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# First report of transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) in pigs from Poland

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## Abstract

Porcine transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) are swine coronaviruses belonging to the genus *Alphacoronavirus* in the family *Coronaviridae*. To date, there are no reports on the prevalence and genetic characterization of these viruses in domestic pigs from Poland. In this study, 828 serum samples were tested by TGEV/PRCV immunoassay to estimate TGEV and PRCV seroprevalence, while 277 nasal swabs and 221 stool samples were tested by real-time PCR to detect viral RNA. Our results revealed that 2.2% (95% CI 1.2, 3.2) of serum samples were positive for anti-TGEV antibodies, while 12.2% (95% CI 9.8, 14.4) of samples were positive for anti-PRCV antibodies. 2.5% (95% CI 1.5, 2.6) and 5.2% (95% CI 3.7, 6.7) of serum samples were inconclusive for TGEV and PRCV, respectively. RNA of TGEV was not detected in any of the tested samples, while PRCV RNA was detected in 6.22% of samples. Genetic and phylogenetic analysis revealed that all Polish PRCV strains were closely related to European and Korean PRCV strains than to American strains. Some of the Polish PRCV strains have a 672 nt deletion at the same position at the 5' end of the S gene as other European and Korean PRCV strains, suggesting that they originated from the same precursor. Other Polish PRCV strains had a 690 nt deletion that differed in size and location from any of the known PRCV strains. This may suggest that these Polish PRCVs may have originated from different ancestor. Furthermore, the Polish PRCV strains showed some unique changes in their sequences, which may reflect their evolution. This study is the first report on the prevalence of TGEV/PRCV in pigs from Poland. In addition, this is the first report on the genetic characterization of Polish PRCV strains, which provide new information on PRCV heterogeneity.

**Keywords** TGEV, PRCV, Coronaviruses, Pig, Real-time RT-PCR, Sequencing

## Introduction

Porcine transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) are swine coronaviruses belonging to the genus *Alphacoronavirus* in the family *Coronaviridae*. TGEV causes transmissible gastroenteritis (TGE), which is on the World Organization for Animal Health (WOAH) list of notifiable diseases. TGE was first reported in the United States in 1946 and subsequently spread worldwide, causing severe diarrhea and vomiting in swine herds. TGEV infects pigs of all age groups, but the disease is most fatal in piglets less than 2 weeks old [23]. However, the occurrence of TGE has gradually declined since 1984 due to the emergence and gradual spread of porcine respiratory coronavirus (PRCV), a natural mutant of TGEV with a large deletion (621–681 nt) in the 5' spike (S) gene and small deletions in the 3/3a and 3 – 1/3b genes. Deletion in the S gene is thought to be associated with differences in tissue tropism between TGEV and PRCV. Unlike TGEV, which replicates mostly in small intestine epithelial cells, PRCV

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replicates almost exclusively in respiratory epithelial cells and causes no or mild respiratory infection symptoms. No diarrhea is observed in PRCV-infected pigs [23, 40].

Both TGEV and PRCV contain a single-stranded positive sense RNA genome of ~28 kb and have four genes encoding structural proteins (the spike (S), envelope (E), membrane (M) and nucleocapsid (N)) and five genes encoding nonstructural proteins. The S protein is the main structural protein of the viral envelope and is involved in recognition and binding to the host receptor. Furthermore, it is the main protein that contains epitopes that induce neutralizing antibodies [12]. The M protein is a transmembrane protein and is involved in virion formation. The E protein is a membrane-associated protein that plays an important role in the early maturation stage of the virus. The N protein binds to genomic RNA to form a nucleocapsid and is involved in the induction of apoptosis [22].

The diagnosis of PRCV and TGEV is possible by isolation of the virus in cell cultures, detection antibodies against the virus and detection of viral nucleic acids or proteins. The isolation of viruses in cell cultures is a long-term process, so serological and molecular methods are most commonly used to detect these viruses. However, the genetic and antigenic similarities between PRCV and TGEV create problems in the diagnosis of these coronaviruses. In protein S, antigenic site A, which is the main inducer of neutralizing antibodies, is highly conserved among TGEV and PRCV strains. Therefore, the classical virus neutralization test and most conventional serologic tests cannot distinguish between pigs infected with TGEV and those infected with PRCV. Differential serological diagnosis is only possible with the implementation of a blocking ELISA with a monoclonal antibody targeting the S protein epitope that is deleted in PRCV [8]. Molecular techniques such as reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR and real-time reverse-transcription polymerase amplification (RT-RPA), which were developed for the direct detection of TGEV and PRCV in clinical samples, also allow differentiation between TGEV and PRCV, but only when the target amplification product is an S gene sequence conserved in TGEV but absent in PRCV [20]. Differentiation between TGEV and PRCV can also be achieved by analyzing PCR products through restriction enzyme cleavage or sequencing.

