Slov Vet Res
DOI 10.26873/SVR-1708-2024
UDC 601.4:575.116.4: 599.731.111
Pages: 263—9

Original Research Article

PCV2 and PCV3 Genotyping in Wild Boars From Serbia

Key words

PCV2;
PCV3;
genotyping;
PCR;
wild boar

Jakov Nišavić[1], Andrea Radalj[1]*, Nenad Milić[1], Isidora Prošić[1], Aleksandar Živulj[2], Damir Benković[3], Branislav Vejnović[4]

[1]Department for Microbiology, Faculty of Veterinary Medicine, University of Belgrade, 11000 Belgrade, [2]Veterinary Specialized Institute „Pančevo", 26000 Pančevo, [3]Veterinary Specialized Institute „Sombor", 25000 Sombor, [4]Department for Economics and Statistics, Faculty of Veterinary Medicine, University of Belgrade, 11000 Belgrade, Serbia

***Corresponding author:** andrea.zoric@vet.bg.ac.rs

Abstract: Porcine circoviruses 2 and 3 (PCV2 and PCV3) are known agents of diseases in domestic pigs and wild boars. PCV2 is an economically important pathogen causing porcine circovirus-associated diseases (PCVAD), while the recently discovered PCV3 is associated with similar disorders. Wild boars can serve as a PCV reservoir for domestic pigs, which is a particular risk for pig farms with low biosecurity. Reports of these infections in Serbia are sporadic, and this study was intended as a follow-up to an earlier study. Our aim was to assess the prevalence and genetic characteristics of PCVs circulating in wild boars in a region in north-eastern Serbia with extensive hunting areas. In our study of 103 samples, 17.48% tested positive for PCV2 and 15.53% for PCV3. The low co-infection rates in 2.94% of the PCR-positive samples, suggests these viruses circulate independently. PCV2 prevalence was lower than in our previous study (40.32% out of 124 samples), but the genetic stability of circulating strains was detected with a clear genotype shift towards PCV2d-2. Moreover, this is the first report of PCV3 occurrence in wild boar in Serbia, and the detected strains were grouped into two genotypes: PCV3-1 and PCV3-3c. The PCV3-1 sequences were clustered with German strains, indicating the prevalence of this genotype in Europe. However, no further geographical correlation could be established, as the PCV3-3c representative was separated within the cluster containing Chinese and Indian strains. Furthermore, there was no correlation between PCV positivity and pathological findings in the sampled animals indicating subclinical infection.

Received: 20 February 2023
Accepted: 22 April 2024

Introduction

Over Porcine circoviruses (PCV) are one of the smallest DNA viruses with a circular genome and belong to the family *Circoviridae* (1). These known pathogens are associated with disease in both domestic pigs and wild boars. In recent years, new PCVs and genotypes of already known viruses have been gradually discovered (2, 3). Porcine circovirus 2 (PCV2) is the best-studied porcine circovirus and causes several diseases collectively known as porcine circovirus-associated disease (PCVAD), including postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), pneumonia, reproductive problems, and so on (3, 4). PCV2 can spread easily in a susceptible population, mainly through direct contact and can be shed over a long period of time, exposing susceptible pigs to contaminated respiratory secretions, feces and urine (3). PCV3 was initially identified in 2016 through metagenomic sequencing within the domestic pig population and this discovery was linked to instances of reproductive failure and PDNS (2, 4, 5). It is widely recognized that factors beyond PCV2 infection are required to cause severe disease, however, PCV2 impacts the immune response in wild boars, which can exacerbate other existing diseases in these animals (3). Similarly, PCV3 has also been found in apparently healthy animals and it is believed that

