsied animals and three corn samples which were searched the different pesticides: organochlorinated (aldrin, dieldrin, endrin, BHC, DDT, HCB, endosulfan, heptachlor, heptachlor epoxid, lindane, methoxychlor, mirex, dicofol), organophosphorus (malathion, parathion-etic, parathion-metilic), carbamates (carbendazim and its metabolism products, benomyl and thiophanate metyl), chloroalquil tio fungicides (captan, folpet), triazols (cyproconazole, chlorothalonil, difenoconazole, propiconazole, tebuconazole), pyrethroids (betacifluthrin, bifenthrin, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, lambda-cyhalothrin, permethrin) and the miscellaneous compound (propargit). The analyze methods utilized varying from each type of pesticide, and were used gas chromatography, high performance liquid chromatography, associated to mass spectrometry with ionic monitoring [13,14].

The search of aflatoxins B1, B2, G1 e G2 were realized in the Laboratory of Mycotoxins from the Ministry of Agriculture, in Belo Horizonte (Minas Gerais), to determine the aflatoxins (B1, B2, G1, G2) and zearalenone content by the method DOU, using high performance liquid chromatography [11,15,16].

In this report, neither pesticides nor aflatoxins were detected in the samples. However, ZEA was

found in the three corn samples and stomach contents of the three necropsied animals with average concentration of 80 µg/g (+/- 16.05 µg and 8.75 µg/g (+/- 8.2 µg) , respectively.

Histological and imunohistochemical exams

Fragments from kidneys, liver and spleen of the three necropsied animals, corresponding none samples and examined by hematoxylin and eosin coloration. The microscopic analysis of the tissues collected from the three necropsied animals showed lymphatic follicles with severe lymphocytolysis and intense macrophage proliferation, mainly histiocytes (Figure 3). The kidneys presented multifocal glomerulonephritis, lymphohistiocytic interstitial nephritis and diffuse tubular nephrosis.

Subsequently, immunohistochemical method (IHC) for the detection of PCV-type 2 in sections from paraffin-embedded tissues was performed using streptavidin-biotinperoxidase, endogenous peroxidases, blockade serum, secondary antibody and streptavidin-peroxidase² [3]. The positive control was performed using paraffin-embedded lymph node fragments with strong positive reaction by in situ hybridization in the La Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB). Blocks of negative lymph nodes to PCV2 were from Brazilian Company for Agricultural Research (EMBRAPA) in pigs and poultry. An intensity of marking in the tissues was ranked from 1 to 4, begging 1 with light focal marking and 4 with intense and diffuse marking in the cytoplasm's mononuclear cells [4]. In this study, the three animals were positive for PCV2 by IHC and the infection was detected in its lymph nodes (++++) (Figure 4), Kidneys (++) and livers (++)



Figure 1. Mummified fetuses, the most frequent alteration observed in the farrowing.

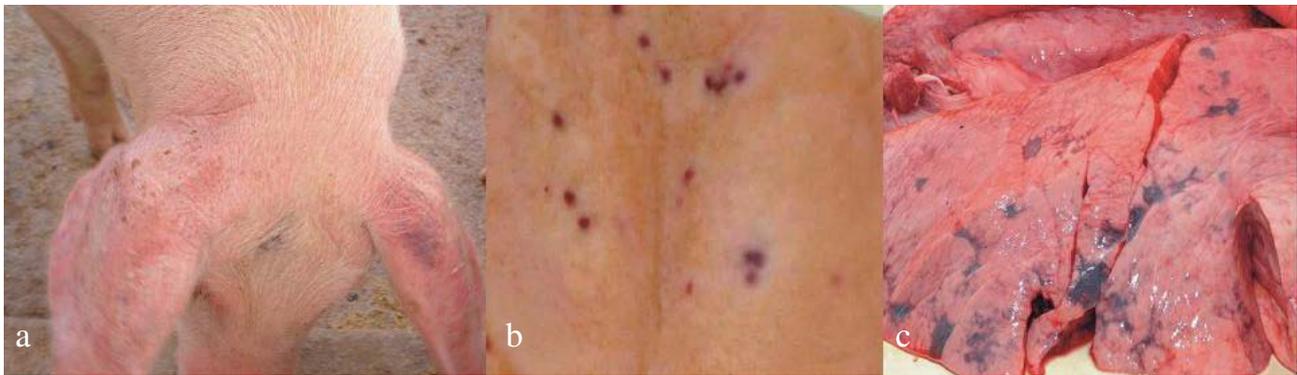


Figure 2. Hemorrhagic skin lesions with round to irregular, red to purple macules (a and b) and lung suffusions (c) in finishing pig.

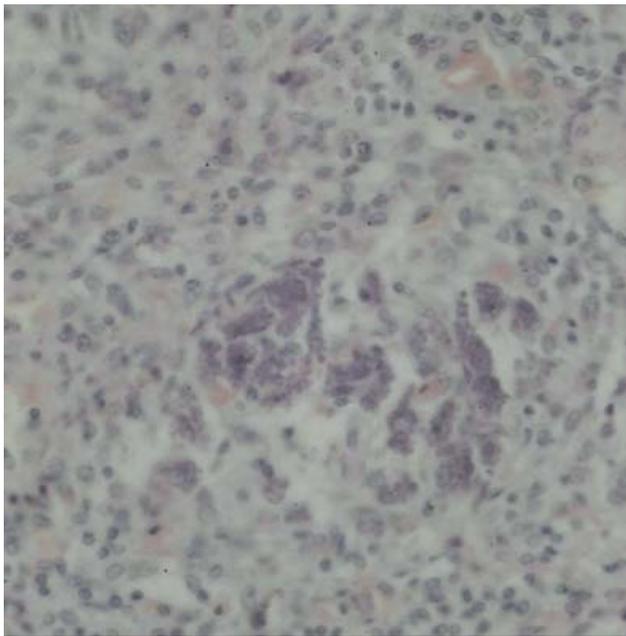


Figure 3. Lymphocytosis, histiocytosis and macrophage proliferation in mesenteric lymph node (400 X).