The spread of PRCV and TGEV increases in high-density herds because the viruses are transmitted via aerosol and oral-fecal routes, respectively. In addition, the incidence of TGEV and PRCV is greater in cold seasons, probably due to the increased survival of the viruses at lower temperatures and less exposure to sunlight, to which they are sensitive [23, 40]. PRCV infection may partially protect against TGEV; therefore, the presence of TGEV in PRCV-immune herds reduces the enteric clinical signs of TGEV and pre-weaning mortality [9]. Currently, TGE is considered a disease of the past. However, sporadic antibodies to TGEV and even outbreaks have been observed in Europe, North America and Asia, indicating that TGEV is still present in swine herds [11, 14, 23, 24, 29, 32, 40, 42].

To date, there are no reports on the circulation of PRCV and TGEV in domestic pigs from Poland. Therefore, the aim of this study was to fill this gap in knowledge. The first objective was to determine the relative prevalence of antibodies to TGEV and PRCV in Poland. The second objective was to genetically characterize the TGEV/PRCV strains circulating in Poland and determine their genetic relatedness to sequences of other PRCV/TGEV strains circulating worldwide. This is the first description of the occurrence of TGEV and PRCV in swine herds in Poland and the first description of PRCV sequences from Polish pigs. This study undoubtedly provides new information on the genetic variability and evolution of currently circulating PRCV strains.

## Materials and methods

### Sample collection

The serum samples used in this study were a fraction of the samples collected by veterinary inspection during official annual sampling to monitor the classical swine fever (CSF) eradication program in Poland. Serum samples were collected from 828 randomly selected pigs, representing 0,0075% of the total pig population in Poland, as estimated by the Central Statistical Office (Poland) and were collected from 13 out of 16 voivodships in Poland (wielkopolskie, podkarpackie, podlaskie, pomorskie, kujawsko-pomorskie, warmińsko-mazurskie, lubuskie, zachodnio-pomorskie, małopolskie, opolskie, łódzkie, śląskie, świętokrzyskie). A summary of the number of animals in each of the voivodships subjected to this study is shown in Table 1. Samples were randomly collected from different herds. No herds were vaccinated against the PRCV or TGEV strains. Furthermore, 277 nasal swab samples and 221 fecal samples collected between 2021 and 2024 were used to detect PRCV/TGEV RNA. The nasal swabs were obtained from animals with suspected respiratory disease, whereas the fecal samples were obtained from pigs with gastrointestinal symptoms. Both, nasal swabs and fecal samples were collected in a noninvasive manner by veterinarians during routine veterinary examinations, therefore ethical review and approval were not required. Verbal informed consent was obtained from all owners prior to sampling.

**Table 1** Overview of the number of serum samples used in this study

| Voivodship | Total number of animals | Numer of tested samples | % of tested samples |
|---|---|---|---|
| Wielkopolskie | 4209713 | 48 | 0,00114 |
| Podkarpackie | 113725 | 96 | 0,08441 |
| Podlaskie | 359254 | 50 | 0,01391 |
| Pomorskie | 788429 | 60 | 0,00761 |
| Kujawsko-pomorskie | 1039095 | 108 | 0,01039 |
| Warmińsko-mazurskie | 581893 | 93 | 0,01598 |
| Lubuskie | 75960 | 99 | 0,13033 |
| Zachodniopomorskie | 208239 | 55 | 0,02641 |
| Małopolskie | 117134 | 50 | 0,04268 |
| Opolskie | 310241 | 50 | 0,01611 |
| Łódzkie | 1011133 | 51 | 0,00504 |
| Śląskie | 182840 | 46 | 0,02515 |
| Świętokrzyskie | 171142 | 22 | 0,01285 |

### TGEV/PRCV ELISA

All sera were tested with a TGEV/PRCV blocking ELISA (SVANOVIR TGEV/PRCV-Ab, Svanova, Biotech AB, Uppsala, Sweden) according to the instructions included in the kit. The ELISA is based on the use of two monoclonal antibodies: a monoclonal antibody specific for TGEV (TGEV mAb), which recognizes an antigen that PRCV does not have, and a monoclonal antibody that recognizes antigens present in both TGEV and PRCV (TGEV/PRCV mAb). The ELISA results were interpreted as positive, negative or inconclusive for PRCV and TGEV.

### RNA extraction

The fecal samples were diluted 1:10 (v/v) with phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na2HPO4 and 1.8 mM KH2PO4), vortexed and centrifuged for 8 min at 6,000× g at 4°C. The clarified supernatants were collected and stored at -80°C for RNA extraction. The viral RNA was extracted from 140 µl of fecal supernatants and nasal swabs using a QIAMP Viral RNA Mini Kit (Qiagen, Hilden, Germany).

