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Distribution and phylogenetic analysis of porcine parvoviruses in the wild boar population of Russia

Alina Komina[1], Nikita Krasnikov[1*], Maria Simakova[1], Valentina Rykova[1], Elena Zhukova[1], Alexander Bulgakov[1] and Anton Yuzhakov[1]

Abstract

Background Porcine parvoviruses (PPVs) are widespread worldwide in the swine population. PPV1 is a significant infectious agent in pig production, causing porcine reproductive failure. The pathogenic potential of novel PPVs has been poorly studied. Since wild boars are a reservoir for PPVs, the aim of this study was to investigate their prevalence and genetic diversity in the wild boar population. Tissue samples (spleen, lungs, and lymph nodes) collected from 108 wild boars from three regions of Russia during 2021–2024 were analyzed.

Results PPV1–7 were found in wild boar populations in Russia, and the most abundant species were PPV7 (59.3%) and PPV3 (49.1%). The research did not reveal any significant relationship between the gender and age of the animals and the prevalence of PPVs. A comparison between the detection rates of PPVs and PCV2/PCV3 revealed the random nature of coinfections. For phylogenetic analysis, complete VP1/VP2 gene sequences of 17 PPV1 isolates were obtained. Most of them belonged to the 27a-like group. Two isolates were in the same cluster as the highly virulent Kresse strain. Isolate BelWB57 had amino acid substitutions that were specific to both the Kresse and 27a-like strains, but it was not classified in either group. Additionally, three sequences for PPV2, PPV3, and PPV7, and one sequence for PPV5 and PPV6 VP1/VP2 genes were obtained. PPV2, PPV3, and PPV7 isolates demonstrated distribution across various clusters with strains from domestic pigs and wild boars from different countries. PPV6 isolate was included in the same clade as the Russian isolate from a domestic pig, whereas PPV5 did not enter any clade with representatives from our country.

Conclusions This is the first work devoted to the study of the PPV1–7 prevalence, as well as the genetic characteristics of isolates circulating among wild boars in various regions of Russia. Our data showed that PPV1–7 is widespread in wild boar populations. Phylogenetic analysis of PPV1 demonstrates a significant prevalence of 27a-like isolates.

Keywords Porcine parvovirus, PPV1–7, Wild boar, Porcine viruses, Phylogenetic analysis

*Correspondence:
Nikita Krasnikov
nick.krasnickoff2011@yandex.ru
[1]Federal State Budget Scientific Institution "Federal Scientific Center VIEV", Moscow, Russia

Introduction

Porcine parvoviruses (PPVs) are small, non-enveloped viruses with linear single-stranded DNA genome. PPVs are members of the *Parvoviridae* family, and can be further subdivided into two subfamilies. The *Parvovirinae* subfamily encompasses three genera with PPVs: *Protoparvovirus* (PPV1 and PPV8), *Tetraparvovirus* (PPV2 and PPV3), and *Copiparvovirus* (PPV4 and PPV6). The *Hamaparvovirinae* subfamily comprises the *Chaphamaparvovirus* genus, which includes PPV7 [1, 2]. PPV5 currently remains unclassified according to the International Committee on Taxonomy of Viruses (ICTV).

PPVs possess a 4–6 kb genome that contains two major open reading frames (ORFs): ORF1 and ORF2. However, additional ORF3 was identified in PPV4, and minor ORF was also predicted in PPV7. ORF1 encodes non-structural proteins (NSPs), and ORF2 encodes viral capsid proteins (VPs) [2–4]. The mutation frequency in the genes encoding the capsid proteins is higher than that of the NSPs (4.71×10^{-5} and 9.71×10^{-6} nucleotides/substitutions/year (nsy), respectively) [5, 6]. Therefore, the VP1/VP2 genes are more appropriate for phylogenetic analysis of PPVs to trace their molecular epidemiology. To date, four different classification systems for PPV1 strains have been suggested based on the diversity of their VP1/VP2 gene sequences [5–9]. According to the most recent classification by Vereecke et al. 2022, all PPV1 strains have been proposed to be divided into four clusters (PPV1a–PPV1d) based on intra and inter-cluster amino acid diversities [5].

Among all PPVs, PPV1 is an economically significant pathogen responsible for reproductive losses in pig production. The virus was first discovered as a contaminant in porcine cell cultures while cultivating classical swine fever virus (CSFV) [10]. Further research has demonstrated its pathogenicity and showed that PPV1 causes reproductive failure in pigs with associated clinical signs such as stillbirth, mummification, embryonic death, and infertility, known as SMEDI syndrome [4]. In addition, the virus has been implicated as the causative agent of nonsuppurative myocarditis, enteritis, and vesicular dermatitis [11–13]. To date, vaccines based on NADL-2, IDT, and PPV014 strains have been widely applied to prevent infection with PPV1. However, the emergence of highly pathogenic strains similar to 27a and Kresse, has raised questions about the effectiveness of these vaccines [5, 14, 15].

Unlike PPV1, there is still no conclusive evidence regarding the pathogenic potential of other PPVs. They were observed in both healthy and diseased pigs with various clinical signs [13, 16–20]. Due to the lack of studies on experimental infections, it is difficult to determine the significance of novel PPVs, leaving the question of their pathogenicity open.

Wild boar (*Sus scrofa*) can serve as a reservoir for various infectious agents, including zoonotic pathogens that can cause diseases in livestock, primarily affecting domestic pigs [21]. It has been speculated that PPV1 is transmitted between wild boars and domestic pigs by direct contact, although the exact role of a wild boar as a potential source of transmission has not been completely investigated [22]. PPV1 is widespread in wild boar herds, and the circulation was reported in the USA, Croatia, Slovenia, Spain, Italy, and Serbia [23–28]. Novel PPVs (PPV2, PPV3, PPV4) have also been found in a number of European countries [29–32]. Additionally, the presence of PPVs (PPV1–PPV7) in wild boars has been reported in South Korea [33].