this virus serves as a contributing factor in intensifying the severity of diseases during concurrent infections (6, 7, 8). These viruses are genetically heterogeneous and PCV2 is currently divided into eight genotypes (PCV2a-PCV2h), while PCV3 is divided into three genotypes and several subtypes (9, 10, 11). Evidence of a global genotype shift of PCV2 is accumulating, as the PCV2d genotype is gradually becoming dominant worldwide (12-15). Frequently, the primary origin of virus spread is rooted in the structure of the domestic pig farming sector. These small units, mostly owned by families, do not implement any biosecurity measures and the pigs are typically raised in a free-range manner, occasionally coming into close contact with wild boars (15, 16). Moreover, high prevalence of PCVs in wild boars and the genetic similarity between PCV3 strains found in domestic and wild populations could imply that wild boars serve as virus reservoirs (17-20). Generally, the occurrence of PCVs in the wild is typically greater in regions with extensive pig farming (15, 21). The estimated density of the wild boar population in Serbia is 0.2 - 1.38 animals per km^2, and the country has the highest density of pigs in the Western Balkans, with around 2.7 million domestic pigs (3). The prevalence of PCVs in the wild boar population varies from country to country. In our recent study, conducted in hunting areas in a region with traditional pig breeding, we confirmed the dominant presence of genotype PCV2d in the wild boar population, but no PCV3-positive animals were detected at that time (15). Aside from our study, the only available literature information dates back to 2012, and demonstrates the presence of PCV2b in the domestic pig population in Serbia (30). Published European studies indicate the presence of PCV3 in over 70% of the tested samples from wild boars (19). Savić et al. (8) reported the prevalence of PCV3 in domestic pig farms and link this virus to the occurrence of PCVAD, which serves as the only data available from Serbia concerning this topic. The aim of this study was to follow up on our previous work and analyse the presence and genetic characteristics of porcine circoviruses in wild boars from the same hunting areas three years later.

Materials and methods

Samples

Samples of lymph nodes and spleen were collected from 103 adult wild boars during the 2021/2022 hunting season in the South Banat district of Vojvodina in north-eastern Serbia. The collection of wild boar samples was carried out as part of regular monitoring for African and classical swine fever organized by the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management. Sampling was performed in the field without a complete pathoanatomical section, and general condition of the sampled animals was noted. The hunting areas covered by this survey included: Opovo (45°3'N, 20°25'E), Plandište (45°22'N, 21°12'E), Bela Crkva (44°87'N, 21°43'E), Alibunar (45°4'N, 20°58'E), Vršac (45°13'N, 21°36'E), Pančevo (44°82'N, 20°63'E), and Kovin (44°44' N, 20°58' E). The samples were transported on ice to the Faculty of Veterinary Medicine, University of Belgrade for further examination. Samples from each animal were pooled and homogenised in phosphate buffered saline (PBS 7.2). Tissue suspensions were centrifuged at 1.677 × g for 10 minutes, and DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA).

PCR, sequencing and phylogeny

PCR detection of PCV2 and PCV3 was performed using primers and protocols described by Castro et al. (22) and Franzo et al. (23). Internal reference strains of PCV2 (GenBank acc. no. MW550043) and PCV3 from the Department of Microbiology, Faculty of Veterinary Medicine, University of Belgrade were used as positive controls. The PCR products that were positive for PCV2 and PCV3 were selected for sequencing. All PCV2-positive samples were sequenced with the PCR primers described previously, and PCV3-positive samples from different hunting areas were sequenced with primers specific for the viral capsid gene (24). The PCV2 and PCV3 nucleotide sequences obtained were compared with analogous sequences from GenBank using the tool BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Generation of consensus sequences, analysis and quality control of the raw sequences were performed using the STADEN package (25).

For phylogenetic analysis, MEGA 11 software (26) was used, and trees were generated by the neighbour-joining algorithm after Kimura-2 parameter correction with 1,000 bootstrap repeats, using appropriate sequences as outgroups. Bootstrap values of more than 70 % were reported. Genotype and cluster representative strains were selected (9-11).

The representative PCV2 and PCV3 sequences obtained in this study were submitted to GenBank and are available under the following accession numbers: OP784785 - OP784793.

Results

The PCR results were as follows: PCV2 DNA was detected in 18/103 animals (17.48%), and 16/103 samples were PCV3 positive (15.53%), with rare mixed infections in 0.97% of the tissues examined (i.e. 2.94% of the PCR-positive samples). The distribution of positive animals among the different hunting areas in this study is shown in Figure 1. PCV3-positive animals were sampled in Plandište (7/16; 43.75 %), Vršac (7/16; 43.75 %) and Pančevo (2/16; 12.5 %), while PCV2-positive samples were from Plandište (5/18; 27.8 %), Kovin (7/18; 38.9 %) and Bela Crkva (6/18; 33.3 %).

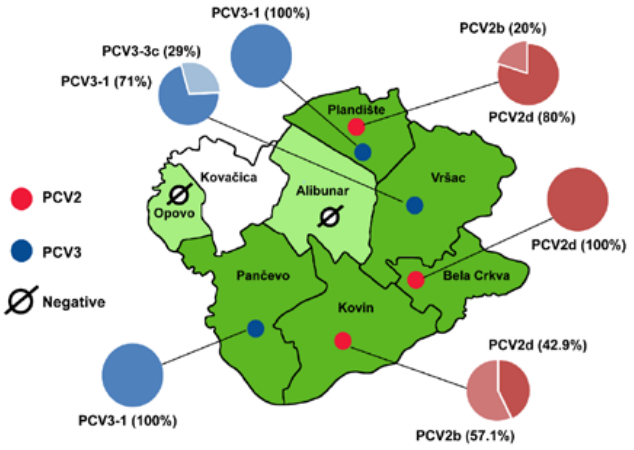

Figure 1: PCV2- and PCV3-positive wild boar samples in different hunting areas and distribution of genotypes.

