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IDENTIFICATION OF PORCINE CIRCOVIRUS TYPE 2 AND PORCINE PARVOVIRUS IN PORCINE STILLBIRTHS AND MUMMIFIED FETUSES FROM SWINE FARMS IN BRAZIL

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ABSTRACT

This study investigated the presence of genome sequences of the porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) in 147 porcine stillbirths and mummified fetuses. These samples, originated from 39 farms located in eight Brazilian states, were collected between 2006 and 2008. Heart and lung fragments were used for extraction of total DNA and later amplification of correspondent fragments of the virus pathogens through polymerase chain reaction (PCR) technique. Out of 147 samples, 74 (50.3%) were positive for PCV2 while nine samples (6.2%) were positive for PCV2 and PPV. None of the samples were positive just for PPV. Out of 39 investigated farms, 21 (53.8%) had fetuses positive for PCV2 while co-infection with PCV2 and PPV was detected in 3 farms (7.7%). These results indicate that PCV2 could be an important infection agent in cases of porcine stillbirths and mummified fetuses in Brazil and must be included in differential diagnostic list.

KEYWORDS: porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), polymerase chain reaction (PCR), reproductive failure, swine.

RESUMO

IDENTIFICAÇÃO DO CIRCOVÍRUS SUÍNO TIPO 2 E DO PARVOVÍRUS SUÍNO EM FETOS SUÍNOS NATIMORTOS E MUMIFICADOS PROVENIENTES DE GRANJAS NO BRASIL

Foi investigada a presença de seqüências genômicas do circovírus suíno tipo 2 (PCV2) e do parvovírus suíno (PPV) em 147 fetos suínos natimortos e mumificados. Estas amostras, provenientes de 39 granjas localizadas em oito estados brasileiros, foram coletadas entre os anos de 2006 a 2008. Foram utilizados fragmentos de coração e pulmão para extração do DNA total e posterior amplificação de fragmentos correspondentes aos patógenos virais pela técnica de reação em cadeia da polimerase (PCR). Entre as 147 amostras, 74 (50,3%) foram positivas ao PCV2 enquanto nove amostras (6,2%) apresentaram co-infecção com o PCV2 e o PPV. Nenhuma amostra foi positiva apenas para PPV. Entre as 39 granjas estudadas, 21 (53,8%) apresentaram fetos positivos ao PCV2 enquanto que co-infecção com o PCV2 e o PPV foi detectada em três (7,7%). Estes resultados indicam que o PCV2 pode ser um importante agente infeccioso causador de morte embrionária e fetal em suínos no Brasil e deve ser incluído na lista de diagnóstico diferencial.

PALAVRAS-CHAVE: suíno, circovírus suíno tipo 2 (PCV2), parvovírus suíno (PPV), falhas reprodutivas, reação em cadeia da polimerase (PCR).

INTRODUCTION

Reproductive failure in pigs, such as return to estrus, abortion, embryonic and fetal deaths, undermine commercial farms´ goals of producing born-alive and / or weaned piglets. Thus, these reproductive failures can cause economic losses in swine production.

The causes of reproductive failure in pigs may be infectious or noninfectious (HOLLER, 1994). Among non-infectious causes, multiple management procedures can influence the reproductive performance of the herd, while infectious complications may be caused mainly by bacterial and viral agents (ALMOND, 2006). Among the most common viral infectious agents detected in stillborn, mummified and aborted fetuses are the porcine parvovirus (KIM et al., 2004) and the porcine reproductive and respiratory syndrome virus (MALDONADO et al., 2005). Recent studies have associated porcine circovirus type 2 (PCV2) with reproductive failures with direct effects on embryo or fetus (WEST et al. 1999; SANCHEZ et al., 2001, JOHNSON et al. 2002; MATEUSEN et al. , 2004; PARK et al. 2005; MATEUSEN et al., 2007, HANSEN et al., 2010). Among these infectious agents mentioned, it is worth noting that Brazil is considered free from the porcine reproductive and respiratory syndrome virus (BRAZIL, 2004).

