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Reproductive failure in Zovawk associated with porcine circovirus-2

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Abstract
One of the main reasons for the decline in the swine population is reproductive failure. Porcine circovirus (PCV), the cause of PCV2-related reproductive failure, is characterised by acute systemic sickness in the dam, abortion, mummified foetuses, stillbirths, or weak-born piglets. The virus primarily attacks lymphoid tissues, causing lymphoid depletion and immunosuppression in pigs. The purpose of this study was to investigate the pathology and molecular diagnosis of porcine circovirus-2-associated reproductive failure in Zovawk. The PCV was detected using primers to amplify the ORF2 region. The most common gross lesions observed in the study were hydrothorax and or ascities, an enlarged and congested liver, enlarged lymph nodes with mild congestion, and pneumonic lungs. Renomegaly and splenomegaly were also observed. Histopathologically, the lungs showed interstitial pneumonia, loss of hepatocytes, hydropic degeneration, and mononuclear infiltration in the liver. The kidneys showed hydropic degeneration of the tubular epithelial cells with vascular congestion and haemorrhages in the inter-tubular spaces. The spleen revealed mild to severe lymphoid depletion.

Keywords: Porcine circovirus, porcine circovirus associated disease, reproductive failure, swine

Introduction
The swine population is important to India's socioeconomic condition, particularly in the north-eastern region. According to the 20th Quinquennial Livestock Census, India has a total pig population of 9.06 million pigs. Various health issues impact the swine population, with reproductive issues being one of the most serious. Although many bacteria and parasites have been shown to cause reproductive diseases in the pig population, viral causes have been found to play a significant role in creating reproductive problems in pigs (Givens and Marley, 2008; Meng, 2013) [1, 2]. Porcine circovirus (PCV), a single-stranded DNA virus from the Circoviridae family, is what causes PCV2-related reproductive failure (Tischer, 1982) [3] and may clinically result in acute systemic illness in the dam, abortion, increased nonviable foetuses at parturition (mummified and stillborn piglets), or weak-born piglets (O'Connor *et al.*, 2001; Park *et al.*, 2005; West *et al.*, 1999) [4, 5, 6]. West and co-workers [6] reported the first incidence of PCV2-associated reproductive failure in pigs. The virus has been reported in many different countries in Asia, Europe, North America, Oceania, and South America (Patterson and Opriessnig, 2010; Li *et al.*, 2021; Franzo *et al.*, 2022; Franzo *et al.*, 2020) [7, 8, 9, 10] and has also been reported recently in India (Sharma, 2007; Rajkhowa, 2008; Bhattacharjee, 2021) [11, 12, 13]. Diagnostic techniques like PCR, virus isolation, immunohistochemistry, and in situ hybridization can be used to detect PCV (Segalés, 2012) [14]. The majority of livestock farmers are dependent on a healthy pig population for their livelihood. Pig rearing is an essential agricultural activity of small farmholders that influences socioeconomic development in all North Eastern states. In view of the above facts, the current study was designed to investigate the pathological and molecular detection of porcine circovirus 2-associated reproductive failure in the Zovawk breed of pig.

Material and Method
Detection of PCV by polymerase chain reaction (PCR)
The ORF2 gene region of PCV2 was amplified using the published primer sequences PCVLF 5'-TAGGTTAGGGCTGTGGCCTT-3'and PCVLR 5'-CCGCACCTTCGGATATACTG-3'amplified a target of fragment size 263bp (Larochelle *et al.*, 1999) [15] and the PCR mixture

as mentioned in Table 1. The contents were properly mixed by brief spinning and incubated in a thermal cycler. Cycling conditions were as follows: one cycle of initial denaturation at 95 °C for 5 min, followed by 30 cycles of each of denaturation at 95 °C for 45 secs, annealing at 60 °C for 45 s, extension at 72 °C for 1min, and a final elongation step at 72 °C for 5 mins, which completed the reaction. Agarose gel electrophoresis using 1.5% agarose gel containing ethidium bromide was used to confirm PCR results.