The overall similarity of the detected PCV2 strains with other strains from GenBank, including previously reported strains from the same hunting grounds (MW550042-MW550059), was 90-100%, while they were 93-100% similar to each other.

Phylogeny revealed that the detected strains belonged to PCV2b (5/18; 27.8%) and PCV2d (13/18; 72.2%) genotypes. The detected PCV2d strains were clustered with PCV2d-2 sequences detected in 2018/2019 in wild boar from the same hunting areas, as well as with analogous strains from Hungary, China and the USA. In addition, the PCV2b sequences from this study also clustered with PCV2b sequences detected in wild boar from the same hunting areas (Figure 2). The PCV3 sequences from this study had an overall similarity of 98-100 % with other sequences examined from GenBank and had a similarity of 98-99 % with a previously reported Serbian PCV3 strain detected in domestic pigs. In addition, the detected PCV3 strains were 99-100 % similar to each other. The results of the phylogenetic analysis showed that the sequences examined belonged to the PCV3-1 (14/16; 87.5%) and PCV3-3c (2/16; 12.5%) genotypes. The PCV3-1 sequences formed a cluster with analogous sequences from Germany, while the PCV3-3c genotype representative in this study did not form a cluster with sequences from India and China belonging to the same genotype (Figure 3). The distribution of the PCV2 and PCV3 genotypes in positive samples from different hunting areas is shown in Figure 1.

Discussion

This study is a continuation of our previous work on wild boar samples from the same hunting area taken in 2018/2019 (15). These results showed a high frequency of PCV2 infection, while PCV3 was not detected. Similarly, the reported detection frequency of PCV3 in South Korean wild boar was also relatively low (27). Our current results are more in line with European studies showing high PCV3 infection

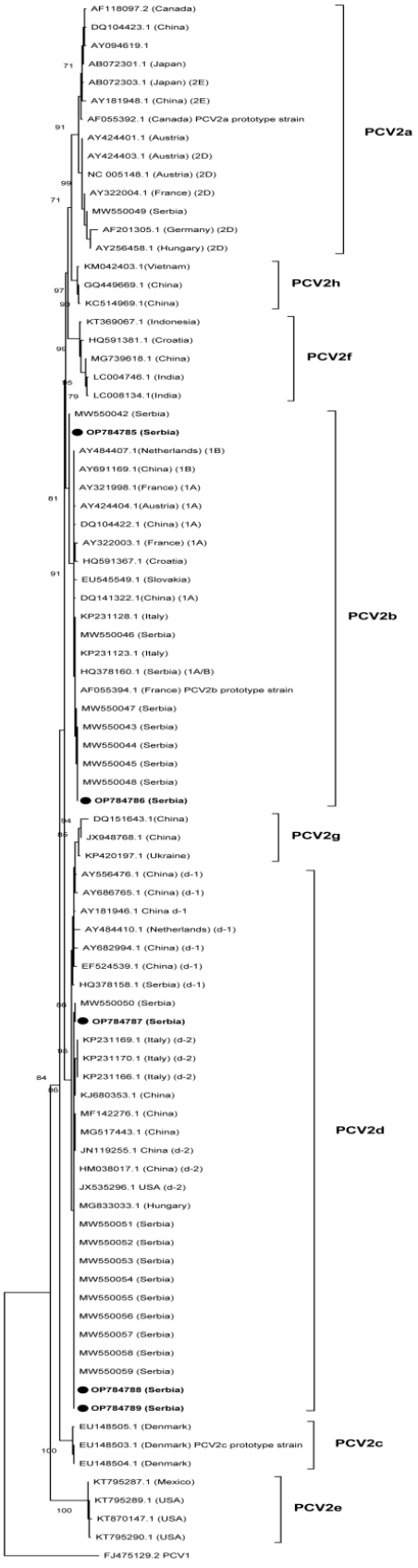

Figure 2: Neighbour-joining tree constructed from wild boar PCV2 sequences (OP784785 - OP784789) and sequence representatives of different genotypes. Previously detected PCV2 strains from the same area are marked MW550042–MW550059. The sequences from this study are marked by a black circle.