The PCV2, a member of the Circoviridae family, is widespread within swine population (SEGAL et al., 2005) and has been associated with other diseases, such as post-weaning multisystemic wasting syndrome or PMWS (ELLIS et al., 1999), dermatitis and nephropathy syndrome (ROSELLE et al., 2000), porcine respiratory disease complex (KIM et al., 2003), increase of pre-weaning mortality in piglets (BRUNBORG et al., 2007) and immune suppression (KAICHUANG et al., 2008).

Due to the variety of PCV2-related diseases, many studies have been performed around the world. In Brazil, the number of studies related to PCV2 has grown since the first report of post-weaning multisystemic wasting syndrome (CIACCI-ZANELLA, 2003). However, only one study regarding PCV2 and reproductive failure has been found in Brazil, and in this study the PCV2 infectious agent was regarded as of little importance (FISHER et al., 2007). On the other hand, in other countries, studies have reported increased rates of miscarriage, return to estrus and mummified and stillborn fetuses related to PCV2 (WEST et al. 1999; BRUNBORG et al., 2007). ZIZLAVSKY et al. (2008) reported PCV2 as the main infectious agent detected in swine fetuses in the Czech Republic.

The aim of this study was to investigate, by polymerase chain reaction (PCR), the presence of DNA of porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) in stillbirths and mummified fetuses.

MATERIAL AND METHODS

To investigate the presence of DNA fragments of PCV2 and PPV 147 stillbirths and mummified fetuses were used. They were collected between June 2006 and June 2008. The samples came from 39 commercial farms in the main regions of swine production in Brazil: Minas Gerais (14), Paraná (12), Santa Catarina (6), Rio Grande do Sul (2), Goiás (2), Bahia (1), Rio de Janeiro (1) and Espírito Santo (1). These farms had herds presenting from 150 to 3000 sows with full production cycle or piglets production unit. The herds in these commercial farms were vaccinated against PPV but not against PCV2.

The fetuses were stored in labeled plastic bags, frozen at -20oC and then sent in isothermal boxes to Microvet Laboratory, located in Viçosa-MG. The transportation period to the laboratory ranged from 24 to 48 hours. In the laboratory, the fetuses were autopsied with sterile equipment to remove fragments of heart and lung.

Total DNA extraction was performed by means of the standard phenol-chloroform method, suggested by Davis et al. (1994), with modifications. Fragments of heart and lung of each fetus were macerated with subsequent addition of 3 to 5 mL of phosphate buffer (0.04 M Na2HPO4, 0.01 M KH2PO4, pH 7.4) for homogenization. Part of the suspension (1.5 ml) was transfered to Eppendorf tubes and centrifuged at 10,000 xg for 10 minutes. The supernatant was discarded and resuspended in 1.0 ml of phosphate buffer

with the addition of 125 µl of SDS (sodium dodecyl sulfate) at 10%, mixed by inversion and incubated in a water bath at 65 oC for 30 minutes.

After this phase, 350 µl of 8 M potassium acetate were added, mixed by repeated inversions and incubated on ice for 60 minutes. The precipitate was centrifuged at 10,000 xg for 15 minutes at 12 ºC. The supernatant was transferred to another Eppendorf tube and a volume of phenol / chloroform (1:1) was added. After homogenization by repeated inversion, the phases were separated by centrifugation for 15 minutes at 10,000 xg, and the aqueous phase was collected and added to a volume of chloroform (Merck), mixed by repeated inversion, and the phases separated by centrifugation for 15 minutes at 10,000 xg one more time. The supernatant was collected and added to two volumes of ethanol to precipitate total DNA. Centrifugation for 10 minutes at 15,000 xg was performed to obtain the sediment, which was washed with 150 µl of 70% ethanol. The ethanol was discarded and the sediment dried at room temperature for 30 minutes. It was resuspended in 30 µl TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). After concentration assessment by absorbance at 260 nm using Ultrospec 1100 pro spectrophotometer (Amersham Biosciences), total DNA was diluted to a concentration of 50 ng / mL and stored at -80 °C until use. An aliquot part of total DNA was applied on 0.8% agarose gel to verify the integrity of the total DNA.