Over the past decade, the number of wild boars in Russia has decreased dramatically. The main impact on the number and density reduction of the wild boar population was exerted by intensive depopulation measures as part of prevention works to combat the spread of African swine fever (ASF). In accordance with the decree of the Russian government, the number of wild boars in most regions of the country currently should not exceed one individual per 4,000 hectares (no more than 2,000 total in each region) [34]. Until now, the comprehensive data regarding the circulation of PPVs in wild boar herds is missing. During the serological analysis conducted in 2007, PPV1-specific antibodies were detected in 45% of wild boars in the Moscow Region [35]. By contrast, no studies about the prevalence and genetic diversity of PPVs in wild boars are present to date. Thus, the aim of this study was to investigate the prevalence of PPV1–PPV7 among wild boar populations from several regions of Russia, as well as to conduct genetic analysis to assess their diversity.

Materials and methods

Sampling

The wild boars come from three regions of European Russia: the Moscow, Tver, and the Belgorod Regions (Supplementary Fig. 1). The Moscow and Tver Regions share a common border, while the Belgorod Region is located further south separately from both. Besides, the Belgorod Region is a leading producer of pork, with the highest number of large-scale industrial pig farms in the country. Between 2021 and 2024, samples from 108 free-living wild boars were collected. The sample size was limited by the number of harvested wild boars, and all available samples were examined. Of these, 48 (48 spleen, 44 lymph nodes, 48 lungs) animals were harvested in the hunting estates in the Moscow Region and 35 (35 spleen, 30 lymph nodes, 15 lungs) in the Tver Region during several hunting seasons. Wild boars were shot by authorized

local hunters, and the samples were collected just after death in compliance with current regulations. Samples from 25 (25 spleen, 18 lymph nodes, 7 lungs) wild boars from the Belgorod Region were obtained during the depopulation action of the ASF eradication plan. All the samples were preliminarily tested for ASF virus (ASFV) and eventually considered as negative. There was no reliable data on the animal health statuses, while gender and age were determined if it was possible. In particular, age was estimated using tooth eruption and replacement patterns. The obtained samples were transported to the laboratory under refrigerated conditions and stored at -70 °C until further processing.

Nucleic acid extraction and real-time PCR (qPCR)

From each organ sample, a piece of tissue (0.5–1 g.) was taken and then homogenized in a 5 mL saline solution, aliquoted in 1.5 mL Eppendorf tubes, and stored at − 70 °C prior to lab work. Nucleic acid was extracted using the commercial kit "RIBO-prep RNA/DNA Kit" (FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, Russia) following the manufacturer's instructions and stored at − 20 °C until further analysis. Samples were primarily analyzed for PPVs (PPV1–7). Additionally, to identify possible co-infections, samples were tested for the presence of porcine circoviruses (PCV2–4), CSFV, and porcine reproductive and respiratory syndrome virus (PRRSV). Real-time PCR assays for PRRSV, CSFV, PCV2, and PPV1 detection were performed using commercial PCR kits (Vetbiochem, Russia). For porcine circovirus 3 (PCV3) and porcine circovirus 4 (PCV4) detection, PCR with specific primers was applied with the subsequent detection by gel electrophoresis [36, 37]. As part of our previous study, 30 out of 108 wild boars from the Moscow Region were tested for PCV2 and PCV3 [38]. To detect PPV2–PPV7 DNA, we used primers and probes from the available published data [39–43]. The PCR protocol has been optimized for the use of 10X Taq Buffer (Alpha ferment, Russia), dNTPs mix (New England Biolabs, USA) and Taq Polymerase (Alpha ferment, Russia) under the following conditions: 1 cycle 95 °C for 5 min; 40 cycles of: 95 °C for 15s, Ta°C (Supplementary Table 1) for 15s, 72 °C for 30s.

Sequencing of VP1/VP2 capsid protein genes

Complete regions of VP1/VP2 capsid proteins were chosen for Sanger sequencing and further phylogenetic analysis. For this purpose, the samples with low Ct-value (< 25) in qPCR were selected from each region. To obtain the final overlapping fragments, specific primers were developed for each PPV species (Supplementary Table 2).

To amplify the fragments, we performed PCR using the following conditions: denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C for 15s, Ta°C (Supplementary Table 2) for 20s, and 72 °C for 40s, and a final extension step at 72 °C for 10 min. The reaction mixture, prepared in a volume of 25 µl, contained 2.5 µl of extracted DNA, 2.5 µl of 10X Taq Buffer (Alpha ferment, Russia), 0.5 µl of dNTPs mix (New England Biolabs, USA), 17.25 µl of nuclease-free water, 0.25 µl Taq Polymerase (Alpha ferment, Russia), and 1 µl each of forward and reverse primers (10 µM). The PCR products were analyzed on a 1% agarose gel containing Tris-acetate buffer solution (pH 8.0) and ethidium bromide (0.5 µg/mL). The amplicons were manually cut and purified using the Cleanup Standard kit (Evrogen, Russia) according to the manufacturer's instructions. Sanger sequencing was performed in both directions with the specified PCR primers using the Big Dye 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, USA) and carried out on the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, USA). The obtained sequence chromatograms were analyzed, trimmed and assembled into consensus sequences using SeqMan Lasergene 11.1.0 software (DNASTAR, Madison, WI, USA).

Phylogenetic analysis

Multiple sequence alignments were performed using the MAFFT method in UGENE (v. 45.1) software. Phylogenetic trees were inferred by the Maximum Likelihood method and the General Time Reversible (G + I) model in MEGA v7.0 [44]. The robustness of the tree topology was evaluated by 1000 bootstrap replications. The genetic distances were calculated with the Kimura-2 parameter at the nucleotide level in MEGA v7.0.