Table 1: PCR mixture

PCR Reagents	Amount
10X PCR buffer	2.5 µl
Forward primer (20 pmol/µl)	1.0 µl
Reverse primer (20 pmol/µl)	1.0 µl
10 mMdNTPs	0.60 µl
DNA	1 µl (approx. 750 ng)
Taq DNA polymerase (5U/µl) (Fermentas)	0.3 µl
Nuclease free water to make	25 µl

Histopathological examination

Tissue samples of aborted foetuses, stillborns and weak-born piglets were collected from samples that were brought to the postmortem hall, Department of Veterinary Pathology, Selesih, Mizoram. The formalin-fixed tissues were processed by the paraffin wax embedding method of tissue sectioning. The sections were cut at 4-5 micron thickness and stained with haematoxyline and eosin (H & E) stain for histopathological examination (Luna, 1968) [16].

Results and Discussion

A PCR based on ORF2 primers flanking a 263-bp region could be detected (Fig. 1) in 4 out of 12 PCV2 suspected cases (33.34%). A similar report on the PCR amplification of the particular sequence of PCV2 using type-specific primers (Boilin *et al.*, 2001; Rudan *et al.*, 2009 [17, 18]. PCV2-associated diseases are now recognised as one of the most economically important global issues affecting growing swine, with up to 20% mortality possible (Segales 2012) [14]. The severity of infection can be complicated by common co-infections with other viruses or bacteria (Bandrick *et al.*, 2022) [19].

The present study showed typical clinical signs of PCV2-associated reproductive failure like abortion and stillborn piglets as the major clinical manifestations in the farm, as has also been reported by other workers (West *et al.*, 1999; Patterson AR and Opriessnig, 2010; Opriessnig, 2007) [6, 17, 20]. A postmortem of 12 PCV2 suspected cases of aborted, stillborn, or weak-born piglets was conducted (Figs. 2 and 3). Ascites and hydrothorax were common findings. In some of the cases, ascites alone was observed and in some, hydrothorax alone (Fig. 4). Mostly the lungs were pale in appearance, but some of them showed congestion with varying degrees of mottling (Fig. 5). In the majority of the cases, the liver was congested, and hepatomegaly was mostly observed (Fig. 6). Kidneys mostly showed congestion, and the capsules of the kidneys were easily peeled off. Renomegaly was also observed in some of the cases (Fig. 7). Splenomegaly was commonly observed, while in some cases the spleen showed mild to severe congestion (Fig. 8). Such findings on the gross lesions on PCV2 associated cases of abortion, stillbirth, weak-born piglets, and other PCVAD were also reported (Sharma, 2007; Rajkhowa, 2008; Segalés, 2012; Segales and Domingo, 2002; O'dea, 2010; Rosell *et al.*, 1999; Brunborg *et al.*, 2004) [11, 12, 14, 21, 22, 23].