The amplification of DNA fragments specific to PCV2 and PPV was performed in duplex PCR assay followed by the method recommended by KIM et al. (2003) (Table 1). Amplifications were performed in 50 µl of reaction mixture containing 50 ng of total DNA, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.25 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 1 µM of each oligonucleotide and 2.5 U Taq DNA polymerase (Invitrogen). The amplification program, in a thermocycler Mastercycler gradient (Eppendorf), consisted of one step of 94 °C for 1 min., followed by 30 cycles of 55 °C for 1 min., 72 °C for 3 min., and ended with an extension step of 72 °C for 4 min. Amplification products were applied on 1.5% agarose gel in the presence of ethidium bromide and submitted to electrophoresis at 60 V and photographed under UV light. The tests were performed with positive and negative controls for validation.

TABLE 1. Oligonucleotides used in amplification of DNA fragments of porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) and its characteristics

Vírus	Oligonucleotides and sequence (5' para 3')	Position in genome	Product Size	Reference
PCV2	D CGGATATTGTAGTCCTGGTCG	1095-1115	481 pb	ELLIS et al., 1999
	R ACTGTCAAGGCTACCACAGTCA	1570-1549		
PPV	D CCAGCAGCTAACACAAGAAAAGGTTATCAC	3708-3730	226 pb	ARNAULD et al., 1998
	R GTCCATGTTGGTAATCCATTGTAAATC	3907-3933		

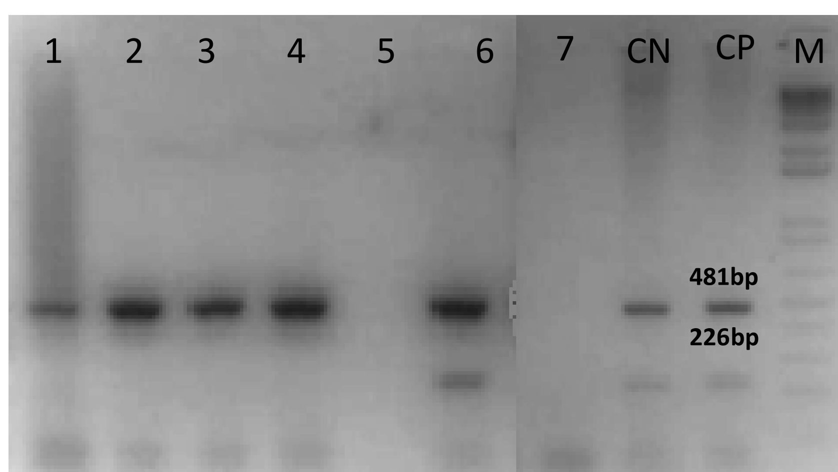
D - Direct R - Reverse

RESULTS AND DISCUSSION

Among the 147 stillbirths and mummified fetuses investigated, the presence of at least one viral agent was detected in 83samples (56.5%), while 64 samples (43.5%) were negative for the infectious agents investigated. PCV2 was detected in 74 samples (50.3%), whereas nine samples (6.2%) had co-infection with PCV2 and PPV. No sample was positive for PPV. Among the 39 commercial farms studied, 21 (53.8%) fetuses were positive for PCV2, and co-infection with PCV2 and PPV was detected in three (7.7%) farms (Table 2).

TABLE 2. Presence of porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) in 147 stillborn and mummified fetuses of pigs from 39 commercial farms in Brazil between 06/2006 and 06/2008.

Viral agent	Samples			Commercial farms		
	Total	Positive	Percentage of positive samples	Total	Positives	Percentage of positive samples
PCV2	147	74	50.3	39	21	53.8
PPV	147	0	0.0	39	0	0.0
PCV2 and PPV	147	9	6.2	39	3	7.7
At leas one viral agent	147	83	56.5	39	24	61.5



Samples 1,2,3,4 = duplex PCR positive for PCV2 and negative for PPV; Sample 5 = duplex PCR negative for PCV2 and PPV; Samples 6, 7 = duplex PCR positive for PCV2 and PPV; PC = positive control duplex PCR for PCV2 and PPV, CN = negative control duplex PCR for PCV2 and PPV, M = molecular marker 1 Kb plus DNA ladder (Invitrogen).

FIGURE 1. Agarose gel electrophoresis of products of polymerase chain reaction (PCR duplex) for porcine circovirus type 2 (PCV2) and porcine parvovirus (PPV) of pieces of heart and lungs of stillborn and mummified fetuses.