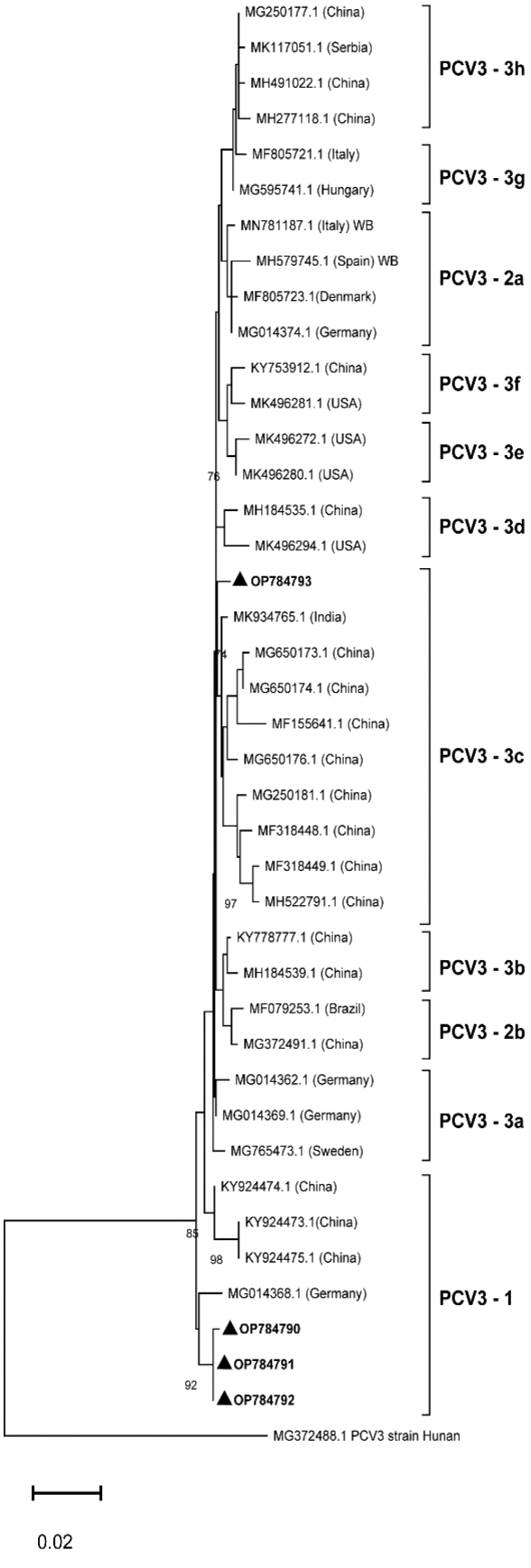

Figure 3: Neighbour-joining tree constructed from wild boar PCV3 sequences (OP784790 - OP784793) and sequence representatives of different genotypes. The sequences from this study are marked by a black triangle.

rates in wild boar ranging from 23% to 71% (6, 7, 19, 28). These results agree with studies confirming the ubiquity of PCV3 in pig farms across Europe (13). Furthermore, Savić et al. (8) suggested that PCV3 is widespread in the pig population in Serbia.

In a study similar to ours, conducted by Dal Santo et al. (29), PCV2 and PCV3 were detected in 57.7% and 15.4%, respectively, of the Brazilian wild boar samples tested. The proportions of European wild boar that are PCV2-positive are variable, ranging from 10 % to 47.3 % in different hunting districts (17, 19, 28). In contrast to our current results, PCV2 was previously detected in 40.32 % of wild boar from this area (15). In general, the higher PCV2 prevalence in wild boar in certain countries and areas is associated with extensive domestic pig farming (15, 21).

Many reports show different co-infection rates of PCV3 and other relevant swine viruses, including PCV2 (2). Prevalence rates of PCV2/PCV3 co-infections detected in wild boar populations in Germany and Italy ranged from 7.5 % to 24.4 %, while these rates are higher in domestic pigs, especially in relation to animals with observed clinical signs (4, 17, 19, 28). Accordingly, the prevalence of PCV3 infections has been reported to be higher in domestic pig farms with PRDC (5). Savić et al. (8) also noted a possible association between PCV3 infection of domestic pigs in Serbia and clinical disease. In contrast, the wild animals in our study were in good condition, and similar to the results reported by Franzo et al. (6), there was no correlation between PCV3 positivity and pathological findings. Our results show that mixed PCV2/PCV3 infections are rare, accounting for 2.94% of all positive samples, comparable to the findings of Saporiti et al. (13) and Dal Santo et al. (29).