On histopathological examination, changes in the lungs were characterised by severe interstitial pneumonia in atelectic lungs. There was fibrinous exudate in the alveolar space with an accumulation of mononuclear cells. Interalveolar septae were thickened due to severe infiltration with mononuclear cells. Congestion and haemorrhages were also observed in the lung sections (Fig. 9). Microscopical changes in the liver included severe hydropic degeneration and loss of hepatocytes, mononuclear infiltration in portal areas, congestion, and haemorrhages (Fig. 10). In the kidneys, hydrophilic degeneration of the tubular epithelial cells with vascular congestion and haemorrhages in the inter-tubular spaces was observed (Fig. 11). Microscopically, the spleen revealed mild to severe lymphoid depletion (Fig. 12). The present study showed typical clinical signs of PCV2-associated reproductive failure like abortion and stillborn piglets as the major clinical manifestations, as also reported by other workers (West *et al.*, 1999; Kim *et al.*, 2004; Opriessnig *et al.*, 2007) [6, 24, 25]. As reported by O'connor *et al.* (2001) [4] some weak-born, non-viable neonatal piglets were also observed; most of the aborted and stillborn piglets showed oedematous swelling with ascites and hydrothorax as a consistent finding. Hydropericardium was also seen in many of the cases. Similar changes were also reported (Segales, 2012; Brunborg *et al.*, 2004; Johnson *et al.*, 2002; [14, 23, 26]. The clinical course of the disease is determined by the timing of infection. According to a study conducted by Sanchez and co-workers (2001) [27] foetuses inoculated at 57 days of gestation had higher viral replication than those infected later in gestation and at 21 days postinoculation, the foetuses showed edoema, enlarged livers, and congestion, whereas foetuses inoculated at 75 and 92 days of gestation failed to produce similar lesions or viral loads. Late-term infections at 86, 92 and 93 days of gestation caused an increase in reproductive abnormalities, including stillbirth, mummified fetuses and weak-born piglets (Johnson *et al.*, 2002) [26].

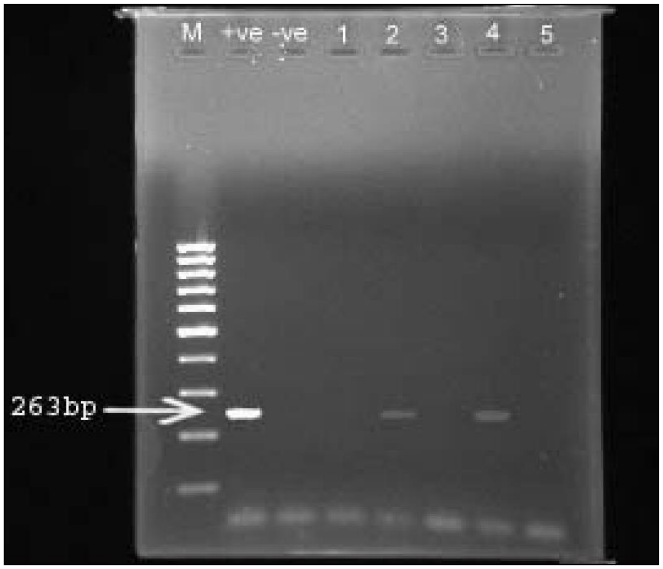

Fig 1: Agarose gel electrophoresis of PCR-amplified ORF2 gene (263 bp) M: DNA 100 bp ladder, Positive control, Negative control, 1-5 test samples