The frequency of PCV2 detection in stillborn, mummified and aborted fetuses in the studies carried out in Brazil and abroad varied considerably. The results of this study are similar to those found by ZIZLAVSKY et al. (2008). In this study performed in the Czech Republic, PCV2 was considered the main infectious agent detected in fetuses between the years 2005 to 2007, with detection frequency ranging from 21.7% to 54.1%. KIM et al. (2004) reported PCV2 frequency of 13.1% of 350 mummified, stillbirths and aborted fetuses in a study performed in South Korea. However, other studies reported lower PCV2 detection frequency in pig fetuses (MALDONADO et al. 2005; FISHER et al., 2007). In Brazil, FISHER et al. (2007) reported only 5.7% of samples positive for PCV2 in a total of 121 fetuses studied. MALDONADO et al. (2005) reported that PCV2 is probably not an important pathogen related to abortions, even in Spain where the weaning multisystemic wasting syndrome is widespread.

Several factors can influence the results of studies of PCV2 frequency in stillborn, mummified and aborted fetuses, such as the technique used for the agents detection (KIM et al. 2004; PARK et al., 2005, HANSEN et al., 2010), the selection of fetuses for diagnosis (PARK et al. , 2005), the fetal organ selected to detect the viral agent (SANCHEZ et al. 2003; KIM et al. 2004; PARK et al., 2005), gestational age (SAN-

CHEZ et al., 2001), the clinical phase of the disease (HANSEN et al., 2010) and the use of vaccination programs against PCV2.

The simultaneous detection of PCV2 and PPV by duplex PCR assay used in this study is an important diagnostic tool, since the PPV infectious agent is the most related to reproductive failure in swine associated with the growing involvement of PCV2 in such cases. According to KIM et al. (2003), the primers for PPV did not affect the response to PCV2 and vice versa.

In this study, heart and lung fragments of stillborn and mummified fetuses of different sizes were used. The concentration of PCV2 and PPV in fetal tissue varies according to the gestational age, as well as among the organs of the same fetus, being the heart and lungs the fetal organs which show greater concentrations of PCV2 and PPV, respectively (Sanchez et al . 2001; SANCHEZ et al. 2003; MENGELING et al., 2006). It is noteworthy that PARK et al. (2005) demonstrated that PCV2 can cause miscarriage; however, some fetuses may not show histopathological lesions and the presence of PCV2 DNA may not be detected.

The results of PCV2 detection in this study demonstrated that vertical transmission may be an important route of infection. Studies have shown that fetuses exposed to PCV2 during pregnancy may be born alive, carry the virus and present the clinical manifestation of post-weaning multisystemic wasting syndrome (SANCHEZ et al. 2004; ROSE et al., 2007). The PCV2 can be transmitted by semen and oocytes of seropositive breeders without clinical signs of post-weaning multisystemic wasting syndrome (LAROCHELLE et al. 2000; BIELANSKI et al., 2004, SCHMOLL et al., 2008). GAVA et al (2008) reported that PCV2 can be transmitted through semen to the fetus during pregnancy. ROSE et al. (2007) demonstrated that vaccination of matrices for the PPV significantly reduces the number of mummified fetuses in matrices experimentally infected with PCV2.

The detection of PCV2 and PPV in mummified and stillborn fetuses observed in this study may indicate that these viruses are the cause of fetal death. Reproductive failure associated with PCV2 and PPV were experimentally reproduced, demonstrating the susceptibility of embryos and fetuses to infection, the dissemination and intrauterine vertical transmission, possibly causing the interruption of pregnancy (SANCHEZ et al. 2001; SANCHEZ et al. 2003; MATEUSEN et al. 2004; PENSAERT et al. 2004; PARK et al. 2005; MENGELING, 2006; MATEUSEN et al. 2007; ROSE et al. 2007; PITTMAN et al., 2008). Authors have demonstrated that co-infection of fetuses with PCV2 and PPV may exacerbate the lesions (FISHER et al., 2007). Histologic changes, such as necrotizing or fibrous myocarditis, can be caused by both PCV2 and PPV, thus only the histopathology does not allow the differential diagnosis (HANSEN et al., 2010). For the diagnosis of reproductive failures associated with PCV2 and PPV, authors suggest to correlate clinical findings such as increased rates of stillborn, mummified and aborted fetuses associated with compatible histopathological lesions, and in situ detection of PCV2 and PPV (PARK et al ., 2005; MENGELING, 2006, SEGAL et al., 2006, HANSEN et al., 2010).