The genetic heterogeneity of PCV2 can be demonstrated by phylogenetic analysis of the corresponding gene segments (10). The strains detected in this study were assigned to PCV2d genotype in 72.2% and to PCV2b genotype in 27.8% of PCV2-positive samples. The latest available data on genetic variability of PCV2 in domestic and wild pigs in Serbia showed that PCV2b was the dominant genotype in the field, which is consistent with results from other countries from that time (20, 30, 31). However, the results of our previous study represented an update showing a visible dominance of PCV2d, consistent with the global PCV2 genotype shift (15). Data from PCV2 strains detected in domestic pigs from different regions of China also show the gradual replacement of PCV2b by the PCV2d genotype (4, 12, 14). A comprehensive analysis of the prevalence of the PCV2 genotype in European domestic pigs by Saporiti et al. (13) also revealed that PCV2d is the most frequently found genotype, followed by PCV2b and 2a. Accordingly, we confirmed the decreasing prevalence of PCV2a in Serbia, as there were no representatives of this genotype in contrast to the 2018/2019 sampling period, when 5.5% were positive, which remains in line with the situation in domestic pigs worldwide (13-15). Further evidence for our

findings is that the PCV2 strains detected in wild boars in Italy in 2021 belong predominantly to PCV2d, followed by PCV2b (19). The representatives of genotypes 2d and 2b in this study were clustered with PCV2d-2 and PCV2b sequences detected in 2018/2019 in wild boar from the same hunting areas, indicating the genetic stability of circulating virus strains in this wild boar population. Overall, the current PCV2d strains are most often representatives of the PCV2d-2 cluster, and our current results confirm the assumption of their dominance in the studied area (9, 15). This is also supported by studies conducted in Italy in both domestic pigs and wild boars (19, 32). Interestingly, Rudova et al. (21) detected genotype f in wild boars, demonstrating the possible PCV2 diversity in the wild. Furthermore, the authors found an accumulation of representatives of the PCV2 genotype in domestic and wild animals, suggesting an ecological interaction. Unfortunately, there are no recent data on PCV2 genotype diversity in Serbian domestic pigs, and the latest available data suggest that domestic pigs are predominantly infected with PCV2b (30, 31).

The PCV3 sequences obtained in this study had a high overall similarity rate of 98-100% to each other and to analogous sequences from GenBank. These data on PCV3 molecular diversity are consistent with other studies showing a relatively high degree of genetic homology between PCV3 strains (2, 4, 5, 7, 13, 14). The phylogenetic analysis of German PCV3 strains performed by Fux et al. (33) describes two main groups of PCV3 strains (PCV3a and b) with corresponding subclusters. This strain classification has also been reported elsewhere (8, 23, 34). Li et al. (34) reported that diversification of PCV3 into PCV3a and b occurred between 2013 and 2014, and also reported the existence of branches PCV3a-1 and PCV3a-2. However, there is no correlation between the distribution of PCV3 strains and their respective geographical origins (2). Mutations within the PCV3 genome are mostly found in the region encoding the cap protein, which can be useful for genotyping strains (4, 35). Accordingly, several authors have proposed the subdivision of PCV3 into three genotypes (PCV3a, PCV3b and PCV3c) (12, 35, 36). According to the PCV3 genotype classification recently proposed by Chung et al. (11), most of the wild boar strains detected in our study are classified as PCV3-1, followed by PCV3-3c (12.5%). A similar study from Italy gave different results and all PCV3 strains from wild boars were classified as subtype 2a, mostly together with other European, Chinese and Brazilian strains (19). PCV3-1 strains from wild boars in Serbia were clustered with German strains, which might be due to the increase of wild boar populations in Europe and their migratory characteristics. Interestingly, the PCV3-3c genotype representative in this study was distantly related within the genotype to strains from India and China, confirming that there is no geographical correlation with the PCV3 genotyping method. Similar results were obtained by Savić et al. (8), who showed clustering of Serbian PCV3 strains in domestic pigs with Chinese strains. When compared with the available analogue sequence of a PCV3 domestic pig strain from Serbia, the overall differences from the wild boar sequences obtained were about 2 %, and the strains did not show clustering, suggesting independent circulation.