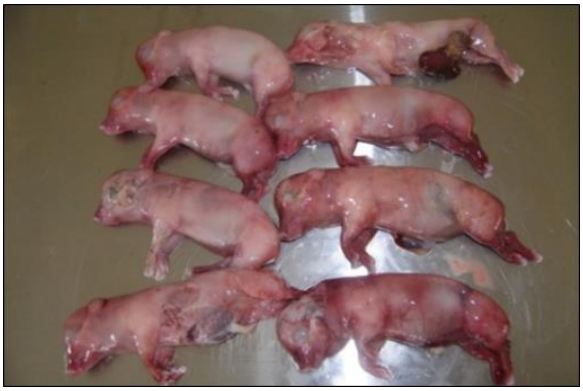

Fig 2: Aborted foetuses

Fig 3: Weakborn piglet

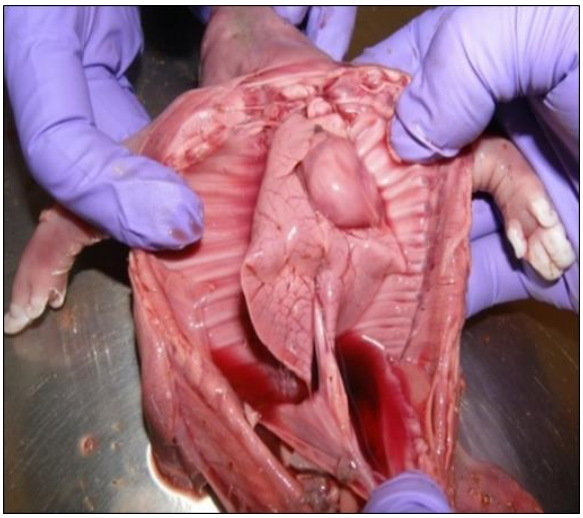

Fig 4: Hydrothorax in aborted foetus

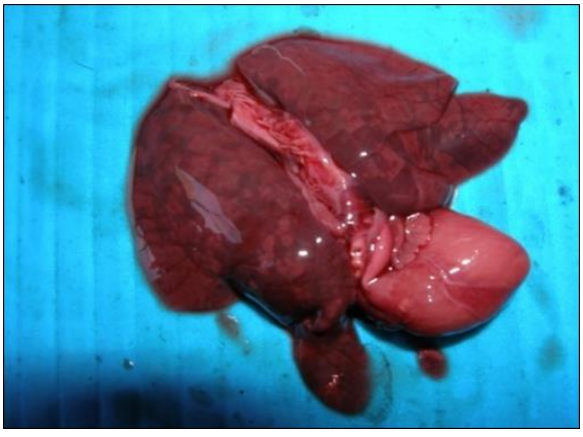

Fig 5: Lungs from aborted foetus showing severe congestion

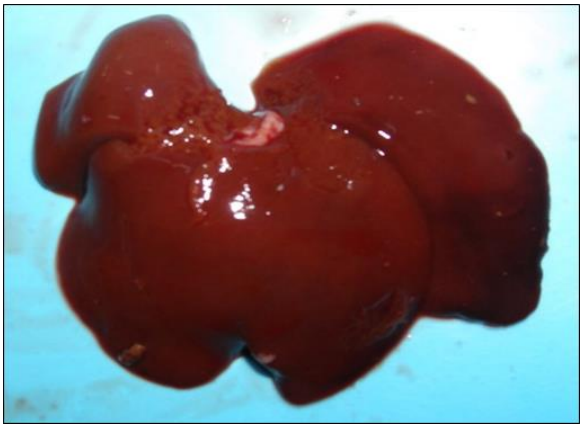

Fig 6: Liver from stillborn piglet showing severe congestion

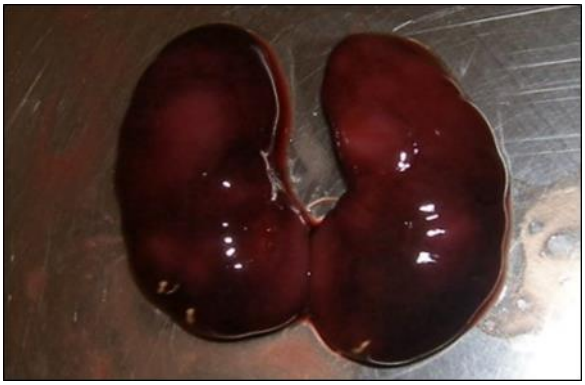

Fig 7: Kidneys from Weakborn piglet-enlarged and congestion

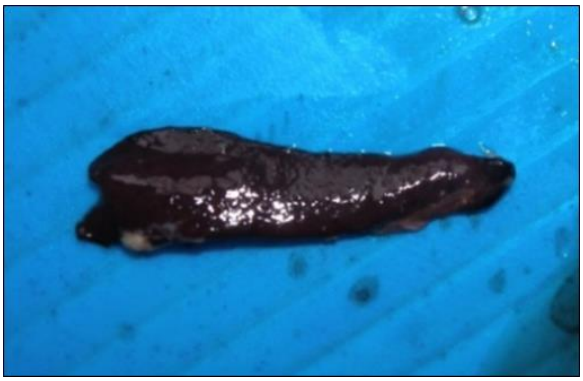

Fig 8: Spleen from weak born piglet-enlarged and congested