The low frequency of PPV detection in this study agrees with other authors´findings (MALDONADO et al. 2005; FISHER et al., 2007). FISHER et al. (2007) reported 2.4% of stillbirths and mummified fetuses positive for PPV in 121 fetuses from nine commercial farms in southern Brazil, while MALDONADO et al. (2005) did not detect the presence of PPV in 293 stillborn and aborted fetuses in Spain. Despite being ubiquitous in the swine population around the world (MENGELING, 2006), authors suggest that the widespread use of vaccine to control PPV infection in many countries is an important tool for the control of reproductive problems caused by this agent (MALDONADO et al. 2005; MENGELING, 2006). Authors reported that these vaccines were effective when tested in field conditions and in experimental infections (MENGELING, 2006). All fetuses evaluated in this study were from farms with vaccination programs for the PPV.

The results observed in this work associated to studies that relate PCV2 to reproductive failure provide information so that PCV2 can be investigated in the list of differential diagnosis of commercial farms with

a history of reproductive failure. On the other hand, the low frequency of PPV detection and the wide use of vaccines to prevent PPV infection by this agent in Brazilian swine herds do not exclude this agent from the list of differential diagnosis, since it is considered endemic in pig population and it has been listed among the most important infectious agents related to reproductive failure for decades.

CONCLUSION

The PCV2 was detected in 56.5% of stillbirths and mummified fetuses and it should be considered in the differential diagnosis of commercial farms with a history of reproductive failure.

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REFERENCES

ALMOND, G. W.; FLOWERS, W. L.; BATISTA, L.; D'ALLAIRE, S. Diseases of the Reproductive System. In: STRAW, B.; D'ALLAIRE, S.; TAYLOR, D.; ZIMMERMAN, J. (Eds.). **Diseases of Swine**. Ames, Iowa: Iowa State Univ Pr, 2006. p. 113-148.

ARNAULD, C.; LEGEAY, O.; LAURIAN, Y.; THIERY, R.; DENIS, M.; BLANCHARD, P.; JESTIN, A. Development of a PCR based-method coupled with a microplate colorimetric assay for the detection of porcine parvovirus and application to diagnosis in piglets tissues and human plasma. **Molecular and Cellular Probes**, v. 12, p. 407-416, 1998.

BIELANSKI, A.; LAROCHELLE, R.; MAGAR, R. An attempt to render oocytes and embryos free from the porcine circovirus type 2 after experimental in vitro exposure. The **Canadian Journal of Veterinary Research**, V. 68, P. 222-225, 2004.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 47, de 18 de junho de 2004. **Diário Oficial [da] República Federativa do Brasil**. Brasília, DF, 2004, n. 119, seção 1.

BRUNBORG, I.; JONASSEN, C.; MOLDAL, J.; BRATBERG, B.; LIUM, B.; KOENEM, F.; SCHONHEIT, J. Association of myocarditis with high viral load of porcine circovirus type 2 in several tissues in cases of fetal death and high mortality in piglets: a case study. **Journal of Veterinary Diagnostic Investigation**, v. 19, p. 368-375, 2007.

CIACCI-ZAENLLA, J. R.; MORES, N. Diagnostic of post-weaning multisystemic wasting syndrome (PMWS) in swine in Brazil caused by porcine circovirus type 2 (PCV2). **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 55, p. 522-527, 2003.

DAVIS, L.; KUEL, W. M.; BATTEY, F. J. Rapid Preparation of Genomic DNA from Tissue or Cultured Cells. In: **Basic methods in molecular biology**. 2. ed. Norwalk: Appleton & Lange, 1994. p. 307-309.