Statistical analysis

Statistical analysis was conducted using Past 4.17 software. Differences in PPV1–PPV7 prevalence rates among different categories were investigated using Fisher's exact test by pairwise comparisons. Results with a p-value of < 0.05 were considered statistically significant.

Results

Distribution and detection rates of PPVs in Russian wild boars

Organ samples from 108 wild boars were obtained between 2021 and 2024 from three regions of Russia. There were 48, 35, and 25 animals from the Moscow, Tver, and Belgorod regions, respectively.

PPV1–7 were detected in the Russian wild boar population, with the highest number of cases for PPV7 (59.3%, 64/108) and PPV3 (49.1%, 53/108) ($p < 0.01$ for all pairwise comparisons). PPV1 was determined in 27 wild boars (25%, 27/108). In the Moscow Region, the PPV7 was the most prevalent species, detected in 62.5% of cases (30/48), which significantly exceeds the detection frequency of other PPVs (according to the results of the

Table 1 Detection rates of PPV1–7 across three Russian regions (2021–2024)

Region (total samples)	PPV1	PPV2	PPV3	PPV4	PPV5	PPV6	PPV7
Moscow Region (48)	10 (20.8%)	13 (27.1%)	15 (31.3%)	1 (2.1%)	1 (2.1%)	4 (8.3%)	30 (62.5%)
Tver Region (35)	9 (25.7%)	12 (34.3%)	22 (62.9%)	0 (0%)	1 (2.9%)	0 (0%)	19 (54.3%)
Belgorod Region (25)	8 (32.0%)	8 (32.0%)	16 (64.0%)	1 (4.0%)	0 (0%)	4 (16.0%)	15 (60.0%)
Total (108)	27 (25.0%)	33 (30.6%)	53 (49.1%)	2 (1.9%)	2 (1.9%)	8 (7.4%)	64 (59.3%)

Table 2 Detection of PPV1–7 in different gender, age, and sample type groups of wild boars

Category (total samples)		PPV1	PPV2	PPV3	PPV4	PPV5	PPV6	PPV7
Gender	Male (47)	12 (25.5%)	15 (31.9%)	22 (46.8%)	2 (4.3%)	1 (2.1%)	0 (0%)	29 (61.7%)
	Female (21)	6 (28.6%)	6 (28.6%)	13 (61.9%)	0 (0%)	1 (4.8%)	5 (23.8%)	14 (66.7%)
Age	Young < 1 year (41)	8 (19.5%)	13 (31.7%)	18 (43.9%)	0 (0%)	0 (0%)	2 (4.9%)	30 (73.2%)
	Adults > 1 year (36)	10 (27.8%)	13 (36.1%)	23 (63.9%)	1 (2.8%)	2 (5.6%)	6 (16.7%)	18 (50.0%)
Sample type	Spleen	10 (62.5%)	13 (76.5%)	19 (90.5%)	1 (50.0%)	1 (100%)	3 (100%)	4 (11.4%)
	Lymph node	14 (87.5%)	11 (64.7%)	18 (85.7%)	2 (100%)	0 (0%)	1 (33.3%)	29 (82.9%)
	Lung	5 (31.3%)	5 (29.4%)	16 (76.2%)	1 (50.0%)	0 (0%)	1 (33.3%)	15 (42.9%)
	Total*	16	17	21	2	1	3	35

* The number of wild boars positive for PPV ($n = 57$) from which all sample types were obtained

Fisher test in paired comparison, $p < 0.01$). The following common species in the Moscow Region were PPV3 (31.3%, 15/48) and PPV2 (27.1%, 13/48). In the Tver Region there was no statistically significant difference in the frequency of PPVs detection; however, the PPV3, PPV7 and PPV2 showed high detection rates that were equal to 62.9% (22/35), 54.3% (19/35), and 34.3% (12/35), respectively. In the Belgorod Region, high detection rates of PPV3 (64%, 16/25) and PPV7 (60%, 15/25) were observed, while PPV1 and PPV2 were detected with the same frequency (32%, 8/25) (Table 1).

In general, the multiple PPV infection rate was higher than the single infection rate. Co-infection with different PPVs was detected in 63 wild boars (Supplementary Table 3). Cases of a combination of four, three, or two PPVs in one individual were observed in 7.4% (8/108), 18.5% (20/108), and 32.4% (35/108), respectively. Each region recorded instances of the simultaneous detection of two to four viral species. The most common combinations were PPV3/PPV7 (13.9%, 15/108) followed by PPV2/PPV3/PPV7 (6.5%, 7/108).

Gender was determined for 68 wild boars: 47 males and 21 females (Table 2). There were no statistically significant distributions of different PPVs within the group of males and the group of females ($p > 0.05$). PPV7 and PPV3 were more common among both genders. However, when compared between males and females, PPV6 was statistically significant ($p = 0.002$), found only in females (23.8%).

The age of 77 wild boars was determined: 41 were young (under one year old) and 36 were adults (over one year old) (Table 2). PPV7 was the most common species among the young animals ($p < 0.05$). There was no statistically significant distribution of different PPVs among adults and between both age groups ($p > 0.05$ for all pairwise comparisons).

Spleen, lung, and lymph node samples were obtained from 57 wild boars, and an incomplete set of organs was achieved for 51 wild boars (Table 2). Statistically significant differences ($p < 0.01$) in the virus detection in different specimen types were noted for PPV7, with dominance in lymph nodes (82.9%). Also, compared with other organs, PPV1 (87.5%) was predominantly detected in the lymph nodes. Uniform detection of virus genomes in the lungs, lymph nodes, and spleen was observed for PPV3 with the following rates: 76.2%, 85.7%, and 90.5%, respectively.