Conclusions

In this paper we have described the occurrence of PCV2 and PCV3 in wild boar, focusing on their genetic characteristics. These viruses are widespread, but there are rare cases of co-infection, suggesting that they mostly occur as single pathogens. There was no correlation between PCV positivity and general condition of the sampled animals, indicative of subclinical infection. Although PCV2 has been detected in wild boar populations in our previous study, this is the first report of PCV3 in these animals in Serbia. The most common genotype of PCV2 found in this study was PCV2d (PCV2d-2 cluster), confirming the previously reported global trend of genotype shift. In addition, we confirmed the circulation of the same PCV2 strains in the sampled hunting areas as in the 2018/2019 season. Most of the PCV3 sequences found were closely related, especially to strains from Germany, suggesting the existence of a single genotype circulating in Europe. Nevertheless, our data are in agreement with the results of other authors showing no geographical correlation with genotype association. Details on the molecular epizootiology of PCV3 are still scarce, and data presented here add to the lack of information on the genetic characteristics of wild boar strains for this part of Europe. The occurrence of PCV2 and PCV3 in wild boar may pose a challenge for commercial pig production and the potential risk of transmission between wild and domestic pigs needs to be considered. In addition, wild boar and domestic pig PCV strains demonstrate phylogenetic clustering, confirming the possibility of pathogen exchange between these species. Therefore, biosecurity measures must be taken in pig farms to ensure distancing from wild populations. As there are large numbers of wild boars in Serbia and extensive domestic pig farming is common, it is important to monitor the occurrence of pathogens in these populations. Since we did not study the local pig population, we do not have information on the genetic diversity of PCV2 and PCV3 in pig farms, so no exact correlation could be established. There are no recent data on PCV2 genotype diversity in Serbian domestic pigs, and none of the detected PCV3 strains from wild boars were clustered with the available domestic pig strain, which could indicate an independent spread of PCV3. However, studies comparing the genetic characteristics of strains from the wild population and from surrounding pig farms could provide more conclusive information. In view of the results presented, we emphasize the importance of screening wild boar samples to contribute to the understanding of the role of porcine circoviruses and epizootiology in different animal populations.

Acknowledgments

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [Contract number 451-03-66/2024-03/200143].

Conflict of interest. The authors declare no conflict of interest.