ELLIS, J.; KRAKOWKA, S.; LAIRMORE, M.; HAINES, D.; BRATANICH, A.; CLARK, E.; ALLAN, G.; KONOBY, C.; HASSARD, L.; MEEHAN, B.; MARTIN, K.; HARDING, J.; KENNEDY, S.; McNEILLY, F. Reproduction of lesions of postweaning multysistemic wasting syndrome in gnotobiotic piglets. **Journal of Veterinary Diagnostic Investigation**, v. 11, p. 3-14, 1999.

GAVA, D.; ZANELLA, E.; MORES, N.; CIACCI-ZANELLA, J. Transmission of porcine circovirus 2 by semen and viral distribution in different piglet tissues. **Pesquisa Veterinária Brasileira**, v. 28, n. 1, p. 70-76, 2008.

HANSEN, S. M.; HJULSAGER, C. K.; HANSEN, B. V.; HAUGEGAARD, S.; DUPONT, K.; HØGEDAL, P.; KUNSTMANN, L.; LARSEN, L. E. Selection of method is crucial for the diagnosis of porcine circovirus type 2 associated reproductive failures. **Veterinary Microbiology**, v. 144, p. 203-209, 2010.

HOLLER, L. D. Diagnosis of swine abortions. **Swine Health and Production**, v. 2, p. 29-31, 1994.

JOHNSON, C.; JOO, H.; DIREKSIN, K.; YOON, K-J.; CHOI, Y. K. Experimental in utero inoculation on late-term swine fetuses with porcine circovirus type 2. **Journal of Veterinary Diagnostic Investigation**, v. 14, p. 507-512, 2002.

KAICHUANG, S.; HUANRONG, L.; XIN, G.; XINNA, G.; HONG, J.; SHIJUN, Z.; HANCHUN, Y. Changes in peripheral blood leukocyte subpopulations in piglets co infected experimentally with porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. **Veterinary Microbiology**, v. 129, p. 367-377, 2008.

KIM, J.; HAN, D.; CHOI, C.; CHAE, C. Simultaneous detection and differentiation between porcine circovirus and porcine parvovirus in boar semen by multiplex seminested polymerase chain reaction. **Journal of Veterinary Medical Science**, v. 65, n. 6, p. 741-744, 2003.

KIM, J.; JUNG, K.; CHAE, C. Prevalence of porcine circovirus type 2 in aborted fetuses and stillborn piglets. **Veterinary Record**, v. 155, p. 489-492, 2004.

LAROCHELLE, R.; BIELANSKI, A.; MULLER, P.; MAGAR, R. PCR detection and evidence of shedding of porcine circovirus type 2 in boar semen. **Journal of Clinical Microbiology**, v. 38, n. 12,

p. 4629-4632, 2000.

MALDONADO, J.; SEGALÉS, J.; MARTINEZ-PUIG, D.; CALSAMIGLIA, M.; RIERA, P.; DOMINGO, M.; ARTIGAS, C. Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain. **Veterinary Journal**, v. 169, p. 454-456, 2005.

MATEUSEN, B.; SANCHEZ, R.; VAN SOOM, A.; MEERTS, P.Ç MAES, D. G. D.; NAUWYNK, H. J. Susceptibility of pig embryos to porcine circovirus type 2 infection. **Theriogenology**, v. 61, p. 91-101, 2004.

MATEUSEN, B.; MAES, D.; VAN SOOM, A.; LEFEBVRE, D.; NAUWYNCK, H. J. . Effect of a porcine circovirus type 2 infection on embryos during early pregnancy. **Theriogenology**, v. 68, p. 896-901, 2007.

MENGELING, W. L. Porcine parvovirus. In: STRAW, B.; D'ALLAIRE, S.; TAYLOR, D.; ZIMMERMAN, J. (Eds.). **Diseases of swine**. Ames, Iowa: Iowa State University Press, 2006. p. 373-385.

PARK, J. S.; KIM, Y.; JUNG, K.; CHOI, C.; LIM, J-K.; KIM, S-H; CHAE, C. Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. **Journal of Comparative Pathology**, v. 132, p. 130-144, 2005.

PENSAERT, M. B.; SANCHEZ, R. E.; LADEKJAER-MIKKELSEN, A. S.; ALLAN, G.; NAUWYNCK, H. J. Viremia and effect of fetal infection with porcine viruses with special reference to porcine circovirus 2 infection. **Veterinary Microbiology**, v. 98, p. 175-183, 2004.