PRRSV, PCV4, and CSFV were not detected in any of the samples, whereas PCV2 and PCV3 were detected in 73 (67.6%) and 48 (44.4%) animals, respectively. The results of the paired detection of the PPVs, PCV2, and PCV3 genomes are presented in Table 3.

The results of the Fisher test showed no significant statistical difference in the frequency of coinfections

Table 3 Detection rates of PPVs in PCV2/PCV3-positive/negative samples from wild boars

	PPV1	PPV2	PPV3	PPV4	PPV5	PPV6	PPV7
PCV2-positive (73)	18 (24.7%)	20 (27.4%)	38 (52.1%)	2 (2.7%)	1 (1.4%)	8 (11.0%)	44 (60.3%)
PCV2-negative (35)	9 (25.7%)	13 (37.1%)	15 (42.9%)	0 (0.0%)	1 (2.9%)	0 (0.0%)	20 (57.1%)
PCV3-positive (48)	13 (27.1%)	14 (29.2%)	31 (64.6%)	1 (2.1%)	0 (0.0%)	2 (4.2%)	27 (56.3%)
PCV3-negative (60)	14 (23.3%)	19 (31.7%)	22 (36.7%)	1 (1.7%)	2 (3.3%)	6 (10.0%)	37 (61.7%)

between PCV2 and PPVs, as well as between PCV3 and PPVs.

Phylogenetic analysis of PPVs

For phylogenetic analysis, complete nucleotide sequences of VP1/VP2 capsid protein genes of PPV1 isolates from 17 wild boars, PPV2, PPV3 and PPV7 from three wild boars, as well as PPV6 from one wild boar were obtained. A nearly complete VP2 nucleotide sequence was obtained from one PPV5 isolate from the Moscow Region. It was not feasible to obtain any PPV4 sequences due to the low concentration of viral DNA in the samples. Sequences were deposited in the NCBI GenBank under accession numbers (Supplementary Table 4). The isolate nomenclature includes abbreviations for the region of origin, as follows: Mos– for Moscow, Tv– for Tver, and Bel– for Belgorod Regions, respectively.

Comparative analysis showed 98.2–99.6% overall nucleotide identities between all sequences and 98.8–100% similarity among the Russian strains. In the Moscow Region, the nucleotide identity between the isolates equalled 98.79–100%; in the Tver Region, 99.26–100%; in the Belgorod Region, 99.22–99.68%. There is also a high nucleotide identity between the regions: Moscow and Tver – 99.0–100%; Moscow and Belgorod 99.0–99.87%; Tver and Belgorod 99.17–99.91%. Phylogenetic analysis, in accordance with the clusterization proposed by Vereecke et al. 2022, revealed that 14 sequenced isolates (except for MosWB27, BelWB57, and BelWB70) belonged to the PPV1b cluster with European 27a-like strains from domestic pigs, Romanian isolate from wild boar JQ249915, and Chinese isolate MN326131. The nucleotide identity between the highly virulent German isolate 27a (AY684871) and our isolates from this cluster was 99.60–99.69%. Isolate MosWB27 belonged to the PPV1d cluster and grouped with strains from South Korea and China, with nucleotide identities of 99.6–99.78% and 99.47–99.6%, respectively. The nucleotide identity between the isolate MosWB27 and the pathogenic Kresse strain included in this cluster was 99.56%. The isolate BelWB70 was located in a separate branch of the PPV1d cluster with isolates from Romanian wild boars: JQ249918, and JQ249913. The isolate BelWB57 formed a distinct branch that was not included in either the cluster with the 27a-like strains (nucleotide identity with the 27a of 99.38%) or the cluster containing the pathogenic Kresse strain (nucleotide identity with the Kresse of 99.60%) (Fig. 1).

Phylogenetic analysis based on the complete nucleotide sequence of the PPV2 capsid protein showed that our isolates were divided into two groups. Isolates from two regions PPV2-TvWB49 and PPV2-MosWB107 belonged to a clade with Polish isolate from a domestic pig and Romanian and Chinese isolates from wild boars. On a separate branch was isolate PPV2-BelWB73, with the highest nucleotide identity to the Serbian (KC701306) and Chinese (NC038883) isolates. The PPV2 isolates were identical to each other with 99.6–99.8% similarity. When considering the phylogenetic dendrogram of PPV3, we also observed two groups of isolates. Isolate PPV3-TvWB41 was part of a "European" clade, which includes strains from European pigs, with similarities ranging from 98.5 to 99.1%. Isolates from distinct regions PPV3-BelWB73 and PPV3-MosWB95 were in a clade containing strains from pigs from Colombia and Hungary and wild boars from China. The nucleotide identity between our isolates equalled 98–99.46%. The PPV6 isolate PPV6-BelWB74 belonged in a clade with Russian porcine isolate Kem-8 from the Kemerovo Region (with a nucleotide identity of 99.69%), Poland (with a nucleotide identity of 99.61–99.86%) and Spain (with the 99.77% identity). Phylogenetic analysis based on the particular nucleotide sequence of the capsid protein PPV5 showed that the isolate PPV5-MosWB28 belonged to a clade with strains from Colombia, South Korea, and the USA. Isolates obtained from pigs in Russia were in two other clades and their nucleotide identity with PPV5-MosWB28 was 99.1–99.3%. The phylogenetic dendrogram of PPV7 showed that our isolates were divided into two groups. Isolates PPV7-BelWB64 and PPV7-TvWB80 were located in a separate branch and their nucleotide identity was 99.6%. Isolate PPV7-MosWB93 belonged to the group with porcine strains from Colombia, China, and Brazil (nucleotide identity of 95.02–97.12%) (Fig. 2).