### Real-time RT-PCR assays

Two real-time RT-PCR assays were used for the detection and differentiation of TGEV and PRCV. First, RNA samples extracted from all fecal and nasal swab samples were tested by real-time RT-PCR using primers and a probe targeting the nucleocapsid (N) gene that reacts with both PRCV and TGEV. Positive samples were then used for differentiation between TGEV and PRCV using real-time RT-PCR with primers and a probe targeting the TGEV spike gene without the expectation of reacting with PRCV. The samples were considered PRCV positive when they yielded positive results with primers and probe targeting the PRCV N gene but negative results with primers and probe targeting the TGEV S gene, whereas the TGEV-positive samples gave positive results with primers and probe targeting both, the N gene of PRCV and the S gene of TGEV. All real-time RT-PCR amplifications were run on a Rotor-Gene Q cycler (Qiagen, Heiden, Germany) using the QuantiTect Probe RT-PCR kit (Qiagen, Hilden, Germany). PCR reactions were performed in a total volume of 20 µl containing 10 µl of 2x QuantiTect Probe RT-PCR Master Mix, 0.4 µl of forward primer at 20 µM, 0.4 µl of reverse primer at 20 µM, 0.4 µl of probe at 10 µM, 0.2 µl of QuantiTect RT Mix, 5.6 µl of nuclease-free water and 3 µl of extracted RNA. Amplification reactions were performed under the following conditions: one cycle at 50°C for 30 min, one cycle at 95°C for 15 min and 40 cycles at 94°C for 15 s and 60°C for 60 s. The PRCV AR310 and Miller TGEV strains were used as positive controls for each PCR. The PCR negative control was ultrapure water instead of sample to confirm the absence of PCR contamination. The sequences of the primers and probes used for real-time RT-PCR are shown in Table 2.

### Conventional RT-PCR, sequencing and sequence analysis

Real-time RT-PCR positive samples were subjected to conventional RT-PCR to detect the partial open reading frame (ORF) 1b and the 5' region of the S gene sequence. RT-PCR was performed using the OneStep RT-PCR Kit (Qiagen, Hilden, Germany) and primers

**Table 2** Sequences of primers and probes used for the detection and differentiation of TGEV and PRCV

| Target | Primer/probe | Sequence (5'-3') | Gene | Reference |
|---|---|---|---|---|
| PRCV and TGEV | F | AGCTATTGGACTTCAAAGGAAGATG | nucleocapsid | [28] |
| | R | CATAGGCATTAATCTGCTGAAGGAA | | |
| | P | FAM-TCACGTTCACACACAAATACCACTTGCCA_BHQ | | |
| TGEV | TGEV-S-F | GTGGTAATATGYTRTATGGCYTACAA | spike | [36] |
| | TGEV-S-R | GCCAGACCATTGATTTTCAAAACT | | |
| | TGEV-S-P | FAM-TTGCTTATTTACATGGTGCYAGT-BHQ1 | | |

(F1 5′ GGGTAAGTTGCTCATTAGAAATAATGG 3′ and R1 5′ CTTCTTCAAAGCTAGGGACTG3′) as described previously [20]. These primers target deletion of the spike gene. The reaction was carried out under the following conditions: 50°C for 30 min, 95°C for 15 min, 35 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, followed by a final elongation step at 72°C for 10 min. Reverse transcription polymerase chain reaction (RT-PCR) products were visualized under ultraviolet (UV) light after electrophoresis in a 1.5% agarose gel containing Simply Safe (EURx, Poland) in 1 × Tris-acetate-EDTA (TAE) buffer (40 mM Tris/acetate buffer and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0). Positive samples were purified using Nucleo Spin Gel and PCR Clean-up (Macherey-Nagel, Germany) and subjected to Sanger sequencing with the F1 and R1 primers. Sequencing was performed by a commercial company (Genomed S.A., Warsaw, Poland). The obtained sequences were edited and assembled via Geneious Pro 5.3 software (Biomatters Ltd., New Zealand). The sequences obtained in this study and other PRCV and TGEV reference sequences retrieved from GenBank were aligned using the Muscle algorithm. A phylogenetic tree was constructed using the maximum likelihood method with the best-fit model (TN93 + G). Nonparametric bootstrap analysis with 1000 iterations was used to evaluate the robustness of the evolutionary relationships. Alignment, tree building and pairwise genetic distance calculations were performed using MEGA software version 6.06 [38]. The sequences obtained in this study were submitted to GenBank under accession numbers: PQ197042-PQ197056.

## Results

### Serological examination

Out of 828 tested sera, 18 (2.2%, 95% CI 1.2, 3.2) were TGEV-antibody positive, and 101 (12.2%, 95% CI 9.8, 14.4) were PRCV antibody-positive, whereas 21 (2.5%, 95% CI 1.5, 2.6) and 43 (5.2%, 95% CI 3.7, 6.7) were inconclusive for TGEV and PRCV, respectively. The prevalence varies by region. The highest prevalence of antibodies against TGEV was found in the Podlaskie (10.0%, 95% CI 1.7, 18.3) and Lubuskie (9.1%, 95% CI 3.4, 14.8) voivodships. In contrast, no positive or inconclusive samples were detected in Wielkopolska, Pomorskie, Małopolskie, Opolskie, Łódzkie, Śląskie or Świętokrzyskie voivodships. With respect to the PRCV, positive or inconclusive results were found in each of the voivodeships studied. The highest prevalence of antibodies against PRCV was found in Pomorskie (20.0%, 95% CI 9.9, 30.1), Warmińsko-mazurskie (25.8%, 95% CI 16.9, 34.7) and Lubuskie (21.2%, 95% CI 13.2, 29.3) voivodships. The lowest prevalence was detected in Podkarpackie, Małopolskie and Śląskie voivodships (Table 3, Figure S1).