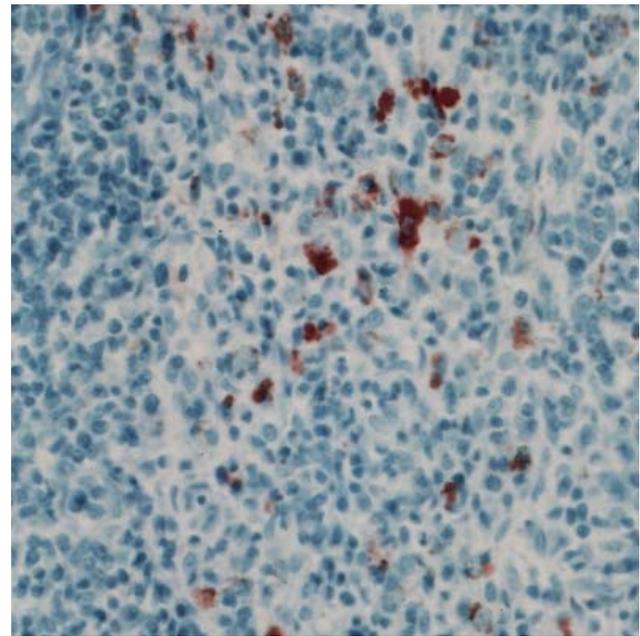


Figure 4. Lymph node with intense positive marking to PCV-2 by immunohistochemical technique (400X).

DISCUSSION

The anamnesis and clinical and histological findings possibly associated with zearalenone contamination and PCV-2 infection in pigs are firstly described in this report. The history, the clinical signs, the necropsy and histological findings suggested a mix of zearalenone mycotoxicosis and PVC-2 syndromes. It was confirmed by PVC-2 tissue detection in immunohistochemical assay, and by food toxicological analysis. Zearalenone concentration of one $\mu\text{g/g}$ cause reproductive alterations in swine [18]. The average founded in stomach contents (8.75 $\mu\text{g/g}$) could produce the reproductive disorders and could has exacerbated the circovirosis. In recently study in Brazil, pre-pu-

bertal gilts fed with 2 $\mu\text{g/g}$ of zearalenone increased significantly the length and weight of the reproductive tract but do not interfered with the performance of animals [2].

Zearalenone contamination can occur in field, transport or storage and the critical period of the alterations, from December 2005 to January 2006, was a summer time, which comprehends corn harvests, concentrated raining and high temperatures. All these factors could favor the fungi growth during this grain storage period, increasing the risks of mycotoxicosis [18].

The most frequent reproductive alteration observed in the farrowing was mummified fetuses that corresponded 66.7% of its products. The several

reproductive disorders as abortion, stillborn, mummified fetuses, estrus returning, vulvae volume and mammary glands enlargement in gilts are frequently related to zearalenone mycotoxicosis [2,18].

In this present report, appetite loss, apathy, wasting, cough and diarrhea and increased mortality and withdrawal rate in growth and post-weaning phases are liked as the several descriptions in the PMWS [6]. Hemorrhagic skin spots and increased kidneys, with multifocal glomerulonephritis, lymphohistiocytic interstitial nephritis suggest several alterations in PDNS [7]. Both syndromes by PCV-2 could be simultaneity occurring in this herd and could have a co-participation of the ZEA ingestion as aggravated factor. Epidemiology studies suggest that PCV-2 infection may also be widespread in the Minas Gerais because of a total 955 tested sera, 935 (96.39%) were reactive in all evaluated livestock [4].

Studies have reported that other microorganism agents as the Porcine Parvovirus (PPV) could be favored the clinical syndromes of PCV-2 [8,12]. In the present case report these virus syndromes could also be considered and the possible viral co-infections, associated with ZEA ingestion, could be exacerbating the reproductive alterations. However before this outbreak were not observed clinical signs of parvovirus in the herd.

Control and preventive measures adopted during the outbreak, as sick animal isolation and withdrawal of sows with reproductive disorders pos-

sibly contributed to a disease control. Meanwhile, after zearalenone ration diagnosis and bad corn to good sorghum (free of zearalenone) exchange at ration, the animals showed clinical improvement and the reproduction returned to normal level. After two months the carcasses or organs were not condemned in slaughters and no lesions by PCV-2 were observed, showing that the ZEA contamination could be as the initial factory to the evolution of the observed lesions.

In this present report was decrypted the detection of these two agents together in the swine herd. The historic and the reported data can suggest the ZEA ingestion as initial or accented factor to PCV-2 syndromes, indicating the importance of the multi-interactions between mycotoxins and viral infections in pigs or other animals.

SOURCES AND MANUFACTURERS

¹Oxitrat, Vallée S.A., São Paulo, SP, Brazil.

²LSAB Kit, Dako, Milan, Italy.

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