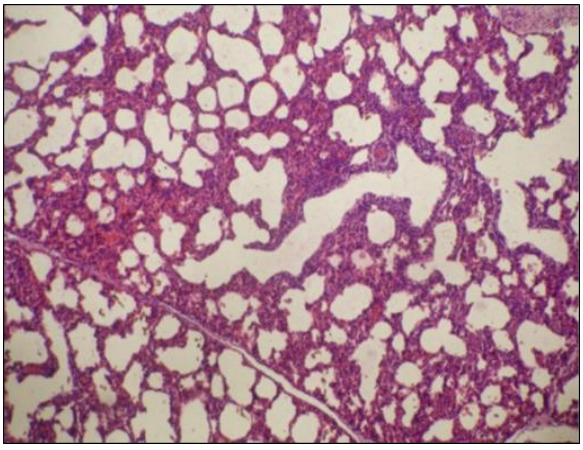

Fig 9: Lungs showing thickening of interstitium due to congestion, infiltration by MNC's and serofibrinous exudates in stillborn piglet (H&E, 100X)

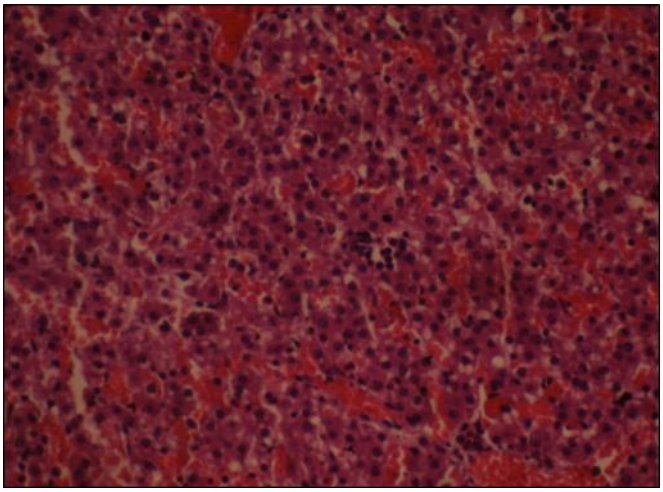

Fig 10: Liver from stillborn piglet showing severe congestion in the sinusoids with foci of infiltration of mononuclear cells (H&E, 400X)

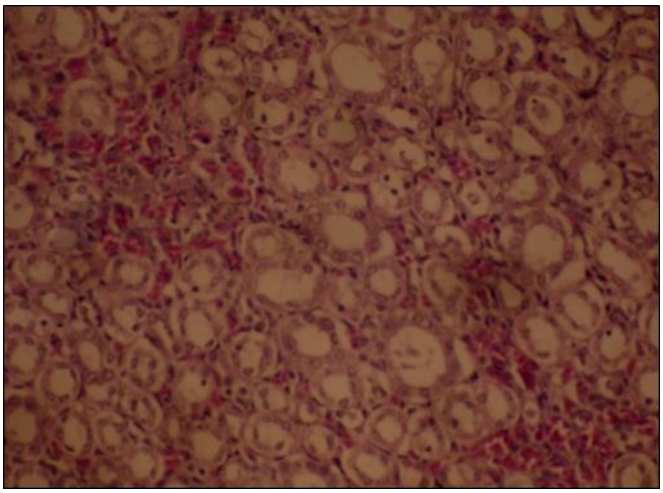

Fig 11: Kidney from weakborn piglet which died after 3 days showing congestion and haemorrhages in the inter-tubular spaces and mild hydropic degenerations in the tubular epithelial cells (H&E, 400X)