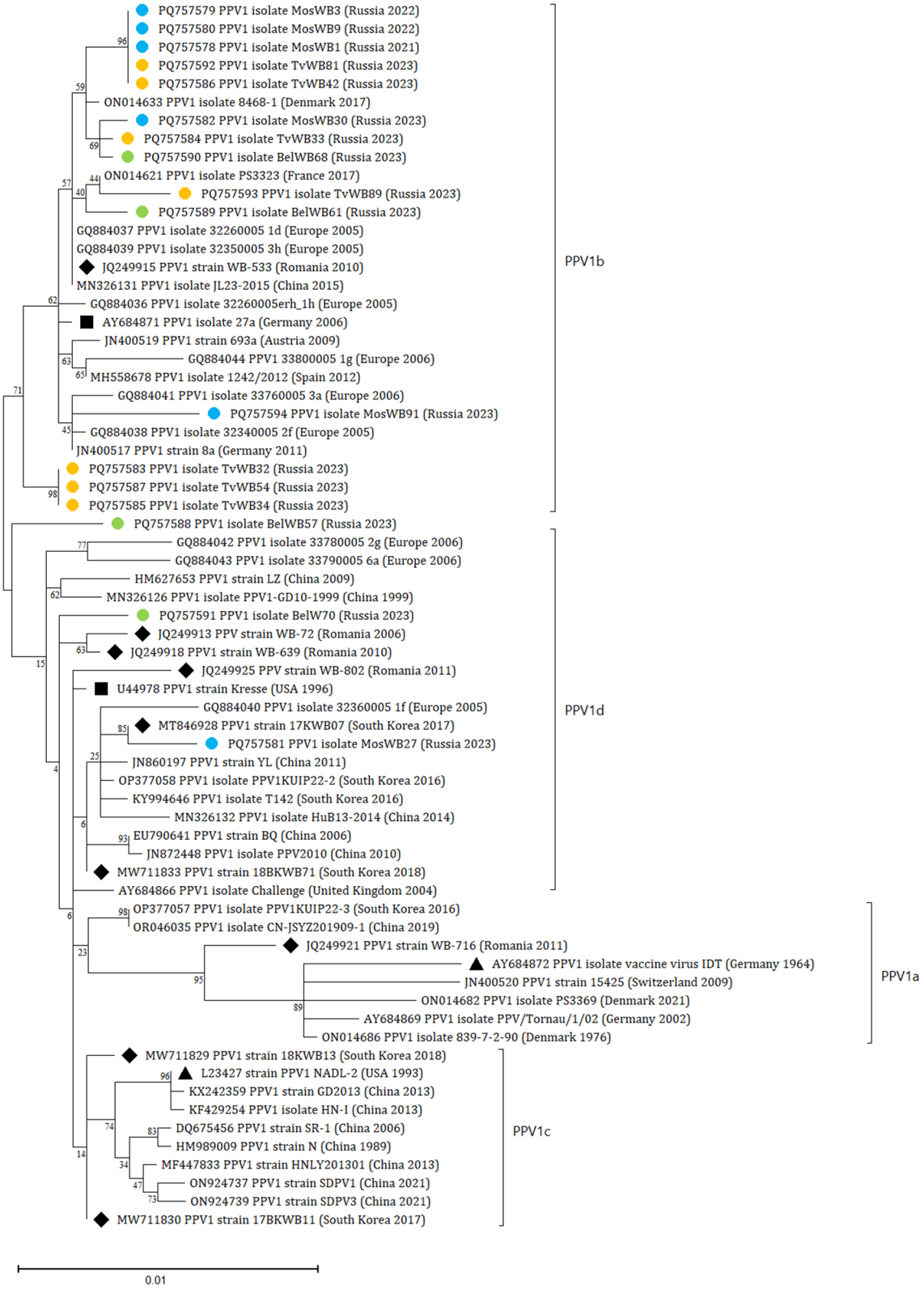

Fig. 1 Phylogenetic tree showing clustering of PPV1 isolates by region and strain type. The obtained isolates are designated by colored circles. Blue circles - Moscow Region, yellow circles - Tver Region, green circles - Belgorod Region. Isolates obtained from wild boars from other countries are designated by ◆, the vaccine strains are designated by ▲, and highly virulent strains are designated by ■. Clusterization of PPV1 strains is given in accordance with the classification of Vereecke et al. [5]