### Molecular examination

Twenty one (7.6%) of the 277 nasal swab samples and 10 (4.5%) of the 221 fecal samples were positive using real-time RT-PCR targeting the nucleocapsid (N) gene, which

**Table 3** Seroprevalence of TGEV and PRCV determined in this study

| Voivodship | TGEV | | PRCV | |
|---|---|---|---|---|
| | Animal seroprevalence (%) (95% CI) | | Animal seroprevalence (%) (95% CI) | |
| | Positive | Inconclusive | Positive | Inconclusive |
| Wielkopolskie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 6.3 (-0.6, 13.1) | 6.3 (-0.6, 13.1) |
| Podkarpackie | 0.0 (0.0, 0.0) | 1.0 (-1.0, 3.1) | 2.1 (-0.8, 4.9) | 1.0 (-1.0, 3.1) |
| Podlaskie | 10.0 (1.7, 18.3) | 2.0 (-1.9, 5.9) | 10.0 (1.7, 18.3) | 2.0 (-1.9, 5.9) |
| Pomorskie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 20.0 (9.9, 30.1) | 5.0 (-0.5, 10.5) |
| Kujawsko-pomorskie | 0.0 (0.0, 0.0) | 1.9 (-0.7, 4.4) | 13.0 (6.6, 19.3) | 6.5 (1.8, 11.1) |
| Warmińsko-mazurskie | 4.3 (0.2, 8.4) | 1.1 (-1.0, 3.2) | 25.8 (16.9, 34.7) | 7.5 (2.2, 12.9) |
| Lubuskie | 9.1 (3.4, 14.8) | 12.1 (5.7, 18.6) | 21.2 (13.2, 29.3) | 7.1 (2.0, 12.1) |
| Zachodniopomorskie | 0.0 (0.0, 0.0) | 7.3 (0.4, 14.1) | 10.9 (2.7, 19.1) | 14.5 (5.2, 23.9) |
| Małopolskie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 2.0 (-1.9, 5.9) |
| Opolskie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 10.0 (1.7, 18.3) | 6.0 (-0.6, 12.6) |
| Łódzkie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 13.7 (4.3, 23.2) | 2.0 (-1.8, 5.8) |
| Śląskie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 2.2 (-2.0, 6.4) | 0.0 (0.0, 0.0) |
| Świętokrzyskie | 0.0 (0.0, 0.0) | 0.0 (0.0, 0.0) | 4.5 (-4.2, 13.2) | 4.5 (-4.2, 13.2) |
| **Total** | **2.2 (1.2, 3.2)** | **2.5 (1.5, 2.6)** | **12.2 (9.8, 14.4)** | **5.2 (3.7, 6.7)** |

*CI* Confidence interval

reacts with both PRCV and TGEV. None of the samples were positive when primers and probe targeting the S gene of TGEV were used, indicating PRCV infection. All samples with positive real-time RT-PCR results ($n=31$) were used for amplification of 5' S gene sequences using conventional RT-PCR. Fourteen (66.6%) of 21 nasal swab samples and 3 (30%) of 10 fecal samples yielded products of the expected size. Fifteen samples (14 from nasal swabs and one from feces) that yielded a strong PCR product were selected for sequencing. Sequences were successfully obtained from all 15 samples. The sequences were isolated from 4 different farms, of which 4 sequences (one derived from feces and 3 derived from nasal swabs) were isolated in 2021 from four different farms, while the remaining 11 sequences derived from nasal swabs were isolated in 2024 from the same farm. Overall, the PRCV sequences were obtained from pigs from three different voivodships (Pomorskie, Mazowieckie and Warmińsko-Mazurskie).

The alignment of the 5' S gene sequences of Polish PRCV obtained in this study with sequences of other PRCV (11 European, 3 Korean and 24 American) and TGEV strains ($n=46$) revealed that Polish PRCV strains can be divided into three groups depending on the size and location of the deletion in the S gene (Figure S2). Strains from the first (I) group (#199/24, #198/24, #191/24, #197/24, #190/24, #181/24, #207/24, #186/24, #193/24, #208/24, #209/24 and K104/21) had a 690 nt deletion in the S gene. The strain from group II (#52/21) had a 687 nt deletion, and strains from group III (#66/21 and #72/21) had a 672 nt deletion, similar to other European and Korean PRCV strains. The sequence of strain #52/21 from group II was similar to that of Polish PRCVs from group I, and differed from them by 9 nucleotide substitutions and a TGC insertion. The sequences from group I were identical and shared 96.1% or 94.4% and 90.9% or 92.6% nucleotide or amino acid (aa) sequence identity with the Polish PRCV sequences from groups II and III, respectively. The sequences of the PRCV strains from groups I and II were closely related to the PRCV 15087/12 III NPTV strain from Italy, showing 94.8% nt (96.0% aa) and 96.2% nt (89.6% aa) sequence identity, respectively. The group III sequences were closely related to the Korean strain KPCRV2403, showing 96.0% nt (94.9% aa) identity. All Polish PRCV sequences were more closely related to European and Korean PRCV strains (92.3% nt on average) than to American strains (84.7% nt on average).