References

1. ICTV. Current ICTV taxonomy release: taxonomy browser. Utrecht: International Committee on Taxonomy of Viruses, 2024. https://talk.ictvonline.org/taxonomy/ (12.02.2023)
2. Ouyang T, Niu G, Liu X, Zhang X, Zhang Y, Ren L. Recent progress on porcine circovirus type 3. Infect Genet Evol 2019; 73: 227–33. doi: 10.1016/j.meegid.2019.05.009.
3. Nišavić J, Radalj A, Milić N, et al. A review of some important viral diseases of wild boars. Biotechnol Anim Husb 2021; 37(4): 235–54. doi: 10.2298/BAH2104235N
4. Guo Z, Ruan H, Qiao S, Deng R, Zhang G. Co-infection status of porcine circoviruses (PCV2 and PCV3) and porcine epidemic diarrhea virus (PEDV) in pigs with watery diarrhea in Henan province, central China . Microb Pathog 2020; 142: 104047. doi: 10.1016/j.micpath.2020.104047
5. Kedkovid R, Woonwong Y, Arunorat J, et al. Porcine circovirus type 3 (PCV3) infection in grower pigs from a Thai farm suffering from porcine respiratory disease complex (PRDC). Vet Microbiol 2018; 215: 71–6. doi: 10.1016/j.vetmic.2018.01.004
6. Franzo G, Tucciarone CM, Drigo M, et al. First report of wild boar susceptibility to porcine circovirus type 3: high prevalence in the Colli euganei regional park (Italy) in the absence of clinical signs. Transbound Emerg Dis 2018; 65(4): 957–62. doi: 10.1111/tbed.12905
7. Klaumann F, Dias-Alves A, Cabezón O, et al. Porcine circovirus 3 is highly prevalent in serum and tissues and may persistently infect wild boar (Sus Scrofa Scrofa). Transbound Emerg Dis 2019; 66(1): 91–101. doi: 10.1111/tbed.12988
8. Savić B, Milicevic V, Radanovic O, et al. Identification and genetic characterization of porcine circovirus 3 on pig farms in Serbia. Arch Virol 2020; 165(1): 193–9. doi: 10.1007/s00705-019-04455-y
9. Xiao C-T, Halbur PG, Opriessnig T. Global molecular genetic analysis of porcine circovirus type 2 (PCV2) sequences confirms the presence of four main pcv2 genotypes and reveals a rapid increase of PCV2d. J Gen Virol 2015; 96(7): 1830–41. doi: 10.1099/vir.0.000100
10. Franzo G, Segalés J. Porcine circovirus 2 (PCV-2) genotype update and proposal of a new genotyping methodology. PLoS One 2018; 13(12): e0208585. doi: 10.1371/journal.pone.0208585
11. Chung H-C, Nguyen VG, Park Y, Park B-K. Genotyping of PCV3 based on reassembled viral gene sequences. Vet Med Sci 2021; 7(2): 474–82. doi: 10.1002/vms3.374
12. Chen N, Huang Y, Ye M, et al. Co-infection status of classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PPRSV) and porcine circoviruses (PCV2 and PCV3) in eight regions of china from 2016 to 2018. Infect Genet Evol 2019; 68: 127–35. doi: 10.1016/j.meegid.2018.12.011
13. Saporiti V, Huerta E, Correa-Fiz F, et al. Detection and genotyping of porcine circovirus 2 (PCV-2) and detection of Porcine circovirus 3 (PCV-3) in sera from fattening pigs of different european countries. Transbound Emerg Dis 2020; 67(6): 2521–31. doi: 10.1111/tbed.13596
14. Xu T, Zhang Y-H, Tian R-B, et al. Prevalence and genetic analysis of porcine circovirus type 2 (PCV2) and type 3 (PCV3) between 2018 and 2020 in central China. Infect Genet Evol 2021; 94: 105016. doi: 10.1016/j.meegid.2021.105016
15. Nišavić J, Milic N, Radalj A, et al. Detection and characterisation of porcine circoviruses in wild boars in northeastern Serbia. Vet Med (Praha) 2022; 67(3): 131–7. doi: 10.17221/32/2021-VETMED
16. Ladoşi I, Păpuc TA, Ladoşi D. The impact of african swine fever (ASF) on romanian pig meat production: a review. Acta Vet 2023; 73(1): 1–12. doi: 10.2478/acve-2023-0001
17. Amoroso MG, Serra F, Esposito C, et al. Prevalence of infection with porcine circovirus types 2 and 3 in the wild boar population in the campania region (southern Italy). Animals (Basel) 2021; 11(11): 3215. doi: 10.3390/ani11113215
18. Nišavić J, Milic N, Radalj A, et al. Genetic analysis and distribution of porcine parvoviruses detected in the organs of wild boars in Serbia. Acta Vet 2021; 71(1): 32–46. doi: 10.2478/acve-2021-0003
19. Fanelli A, Pellegrini F, Camero M, et al. Genetic diversity of porcine circovirus types 2 and 3 in wild boar in Italy. Animals (Basel) 2022; 12(8): 953. doi: 10.3390/ani12080953
20. Franzo G, Grassi L, Tucciarone CM, et al. A wild circulation: high presence of porcine circovirus 3 in different mammalian wild hosts and ticks. Transbound and Emerg Dis 2019; 66(4): 1548–57. doi: 10.1111/tbed.13180
21. Rudova N, Buttler J, Kovalenko G, et al. Genetic diversity of porcine circovirus 2 in wild boar and domestic pigs in Ukraine. Viruses 2022; 14(5): 924. doi: 10.3390/v14050924
22. Castro AMMG, Castro Jr. FG, Budiño FEL, et al. Detection of genetic characterization of porcine circovirus 2 (PCV2) in Brazilian wildlife boars. Braz J Microbiol 2012; 43(3): 1022–5. doi: 10.1590/S1517-838220120003000025
23. Franzo G, Legnardi M, Hjulsager CK, et al. Full-genome sequencing of porcine circovirus 3 field strains from denmark, Italy and Spain demonstrates a high within-Europe genetic heterogeneity. Transbound Emerg Dis 2018; 65(3): 602–6. doi: 10.1111/tbed.12836
24. Wen S, Sun W, Li Z, et al. The detection of porcine circovirus 3 in Guangxi, China. Transbound Emerg Dis 2018; 65(1): 27–31. doi: 10.1111/tbed.12754
25. Staden R, Beal KF, Bonfield JK. The Staden Package, 1998. Methods Mol Biol 2000; 132: 115–130. doi: 10.1385/1-59259-192-2:115
26. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 2021; 38(7): 3022–7. doi: 10.1093/molbev/msab120
27. Dhandapani G, Yoon S-W, Noh JY, et al. Detection of porcine circovirus 3 from captured wild boars in Korea. Vet Med Sci 2021; 7(5): 1807–14. doi: 10.1002/vms3.518
28. Prinz C, Stillfried M, Neubert LK, Denner J. Detection of PCV3 in German wild boars. Virol J 2019; 16(1): 25. doi: 10.1186/s12985-019-1133-9
29. Santo ACD, Gressler LT, Costa SZR, et al. Porcine circovirus 2 and 3 in wild boars in southern Brazil. Ciência Rural 2022; 52(2): e20210209. doi: 10.1590/0103-8478cr20210209
30. Savić B, Milićević V, Jakić-Dimic D, et al. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) in Serbia. Arch Virol 2012; 157(1): 21–8. doi: 10.1007/s00705-011-1130-9
31. Toplak I, Lazić S, Lupulović D, et al. Study of the genetic variability of porcine circovirus type 2 detected in Serbia and Slovenia. Acta Vet Hung 2012; 60(3): 409–20. doi: 10.1556/AVet.2012.035