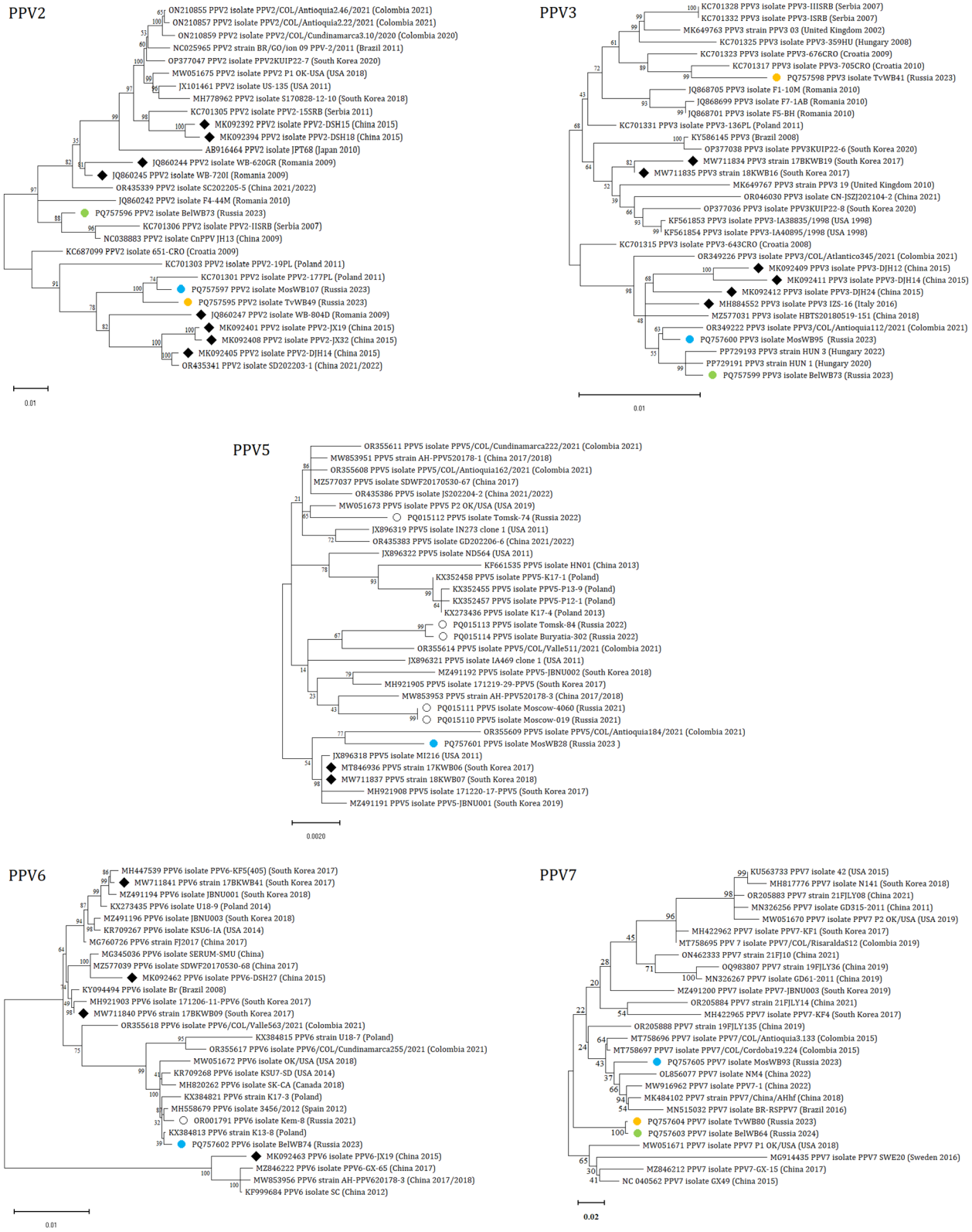

Fig. 2 Phylogenetic trees showing the relationship of novel PPV isolates obtained in this study with strains from other countries available in the GenBank. The obtained isolates are designated by colored circles. Blue circles - Moscow Region, yellow circles - Tver Region, green circles - Belgorod Region. Isolates obtained from wild boars from other countries are designated by ◆. Isolates obtained from domestic pigs in Russia are designated by ○

Amino acid substitutions in VP1/VP2 genes of PPV1 isolates

At the level of the VP2 amino acid sequence, all the obtained isolates showed the following substitutions compared to the NADL-2 vaccine strain: Thr_{45}→Ser, Ile_{215}→Thr, Asp_{378}→Gly, His_{383}→Gln, Arg_{565}→Lys. In addition, substitutions Gln_{228}→Glu, Ala_{414}→Ser, Glu_{419}→Gln, Ser_{436}→Thr were found in isolates MosWB1, MosWB3, MosWB9, MosWB30, TvWB33, TvWB42, BelWB61, BelWB68, TvWB81, TvWB89 and MosWB91. Isolates TvWB32, TvWB34, TvWB54, and BelWB57 were characterized by Ala_{414}→Ser and Ser_{436}→Thr substitutions. Nucleotide changes leading to unique amino acid replacements were found in the VP1/VP2 gene sequence of isolate MosWB27: Arg_{114}→Lys (VP1), Ile_{320}→Thr, Asn_{370}→Asp. The isolate also had the Ser_{436}→Pro substitution. The unique substitutions Glu_{29}→Lys (VP1), Thr_{98}→Ile (VP1), Ala_{13}→Thr, Arg_{82}→Lys were presented in isolate MosWB91 and in isolate BelWB70 Ser_{436}→Ala. Amino acid substitutions in the VP1/VP2 sequences of isolates obtained from wild boars are presented in Table 4.

Discussion

Wild boars are widespread throughout the world and transmit plenty of porcine viruses (ASFV, PRRSV, PCVs, and PPVs) [21]. The food accessibility attracts them to settlements and agricultural lands, which increases the likelihood of contact between them and domestic pigs. In this work, we studied the prevalence and genetic diversity of PPV species circulating in wild boar populations in three regions of Russia. Our results showed that seven PPV species were detected among wild boars, with at least one of them observed in 98 out of 108 individuals (90.7%). The most common species were PPV7 (59.3%) and PPV3 (49.1%), followed by PPV2 (30.6%) and PPV1 (25%). PPV7 was prevalent in the Moscow Region ($p < 0.01$). High detection rates of PPV3 and PPV7 were registered in the Tver and Belgorod Regions. The number of studies on the circulation of novel PPVs in wild boars is limited. Our results on the detection of PPV2 and PPV3 are within the range between the values presented by European colleagues. Previously, PPV3 detection has been reported in Romania (22.8 and 50.5%) [30], Germany – 32.7% [29], and Slovakia – 19.1% [32], as well as PPV2–3 in Serbia (21.7/69.6%) [31]. At the same time, Park et al. reported a low detection rate or complete absence (PPV2) of novel PPVs in South Korea [33]. Since PPV1 has a confirmed association with SMEDI, more data is available on its distribution among wild boar herds. Our research showed that PPV1 is widespread in Russia, with detection in 20.8%, 25.7%, and 32% of wild boars from the Moscow, Tver, and Belgorod Regions, respectively. By contrast, this detection rate was lower than in Italy – 44.4% [23] and Serbia – 56% [24], but higher than in Romania (5.2%) [7] and South Korea (5.4%) [33]. The limitation of the research consists of the low number of samples from the Belgorod Region, which were obtained during the depopulation measures to control the spread of ASF.