The sequence of Polish PRCV strains from group I had unique nucleotide mutations at positions 16 (G/T), 56 (T/G), 774 (T/C), 788 (C/A), and 852 (C/T) compared with the sequences of other PRCV and TGEV strains from around the world. In addition, sequences from group I had an additional unique mutation at positions 750 (T/C) and 751 (T/G), which was also observed in the Polish strain from group II. The group III sequences had specific mutations at position 747 (C/T) and at position 889 (T/C). In addition, all Polish strains had T instead of G at position 19, similar to Korean strains, and T instead of C at position 870 and C instead of A at position 899, the same as the Korean and Italian strains. Furthermore, all Polish strains had a T/C substitution at position 21, a G/T at position 906 and a T/C at position 907, which was also observed in all European and Korean strains. Additional mutations specific to European and Korean strains were also observed in Polish strains from group III ($T/G^{54}$, $T/A^{736}$, $T/C^{855}$) and group II ($T/C^{855}$). The sequences from groups III and II had additional mutations, $C/T^{738}$ and $T/C^{765}$, which was observed only in the Korean and Italian strains. The sequences from group III had T instead of C at position 867, observed only in the Korean strains, whereas the Polish group I and II strains had mutations at positions 786 (G/A), 787 (G/A), and 789 (T/C) and a CAT insertion at positions 761–763, which was specific only to the Italian PRCV strain (Figure S2).

A comparison of the amino acid sequences of the PRCV strains and the amino acid sequence of the reference Purdue TGEV strain showed that 13/15 Polish PRCV strains had a unique substitution of valine (V) for leucine (L) at position 6, whereas 12/15 strains had a unique substitution of alanine (A) for asparagine (N) at position 260. All Polish PRCV strains had phenylalanine (F) instead of valine (V) at position 7, as did the Korean strains and strain ISU20-92330 from the US, and threonine (T) instead of lysine (K) at position 297, also observed in the Korean and Italian PRCV strains. Furthermore, strains #66/21 and #72/21 from group III had a unique substitution of asparagine (N) instead of aspartic acid (D) at position 17, as well as lysine (K) instead of asparagine (N) at position 18 and serine (S) instead of asparagine/threonine (N/T) at position 246, the same like in other European and Korean strains. In addition, all Polish strains had cysteine (C) instead of threonine (T) at position 245, asparagine (N) instead of threonine/serine (T/S) at position 246, threonine (T) instead of valine/cysteine (V/C) at position 248, and the NCT deletion also observed in the PRCV 15087/12 III NPTV strains from Italy (Fig. 1).

The phylogenetic tree revealed that the PRCV strains were grouped into three different clades (Fig. 2). Seven PRCV strains from Canada were grouped together with TGEV strains belonging to group Ib (traditional genotype), and other American PRCV strains were in the same cluster as TGEV strains representing group II (variant genotype), whereas Polish PRCV strains and other European and Korean PRCV strains clustered together