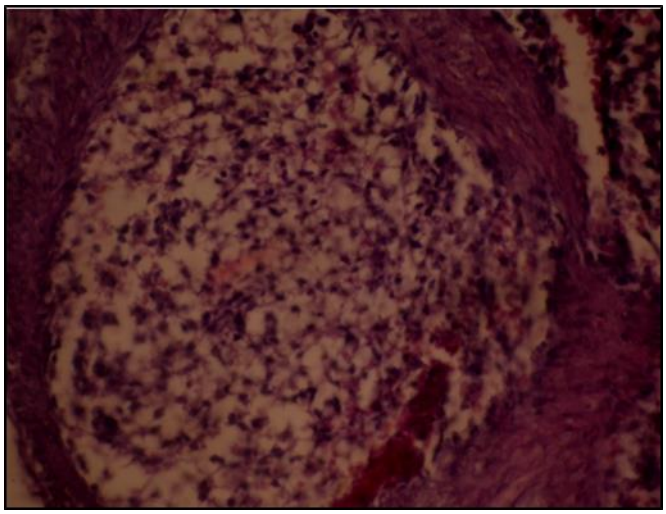

Fig 12: Spleen from a weakborn piglet died at 2 days, showing severe lymphoid depletion (H&E, 400X)

Conclusion

Molecular diagnostics is the gold standard for detecting PCV-2 and differentiating it from other infectious diseases because clinical manifestation and postmortem investigation make it practically impossible to distinguish between the multiple viral viruses affecting pigs. Although significant progress has been achieved in understanding PCV2 pathogenesis and immunological interaction, many fundamental questions remain unanswered. There is a considerable risk of the introduction of transboundary illnesses due to the enormous growth in global trade, human travel, and animal transit. Therefore, a future study on the molecular epidemiology of the circulating PCV is required, which will help in the modification of management practises and the development of vaccination strategies to prevent the PCV infection.

References

1. Givens MD, Marley MSD. Infectious causes of embryonic and fetal mortality. Theriogenology. 2008;70:270-285.
2. Meng XJ. Circoviridae. In Fields Virology, ed. DM Knipe. Philadelphia PA: Lippincott Williams & Wilkins. 6th ed. In press; c2013.
3. Tischer I, Gelderblom H, Vettermann W, Koch MA. A very small porcine virus with circular single-stranded DNA. Nature. 1982;295:64-66.
4. O'Connor B, Gauvreau H, West K, Bogdan J, Ayroud M, Clark EG, *et al*. Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. Canadian Veterinary Journal. 2001;42:551-553.
5. Park JS, Kim J, Ha Y, Jung K, Choi C, Lim JK, *et al*. Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. Journal of Comparative Pathology, 2005;132:139-144.
6. West KH, Bystrom JM, Wojnarowicz C, Shantz N, Jacobson M, Allan GM, *et al*. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. Journal of Veterinary Diagnostic Investigation. 1999;11(6):530-532.
7. Patterson AR, Opriessnig T. Epidemiology and horizontal transmission of porcine circovirus type 2 (PCV2). Animal Health Research Reviews. 2010;11:217-234.
8. Li N, Liu J, Qi J, Hao F, Xu L, Guo K. Genetic Diversity and Prevalence of Porcine Circovirus Type 2 in China During 2000-2019. Frontiers in Veterinary Science. 2021;15(8):788-172. Doi: 10.3389/fvets.2021.788172. PMID: 34977219; PMCID: PMC8717868.
9. Franzo G, Settypalli TBK, Agusi ER, Meseko C, Minoungou G, Ouoba BL, *et al*. Porcine circovirus-2 in Africa: Identification of continent-specific clusters and evidence of independent viral introductions from Europe, North America and Asia. Transboundary and Emerging Diseases. 2022;(4):e1142-e1152. Doi: 10.1111/tbed.14400. Epub 2021 Dec 3. PMID: 34812571.
10. Franzo G, Tinello S, Grassi L, Tucciarone CM, Legnardi M, Cecchinato M, *et al*. Free to Circulate: An Update on the Epidemiological Dynamics of Porcine Circovirus 2 (PCV-2) in Italy Reveals the Role of Local Spreading, Wild Populations and Foreign Countries. Pathogens 2020;9(3):221. Doi: 10.3390/pathogens9030221. PMID: 32192210; PMCID: PMC7157736.
11. Sharma R. Studies on pathology and diagnosis of important viral diseases causing reproductive failure and preweaning mortality in swine. Ph.D. Thesis submitted to