Since gender and age were determined, we took into account the frequency of PPVs detection in these categories. The distribution of viruses between the genders was uniform except for PPV6, but this could be influenced by its low total detection rate. No statistically significant PPVs distribution was found separately within the group of males and females. According to the results, PPV7 and PPV3 were the most common in both males and females, which aligns with the results of the virus spread in the country. PPV1 was evenly distributed between males and females (25.5/28.6%). The absence of a connection between gender and the PPV1 detection rate was also evidenced in the Serbian and Italian wild boar populations [23, 24].

Considering the age category, there was no statistically significant difference connected with the prevalence of each PPV species between adult and young wild boars. Our conclusion is consistent with the results of studies on the relationship between age and detection rate of PPV1 conducted in Italy [23] and Serbia [24] and PPV3 in Slovakia [32]. However, PPV7 was significantly more common in young animals (up to a year old) compared to other PPVs.

According to our analysis of PPV1–PPV7 in various organs (spleen, lymph nodes, and lungs), it was found that for the majority of PPV species (PPV1–PPV6) there was no statistically significant difference in the distribution of viruses between these organs. This result is consistent with previous studies and indicates that these viruses can infect different tissues with similar frequency, without exhibiting organ specificity [13, 30, 31]. However, PPV7 was more often detected in lymph nodes (82.9%), which may be explained by the immune system function or indicate its propensity to localize in the lymphatic system, as it happens in cases with other PPVs, but further studies are required [19].

To identify the distribution between PPVs and PCV2/PCV3 in wild boars, we determined the viral DNA presence in the PCV2/PCV3 positive and negative samples. Despite the fact that some authors noted the connection of PPV1–7 with PCV2 [12, 17, 32, 45–47], there is no common opinion on how these viruses interact with each other. In our research, there were no statistically significant results of PPVs detection in wild boars, positive and negative for PCV2/PCV3, which indicates the random nature of coinfections in the studied wild boar population.

Table 4 Amino acid substitutions in the VP1/VP2 sequences of PPV1 isolates from wild boars relative to the NADL-2 vaccine strain. Dashes indicate the identity of amino acids

PPV1 isolate	Position in the amino acid sequence of PPV1																
	VP1				VP1/VP2												
	29	82	98	114	163 13	195 45	232 82	365 215	378 228	470 320	520 370	528 378	533 383	564 414	569 419	586 436	715 565
NADL2	Glu	His	Thr	Arg	Ala	Thr	Arg	Ile	Gln	Ile	Asn	Asp	His	Ala	Glu	Ser	Arg
Kresse	-	-	-	-	-	Ser	-	Thr	-	-	-	Gly	Gln	-	-	Pro	Lys
27a	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
MosWB1	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
MosWB3	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
MosWB9	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
MosWB27	-	-	-	Lys	-	Ser	-	Thr	-	Thr	Asp	Gly	Gln	-	-	Pro	Lys
MosWB30	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
TvWB32	-	-	-	-	-	Ser	-	Thr	-	-	-	Gly	Gln	Ser	-	Thr	Lys
TvWB33	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
TvWB34	-	-	-	-	-	Ser	-	Thr	-	-	-	Gly	Gln	Ser	-	Thr	Lys
TvWB42	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
TvWB54	-	-	-	-	-	Ser	-	Thr	-	-	-	Gly	Gln	Ser	-	Thr	Lys
BelWB57	-	-	-	-	-	Ser	-	Thr	-	-	-	Gly	Gln	Ser	-	Thr	Lys
BelWB61	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
BelWB68	-	Tyr	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
BelWB70	-	-	-	-	-	Ser	-	Thr	-	-	-	Gly	Gln	-	-	Ala	Lys
TvWB81	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
TvWB89	-	-	-	-	-	Ser	-	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys
MosWB91	Lys	-	Ile	-	Thr	Ser	Lys	Thr	Glu	-	-	Gly	Gln	Ser	Gln	Thr	Lys

Over the past 20 years, numerous studies have focused on the genetic diversity of PPV1 in domestic pigs and wild boars. Streck et al. and Cadar et al. proposed to classify PPV1 strains into six clusters (A-F) based on the genetic heterogeneity of the VP1/VP2 gene sequences [7, 8]. Following this classification, most of the isolates, except for MosWB27, BelWB57, and BelWB70, belonged to cluster B, which is represented by 27a-like PPV1 strains. However, isolate MosWB91 was characterized by Arg_{82}→Lys replacement in the VP2, which is typical of representatives of cluster A. Isolate MosWB27 belonged to cluster E, which also included virulent strains Kresse and Challenge. Isolate BelWB70 was placed along with isolates from Romanian wild boars into cluster D, which is characterized by the replacement of Ser_{436}→Ala at the critical point of the mutation involved in tissue tropism [48]. However, according to this classification, isolate BelWB57 did not belong to any group. Recently, a novel classification system of PPV1 strains has been proposed with a division into four distinct clusters (PPV1a–PPV1d) based on amino acid differences within and between them [5]. According to this system, most of the isolates were assigned to the PPV1b cluster with 27a-like strains. Furthermore, among 27a-like strains, a monophyletic clade comprising isolates from bordered Moscow and Tver Regions stands out, showing phylogenetic proximity supported with an excellent bootstrap value (96%). Isolates MosWB27 and BelWB70 were part of the PPV1d cluster. It was difficult to assign isolate BelWB57 to any specific cluster as well as in the classification by Cadar et al.