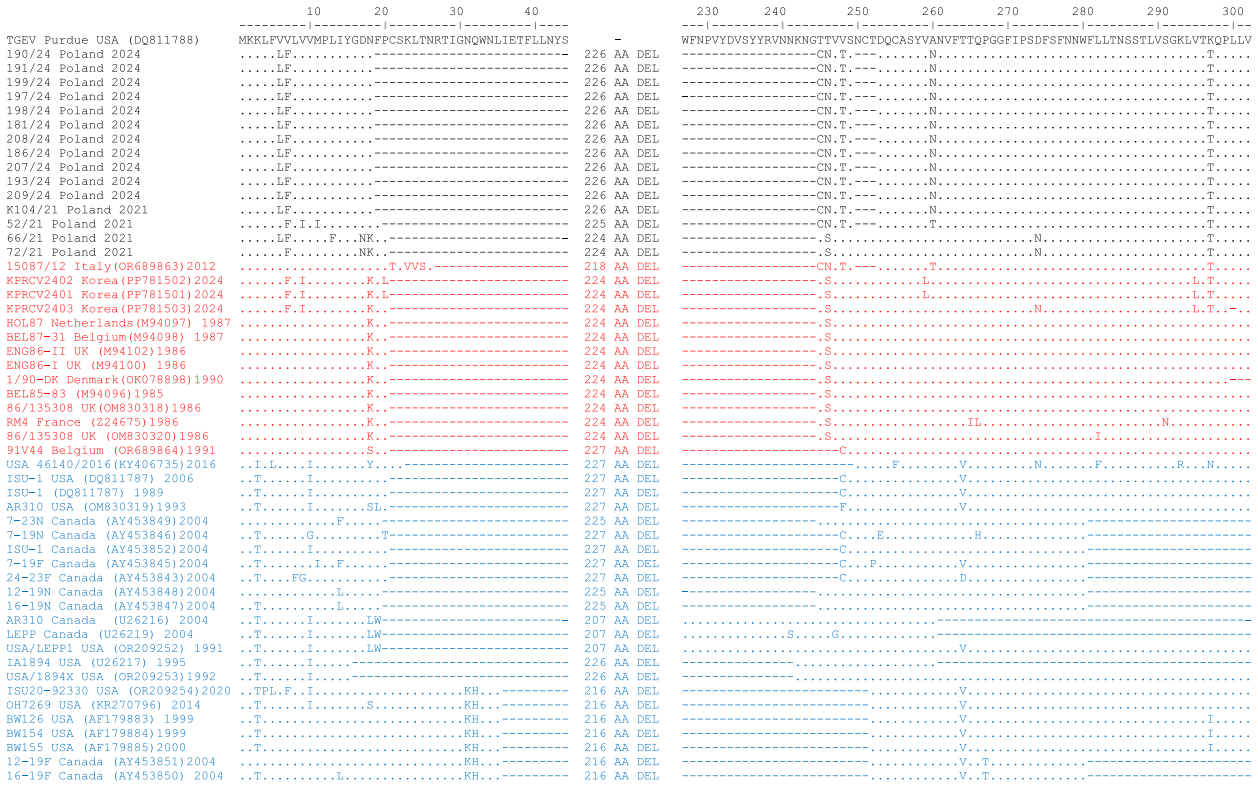

**Fig. 1** Amino acid sequence alignment of the N-terminal region of the S gene of Polish PRCV strains and 37 other PRCV strains as well as the TGEV Purdue strain. Dots represent the same amino acid residues. PRCV strains are indicated in red, TGEV strains in blue and Polish PRCV strains in black

with TGEV strains of genotype Ia. In detail, Polish PRCV strains and other European and Korean PRCV strains formed separate subcluster while TGEV strains of group Ia formed separate subcluster. Phylogenetic analysis confirmed that Polish PRCV sequences from groups I and II were closely related to the Italian PRCV 15087/12 III NPTV strain, whereas those from group III were closely related to Korean strains.

## Discussion

Despite the occurrence of porcine coronaviruses worldwide, there is little information on the distribution of these viruses in pigs in Poland. Seven coronaviruses can infect pigs (TGEV, PRCV, porcine epidemic diarrhea virus (PEDV), swine acute diarrhea syndrome coronavirus (SADS-CoV), porcine hemagglutinating encephalomyelitis virus (PHEV), deltacoronavirus (PDCoV) and swine enteric coronavirus (SeCov), the chimeric virus between PEDV and TGEV/PRCV). PEDV, TGEV and PRCV have been described in European pigs [3, 5, 6, 13, 14, 18, 23, 24, 27, 35, 40, 42]. There are only three papers on the occurrence of PEDV in pigs and wild boars from Poland [1, 2, 30]. This study represents the first investigation of the circulation of PRCV and TGEV in domestic pigs from Poland.

In this study, we estimated the seroprevalence of PRCV and TGEV using a commercial ELISA that can differentiate between TGEV and PRCV. The seroprevalence of TGEV in pigs from Poland was determined to be 2.2%, which was lower than that reported in Italy (5.5%) [14] and Belgium (7.6%) [7], but higher than that reported in Hungary (0.1%) [42]. Therefore, our results confirmed previously obtained results indicating a low seroprevalence of TGEV in European countries. In America, TGEV has been reported in Colombia [33], Venezuela [25], Bolivia, Brazil [26], Argentina [32] and the United States [9]. In the United States, the overall incidence of TGEV was 2.3% and ranged from 0.1–6.8% between 2008 and 2016, reaching its lowest value in 2015–2016 [9]. An investigation of PRCV prevalence in Argentina in 2014–2015 revealed that only 3 out of 87 tested samples were positive for TGEV [47]. In Asia, TGEV has been reported in Japan, China and South Korea. Antibodies against TGEV were detected in 4.3% and 1.2% of tested sera in South Korea [21] and Japan [29], respectively. In China, TGEV detection rates are generally low, but in some regions the prevalence of TGEV is widespread. An analysis by Chen et al. showed that the prevalence of TGEV in China ranged from