32. Dei Giudici S, Lo Presti A, Bonelli P, et al. Phylogenetic analysis of porcine circovirus type 2 in Sardinia, Italy, shows genotype 2d circulation among domestic pigs and wild boars. Infect Genet Evol 2019; 71: 189–96. doi: 10.1016/j.meegid.2019.03.013

33. Fux R, Söckler C, Link EK, et al. Full genome characterization of porcine circovirus type 3 isolates reveals the existence of two distinct groups of virus strains. Virol J 2018; 15(1): 25. doi: 10.1186/s12985-018-0929-3

34. Li G, He W, Zhu H, et al. Origin, genetic diversity, and evolutionary dynamics of novel porcine circovirus 3. Adv Sci (Weinh) 2018; 5(9): 1800275. doi: 10.1002/advs.201800275

35. Qi S, Su M, Guo D, et al. Molecular detection and phylogenetic analysis of porcine circovirus type 3 in 21 provinces of china during 2015–7. Transbound Emerg Dis 2019; 66(2): 1004–15. doi: 10.1111/tbed.13125

36. Fu X, Fang B, Ma J, et al. Insights into the epidemic characteristics and evolutionary history of the novel porcine circovirus type 3 in southern China. Transbound Emerg Dis 2018; 65(2): e296–e303. doi: 10.1111/tbed.12752

Genotipizacija PCV2 in PCV3 pri divjih prašičih iz Srbije

J. Nišavić, A. Radalj, N. Milić, I. Prošić, A. Živulj, D. Benković, B. Vejnović

Izvleček: Prašičja cirkovirusa 2 in 3 (PCV2 in PCV3) sta znana povzročitelja bolezni pri domačih in divjih prašičih. PCV2 je gospodarsko pomemben patogen, ki povzroča bolezni, povezane s prašičjimi cirkovirusi (PCVAD), medtem ko je nedavno odkriti PCV3 povezan s podobnimi boleznimi. Divji prašiči so lahko rezervoar PCV za domače prašiče, kar predstavlja tveganje zlasti za prašičerejske farme z nizko stopnjo biološke varnosti. Poročila o teh okužbah v Srbiji so sporadična, ta študija pa je bila zasnovana kot nadaljevanje predhodne študije. Naš cilj je bil oceniti razširjenost in genetske značilnosti PCV, ki krožijo med divjimi prašiči v regiji severovzhodne Srbije z obsežnimi lovišči. Testirali smo 103 vzorce, od katerih je bilo 17,48 % pozitivnih na PCV2 in 15,53 % na PCV3. Nizka stopnja sočasne okužbe pri 2,94 % vzorcev, pozitivnih na PCR, kaže, da ti virusi krožijo neodvisno. Prevalenca PCV2 je bila nižja kot v naši prejšnji študiji (40,32 % od 124 vzorcev), vendar je bila ugotovljena genetska stabilnost krožečih sevov z jasnim premikom genotipa v smeri PCV2d-2. To je tudi prvo poročilo o pojavu PCV3 pri divjih prašičih v Srbiji, odkriti sevi pa so bili razvrščeni v dva genotipa: PCV3-1 in PCV3-3c. Sekvence PCV3-1 so bile povezane z nemškimi sevi, kar kaže na razširjenost tega genotipa v Evropi. Vendar nadaljnje geografske povezave ni bilo mogoče ugotoviti, saj je bil predstavnik PCV3-3c ločen v skupini kitajskih in indijskih sevov. Poleg tega ni bilo povezave med pozitivnostjo PCV in patološkimi ugotovitvami pri vzorčenih živalih, kar kaže na subklinično okužbo.

Ključne besede: PCV2; PCV3; genotipizacija; PCR; divji prašiči