The phylogenetic analysis, conducted in accordance with several classification systems, revealed that the obtained Russian PPV1 isolates mainly belonged to the 27a-like group. This group has emerged and spread in the swine population in Europe since the early 2000s, and, to some opinions, it was the result of viral adaptation to widely used commercial vaccines over the last few decades [49]. It has previously been shown that PPV1 in the European wild boar populations appears to be more diverse compared to the viruses from domestic pigs. Fourteen viral isolates from Romanian wild boars were diversified into five clusters that grouped together with various porcine isolates [7]. However, the predominance of 27a-like PPV1 isolates in Russian wild boar herds raises questions about this hypothesis. Due to the fact that vaccination is not currently performed in wild boar populations, there is a question about the direction of the virus transmission pathway, as well as the vaccination's role as a driving force in the PPV1 evolution. Additionally, more sequencing data from other European countries need to be obtained in order to fully understand the genetic composition of currently prevalent PPV1 strains in wild boar herds.

It was experimentally demonstrated that only a few changes in amino acid positions in the VP1/VP2 sequences differentiate highly virulent PPV1 strains from non-pathogenic ones [4, 7, 49, 50]. At the same time, most of the known distinctions in the NSP domain are silent [4, 14]. During the work, it was established that isolates MosWB27 and BelWB70 possessed six amino acid substitutions that distinguish the highly pathogenic Kresse strain from the non-pathogenic NADL-2: Thr_{45}→Ser, Ile_{215}→Thr, Asp_{378}→Gly, His_{383}→Gln, Ser_{436}→Pro/Ala and Arg_{565}→Lys. In previous works it was determined that positions 378, 383, and 565 are crucial for the host range and the hemagglutination capability, position 215 is necessary for antibody recognition, and position 436 is responsible for tissue tropism [7, 48–50]. However, isolate MosWB27 also contained substitutions other than the Kresse and NADL-2 strains: Arg_{114}→Lys (VP1), Ile_{320}→Thr, Asn_{370}→Asp.

The prevalent 27a-like strains in European domestic pig herds are additionally characterized by unique amino acid replacements, such as Gln_{228}→Glu, Ala_{414}→Ser, Glu_{419}→Gln, Ser_{436}→Thr [7, 49, 51]. All our isolates within the group with 27a-like strains also contained most of these hallmark substitutions. However, in the isolates TvWB32, TvWB34, TvWB54, and BelWB57, Gln_{228}→Glu and Glu_{419}→Gln substitutions were not detected. It should be noted that the isolate BelWB57 was characterized not only by the distinctive changes associated with pathogenicity but also by characteristic amino acid substitutions for the Kresse (Gln_{228} and Glu_{419}) and the 27a (Ser_{414} and Thr_{436}) strains. In general, we can assume that the occurrence of identical substitutions in PPV1 from different countries takes place due to a cognate evolutionary direction, but the appearance of untypical mutations or their combinations in our isolates and their biological consequences are not yet clear and require further *in vitro* studies.

The PPV2, PPV3, and PPV7 isolates were assigned to different clades with strains from both wild and domestic animals. Due to the limited number of isolates, it is difficult to assess the connection between clustering and the geographical distribution of strains obtained from wild boars; however, it is worth noting the absence of clusters with isolates obtained only from wild boars. The PPV6 isolate was included in the same group as the Russian isolate Kem-8 obtained from a domestic pig in the previous study [52], and showed high nucleotide similarity with it. At the same time, the PPV5 isolate was not included in any group with representatives from our country but was close to isolates from Colombia, the USA, and wild and domestic pigs from South Korea.

Conclusions

This is the first study that emphasizes the genetic diversity of PPV1 and the circulation of novel PPVs in the wild boar population in Russia. Our results delineate the presence of seven PPV species in the country and demonstrate that age, gender, and the presence of coinfection with PCV2/3 do not affect the detection rate of PPVs in wild boars. Phylogenetic analysis of PPV1 revealed that most of our isolates were assigned to the group with 27a-like strains from Europe. Isolates MosWB27 and BelWB70 were part of a cluster with a highly virulent Kresse strain, but they contained a number of amino acid substitutions that were not typical for this group. Isolate BelWB57 demonstrated amino acid substitutions characteristic of both Kresse and 27a strains and was not assigned to any group. The novel PPVs isolates were distributed in numerous clusters with isolates from wild boars and domestic pigs from different countries. Future studies will focus on ongoing monitoring of a broader range of samples to explore the circulation of PPVs both in wild boar and domestic pig populations to comprehensively analyze the current epidemiological situation. Additionally, phylogenetic and recombination studies are necessary in order to comprehend the evolution of PPVs and determine the direction of infection transmission routes.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

A. K. performed qPCRs, Sanger sequencing, phylogenetic and statistical analyzes, and wrote the original draft of the manuscript. N. K., M. S., and V. R. performed Sanger sequencing. M.S. and V. R. performed qPCRs. E. Z. performed the sample preparation. A. K., N. K., and A. Y. were involved in the formulation and design of the study. A. Y. participated in the organization of the sampling process, funding arrangement and supervised the study execution. A. K., N. K., A. B., and A. Y. analyzed and interpreted the data and reviewed the final manuscript. All authors read and approved the final manuscript.

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Data availability

Viral nucleotide sequences are available at the NCBI GenBank under the accession numbers PQ757578-PQ757605.

Declarations

Ethical approval

The animal study was approved by the Ethical and Animal Welfare Committee of the Federal State Budget Scientific Institution "Federal Scientific Center VIEV," (Moscow, Russia). Approval number 662/22 from 17 June 2021. The study was conducted in accordance with the local legislation and institutional requirements.

Competing interests

The authors declare no competing interests.

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