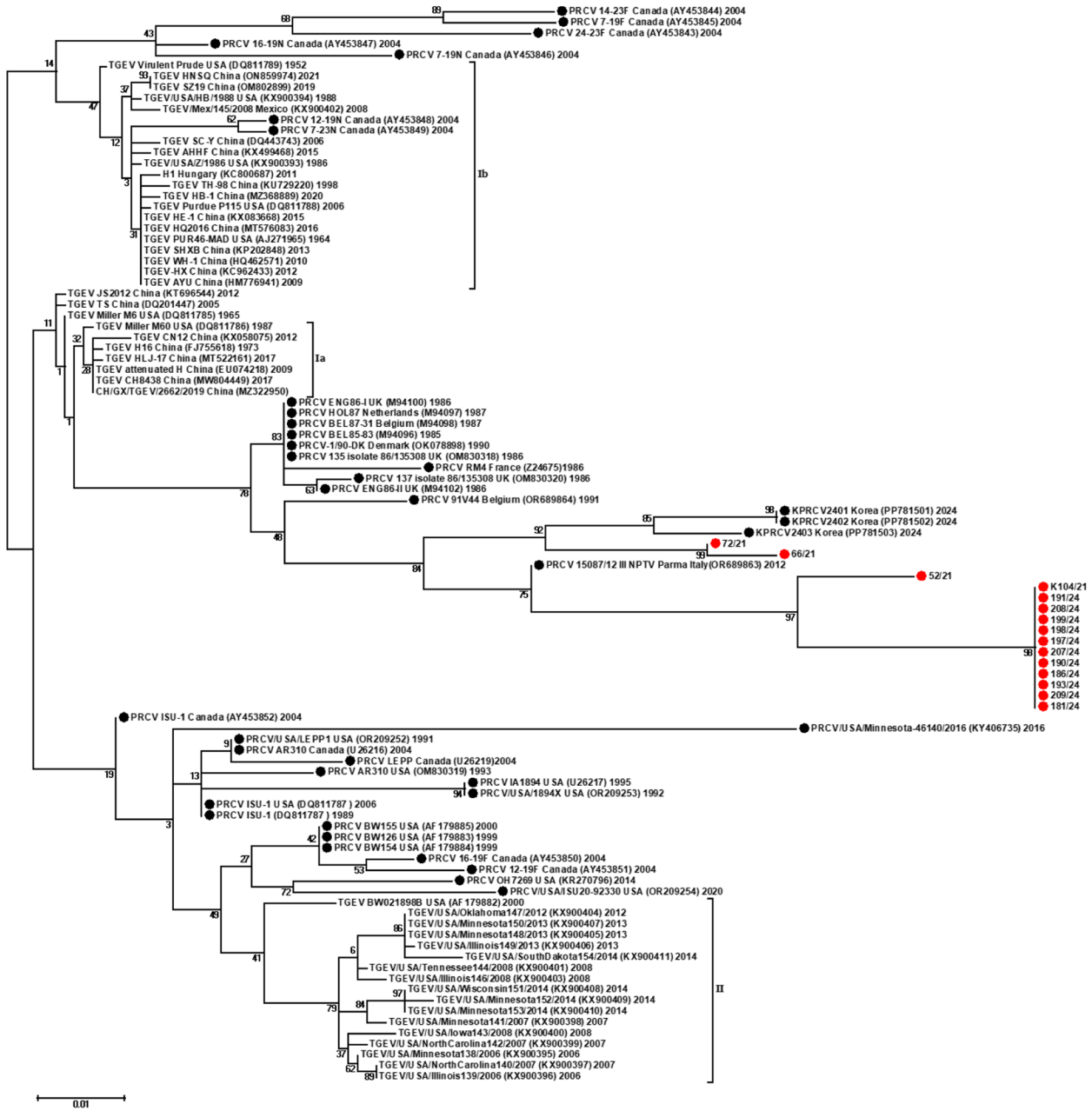


**Fig. 2** Maximum likelihood (ML) tree based on the 5′ S gene sequences of the TGEV ($n = 46$) and PRCV strains ($n = 53$). The PRCV sequences obtained in this study are marked with black circles. Other PRCV sequences are marked with red circles

1–67%, depending on the region. The average prevalence was estimated at 10% [11].

Several studies have suggested that the low incidence of TGEV may be due to widespread PRCV infection [9, 40]. PRCV infection is subclinical and generally beneficial by providing partial immunity to TGEV. Therefore, PRCV can act as a natural live vaccine against TGEV and induce active immunity in pregnant sows, which is passively transmitted to their offspring [45, 44]. Our results showed that the PRCV seroprevalence in Poland was more than five times higher than the TGEV seroprevalence, which may support this thesis. The PRCV seroprevalence in Polish pigs was determined to be 12.2%. It seems that this virus is widely present throughout Poland, as antibodies to PRCV were detected in all the voivodships studied. The results of our study are comparable to those recently obtained in Hungary, where antibodies to PRCV were detected in 139 (15.4%) of 906 sera tested [43]. A higher

seroprevalence of PRCV was observed in Norway (26.6%) [16], Spain and Portugal (48%) [27], Belgium (90.6%) [31], South Korea (63.7%) [20] and Japan (90.2%) [41]. Overall, our results indicate that TGEV circulates at low levels in Polish pigs despite the widespread occurrence of PRCVs. This may be because PRCV-induced antibodies provide only limited protection against TGEV. PRCV-induced neutralizing antibodies are absent or minimal one year after infection [46]. Therefore, animals can be reinfected with TGEV after this time. In Japan and England, TGE outbreaks have occurred on farms where PRCV infection was present [29, 34]. A similar situation was observed in Hungary, where TGEV was present in herds with clinical signs of TGE along with PRCV [24]. The low status of TGEV in Poland may also be due to the increased biosecurity to prevent and control ASF infection.