PESCADOR, C.; BANDARRA, P.; CASTRO, L.; ANTONIASSI, N. A. B.; RAVAZZOLO, A. P.; SONNE, L.; CRUZ, C. E. F.; DRIEMEIER, D. Co-infection by porcine circovirus type 2 and porcine parvovirus in aborted fetuses and stillborn piglets in southern Brazil. **Pesquisa Veterinária Brasileira**, v. 27 n. 10, p. 425-429, 2007.

PITTMAN, S. J. Reproductive failure associated with porcine circovirus type 2 in gilts. **Journal of Swine Health and Production**, v. 16, n. 3, p. 144-148, 2008.

ROSE, N.; BLANCHARD, P.; CARIOLET, R.; GRASLAND, B.; AMENNA, N.; OGER, A.; DURAND, B.; BALASCH, M.; JESTIN, A.; MADEC, F. Vaccination of porcine circovirus type 2 (PCV2) infected sows against porcine parvovirus (PPV) and erysipelas: effect on post-weaning multisystemic wasting syndrome (PMWS) and on PCV2 genome load in the offspring. **Journal of Comparative Pathology**, v. 136, p. 133-144, 2007.

ROSELL, C.; SEGALÉS, J.; FOLCH, J. M.; RAMOS-VARA, J. A.; RODRIGUEZ-ARRIOJA, G. M.; DOMINGO, M.; DURAN, C. O.; BALASCH, M.; PLANA-DURAN, J. Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nefropathy syndrome. **Veterinary Record**, v. 146, p. 40-43, 2000.

SANCHEZ, R. E.; MEERTS, P.; NAUWYNCK, H. J.; ELLIS, J. A.; PENSAERT, M. B. Characteristics of porcine circovirus 2 replication in lymphoid organs of pigs inoculated in late gestation or postnatally and possible relation to clinical and pathological outcome of infection. **Journal of Veterinary Diagnostic Investigation**, v. 16, p. 175-185, 2004.

SANCHEZ, R. E.; MEERTS, P.; NAUWYNCK, H. J.; PENSAERT, M. B. Change of porcine circovirus 2 target cells in pigs during development from fetal to early postnatal life. **Veterinary Microbiology**, v. 95, p. 15-25, 2003.

SANCHEZ, R. E.; NAUWYNCK, H.; MCNEILLY, F.; ALLAN, G.; PENSAERT, M. B. Porcine circovirus 2 infection in swine fetuses inoculated at different stages of gestation. **Veterinary Microbiology**, v. 83, p. 169-176, 2001.

SCHMOLL, F.; LANG, C.; STEINGIGL, A. S.; SCHULZE, K.; KAUFFOLD, J. Prevalence on PCV2 in Austrian and German boars and semen used for artificial insemination. **Theriogenology**, v. 69, p. 814-821, 2008.

SEGALÉS, J.; ALLAN, G. M.; DOMINGO, M. Porcine circovirus diseases. **Animal Health Research Reviews**, v. 6, p. 119-142, 2005.

SEGALÉS, J.; ALLAN, G. M.; DOMINGO, M . Porcine circovirus diseases. In: STRAW, B.; D'ALLAIRE, S.; TAYLOR, D.; ZIMMERMAN, J. (Eds.). **Diseases of swine**. Ames, Iowa: Iowa State University Press, 2006. p. 299-307.

WEST, K. H.; BYSTROM, J. M.; WOJNAROWICZ, C.; SHANTZ, N.; JACOBSON, M.; ALLAN, G.; HAINES, D. M.; CLARK, E.; KRAKOWKA, S.; McNEILLY, F.; KONOBY, C.; MARTIN, K.; ELLIS, J. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. **Journal of Veterinary Diagnostic Investigation**, v. 11, p. 530-532, 1999.

ZIZLAVSKY, M.; CZANDERLOVA, L.; GAMPOVA, M.; KELLNEROVA, D.; TYDLITAT, D.; DRABEK, J. Significant role of PCV2 in reproductive disorders of sows. In: INTERNATIONAL PIG VETERINARY SOCIETY CONGRESS, 20., 2008, Durban, África do Sul. **Anais**… Durban: IPVS, 2008.

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