Deemed University, Indian Veterinary Research Institute, Izatnagar; c2007.
12. Rajkhowa TK. Studies on pathology and diagnosis of PCV2 associated diseases. Ph.D. Thesis submitted to Deemed University, Indian Veterinary Research Institute, lzatnagar; c2008.
13. Bhattacharjee U, Sen A, Sharma I. A retrospective study reveals the Porcine circovirus-2f genotype predominant in the indigenous pig population of North-eastern India. Infection, Genetics and Evolution. 2021;96:105-100. Doi: 10.1016/j.meegid.2021.105100. Epub 2021 Oct 5. PMID: 34619393.
14. Segalés J. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Research. 2012;164:10-19.
15. Larochelle R, Antaya M, Morin M, Magar R. Typing of porcine circovirus in clinical specimens by multiplex PCR. Journal of Virological Methods. 1999;80(1):69-75.
16. Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. 3rd ed. McGraw Hill Book Co., New York, 1968.
17. Bolin SR, Stoffregen WC, Nayar GP, Hamel AL. Postweaning multisystemic wasting syndrome induced after experimental inoculation of cesarean-derived, colostrum deprived piglets with type 2 porcine circovirus. Journal of Veterinary Diagnostic Investigation. 2001;13(3):185-194.
18. Rudan N, Hjulsager CK, Dupont K, Baanek B, Markovic E, Madic J. Genetic characterization of porcine circovirus type 2 (PCV2) isolated Genetic characterization of porcine circovirus type 2 (PCV2) from different pig-farms in Croatia. Veterinarski Arhiv. 2009;79:361-369.
19. Bandrick M, Balasch M, Heinz A, Taylor L, King V, Toepfer J, *et al*. A bivalent porcine circovirus type 2 (PCV2), PCV2a-PCV2b, vaccine offers biologically superior protection compared to monovalent PCV2 vaccines. Vet Res. 2022;53:12. https://doi.org/10.1186/s13567-022-01029-w
20. Opriessnig T, Meng XJ, Halbur PG. Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis and intervention strategies. Journal of Veterinary Diagnostic Investigation. 2007;19:591-615.
21. Segales J, Domingo M. Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. Veterinary Quarterly. 2002;24:109-124.
22. O'dea MA. Porcine circovirus infection. Australia & New Zealand Standard Diagnostic Procedure, 2010, 1-20.
23. Brunborg IM, Moldal T, Jonassen CM. Quantitation of pocinecircovirus type 2 isolated from serum/plasma and tissue samples of healthy pigs with postweaning multisystemic wasting syndrome using a Taq Man-based real-time PCR. Journal of Virological Methods. 2004;122:171-178.
24. Kim J, Jung K, Chae C. Prevalence of porcine circovirus type 2 in aborted fetuses and stillborn piglets. Veterinary Record. 2004;155:489-492.
25. Opriessnig T, Meng XJ, Halbur PG. Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. Journal of Veterinary Diagnostic Investigation. 2007;19:591-615.
26. Johnson CS, Joo HS, Direksin K, Yoon KJ, Choi YK. Experimental in utero inoculation of late-term swine fetuses with porcine circovirus type 2. Journal of Veterinary Diagnostic Investigation. 2002;14(6):507-12. Doi: 10.1177/104063870201400612. PMID: 12423036.
27. Sanchez Jr RE, Nauwynck HJ, McNeilly F, Allan GM, Pensaert MB. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. Veterinatry Microbiology. 2001;83:169-176.