Serum neutralization antibodies against TGEV and PRCV can be detected from approximately 7–10 days after primary infection and last through 42 days. Then, the antibody response decreases [46]. Experimental studies revealed that PRCV infected pigs excrete the virus in nasal secretions for around 1–2 weeks [19]. Earlier findings have shown that 10 days after infection, excretion, lung lesions and clinical symptoms resolve and the titer of virus-neutralizing antibodies against the virus increases [17]. TGEV has not been found in the faces of pigs for more than two weeks after infection. Therefore, antibodies are more easily detected in serum than in RNA in clinical samples. In this study, we were unable to detect RNA of TGEV in any of the tested samples. The same results were obtained in previous studies from Europe [4, 15, 35, 37]. On the other hand, 7.6% (21/277) of nasal swab samples and 4.5% (10/221) of fecal samples turned out to be positive for PRCV using real-time RT-PCR assay (overall, 6.22%). We successfully obtained sequences of PRCV strains and performed molecular analysis.

A phylogenetic tree was constructed to investigate the global evolutionary relationships between Polish PRCV strains and other PRCV and TGEV strains. Previous studies [9, 36] have divided TGEV sequences into two clades (variant and traditional TGEV strains). In our study, we used the classification adopted by Cheng et al. because the TGEV sequences in our tree formed three clusters. The variant genotype represents genotype II, whereas the traditional genotype has been divided into two genotypes, Ia and Ib [10]. The same TGEV classification system was recently used by Bedsted et al. [3]. The global PRCV strains are mainly divided into two groups, American and European, and it has been suggested that each group is derived from a different ancestor [3]. Our phylogenetic analysis clearly indicated that Polish PRCV strains clustered together with other European PRCV strains. Interestingly, this group also includes strains from Korea. Kim et al. [21] reported that Korean PRCV strains were genetically more similar to European than to American PRCV strains, but grouped a little separately from European PRCV strains. Our analysis clearly indicated that Korean strains clustered together with other European strains; however, together with Polish and Italian strains they formed separate subcluster supported by a high bootstrap value of 94%. It has been suggested that Korean strains are derived from European PRCVs and evolved independently when they were introduced into Korea. The close relationship between the Korean, Polish and Italian strains may contradict this theory. Our results suggest that European (including Polish) and Korean PRCV strains may originate from TGEV genotype Ia viruses. Furthermore, our study revealed that American PRCV strains grouped together with TGEV strains belonging to genotypes II and Ib. Previous studies have shown that American PRCV strains were located only in the same clade as genotype II TGEV strains. This discrepancy is most likely because our work included strains that have not been analyzed by other authors.

Our study revealed that genetically different strains of PRCV coexist in Poland. Two of the 15 Polish PRCV strains were closely related to the Korean strains, whereas 12 strains were closely related to the PRCV strain from Italy. This is observed in the phylogenetic tree. Furthermore, this finding was confirmed by genetic analysis of the S gene deletion fragment. The most obvious difference between PRCV and TGEV is a deletion within the S gene of variable size (621–681 nt) [3]. American PRCV strains have deletions of different sizes (621–681 nt) located at different positions, implying that they arose independently. Two Polish, all European and Korean PRCV strains have a 672 nt deletion at the same position on the 5' end of the S gene, suggesting that they originated from the same precursor. Moreover, 12 out of 15 Polish PRCV strains had a 690 nt deletion in the S gene, which differed in size and location from those of European, American and Asian PRCV strains. These findings and the presence of different nucleotide and amino acid changes specific to each group may suggest that Polish PRCV may have originated from different ancestors. Gene deletions are often involved in tissue tropism changes. Additionally, the size and location of the deletions in the S gene may lead to variable biological outcomes [39]. Whether the new truncation observed in Polish PRCV alters the biological function of the protein is unknown. Further genetic analysis of PRCV strains and in vivo studies to investigate their pathogenesis are needed. The occurrence of mutations specific only to

strains from Poland undoubtedly indicates that PRCV is still evolving, which is typical for coronaviruses.

In summary, in this study, we clearly demonstrated the presence of antibodies against TGEV and PRCV using ELISA, which indicates that these viruses circulate in Polish herds. TGEV is circulating at a low level, while PRCV is quite widespread. However, it should be noted that our results do not represent the overall true seroprevalence, as the number of tested samples used in the study is not representative of the whole pig population in Poland. Therefore, the epidemiology of TGEV and PRCV in Poland requires further studies, which should include more samples. In the present study, we obtained, for the first time, sequences of Polish PRCV strains and evaluated their genetic characteristics. Genetic and phylogenetic analyses revealed that the Polish PRCV strains were closely related to the European and Korean PRCV strains. In addition, the Polish PRCV strains showed some unique changes in their sequences, which may reflect their evolution. Our results add to the knowledge of the epidemiology and molecular biology of currently circulating PRCV.

## Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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**Authors' contributions**
"Conceptualization: MO. Data curation: MO and MA. Formal analysis: MO and MA. Investigation: MO and MA. Methodology: MO and MA. Resources: MO and MA. Writing—original draft: MO and MA. Writing—review and editing: MO and MA. All the authors read and approved the final manuscript."

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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