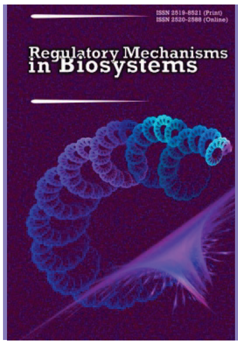

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Molecular characterization and phylogenetic analysis of pseudorabies virus isolated from pigs in Ukraine

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The article presents the results of a molecular genetic study of two isolates of the *Pseudorabies virus* that were isolated from pigs in Ukraine. Bioinformatic analysis of the gE gene fragment of *Aujeszky's disease virus* (*Pseudorabies virus*) isolates was carried out in order to determine the phylogenetic relationships and homology of nucleotide sequences. Fragments of the *Aujeszky disease virus* genome corresponding to the C-terminal region of the gE gene were selected for sequencing and further analysis. As a result of the conducted studies, it was demonstrated that the nucleotide sequences of the analyzed samples differ from each other by the presence of ACG insert in the tandem repeats region. Comparison of the studied sequences with the sequences of strains/isolates of the *Aujeszky's disease virus* found in Europe and Asia, presented in the GenBank database, indicates that such an insert is characteristic for the Min-A and HNJZ strains (position 1487 in the gE gene) isolated in Asia. Analysis of the homology of nucleotide sequences showed that the sequence of the gE gene fragment of sample No. 1 is 100% identical to the sequences of strains 89V87 and 00V72 isolated in Belgium. The homology of the nucleotide sequence of the gE gene fragment of sample No. 3 with strains 89V87 and 00V72 was 99.13%. In order to clarify the analyzed samples belonging to a particular genogroup (genetic cluster), a phylogenetic dendrogram was constructed. This demonstrates the phylogenetic relationships between strains/isolates of the *Aujeszky's disease virus*. It was found that the analyzed samples belong to the genetic cluster uniting European strains/isolates, and the studied isolates are most genetically close to strains 89V87 and 00V72.

Keywords: Aujeszky's disease virus; genogroup; gE gene; nucleotide sequences; phylogenetic dendrogram.

Introduction

Pseudorabies (PR) (Aujeszky's disease) is an infectious disease caused by the virus – *Suid alphaherpesvirus 1* (SuHV-1), better known as *Pseudorabies virus* (PRV) or *Aujeszky's disease virus* (ADV). This virus affects many mammalian species, including pigs, small and large ruminants. Although the virus does not have a pronounced host specificity, the main attention of researchers is focused on the problem of morbidity in pig farms, when domestic pigs themselves are carriers of this pathogen, and such farms have significant economic losses. In addition, wild boars are also described as the natural reservoir of this pathogen (Liu et al., 2022; Zheng et al., 2022).

Wild and domestic pigs are the only natural hosts of PRV (reservoir of infection). Often this virus is isolated from wild pigs even in those countries where the eradication of this disease among domestic pigs has already been carried out. The classical PRV strains have been eradicated from domestic pigs in most developed countries, where eradication programmes have been implemented. Aujeszky's disease remains endemic in Eastern and South-Eastern Europe, Latin America and Asia. Variant strains of this virus currently also circulate in China. It is reported that about 20% of feral pigs in the USA are seropositive. The prevalence of pseudorabies in wild pigs in European countries ranges from 0–60% (Hahn et al., 1997; Romero et al., 1997; Corn et al., 2004; Ai et al., 2018; Carr et al., 2018; Bo & Li, 2022). The seroprevalence ranges of wild pigs to *Aujeszky's disease virus* are on the levels from 12.3% to 18.8% in Ukraine (Sytyuk, 2012a, 2012b, 2012c, 2012d, 2012e).

The full genome and partial (*gC-encoding gene*) DNA-sequencing of pseudorabies virus revealed the presence of two genotypes: I and II, and the prevalence of the latter depends on geographical conditions (Europe/America or Asia) (Corn et al., 2004; Carr et al., 2018).

"Variant" strains of *Pseudorabies virus* deserve special attention. While the so-called "classical" strains of this virus were isolated at the beginning of the last century, "variant" strains were detected in pigs in China in 2011. Genomic sequencing and phylogenetic analysis showed that variant strains form a new branch and are quite distant from the classical PRV strains (Wang et al., 2015; Fan et al., 2016).

PRV strains isolated in China demonstrated sequence differences compared to European and American strains (Ye et al., 2015). The latter indicates that the differences between the two genotypes could be due to long independent evolution, which also explains the low efficacy of vaccines from the Bartha strain to protect against variant strains of PRV genotype II. Ye et al. (2016) also indicated that PRV strains from different geographical regions show the genetic diversity. In a study on phylogenetic analysis based on full-length genome sequences, it was proved that

Chinese strains belong to genotype II (Liu et al., 2020). Bo et al. (2021) indicate that the prototypes of modern Chinese strains could be both viruses of both genotypes I and II. Moreover, the authors proved that even vaccine strains of the virus can be genetic donors of PRV genomic recombination *in vivo*. Therefore, in the fight against this infection in the direction of eradication, the identification and genetic analysis of PRV virus strains is of primary importance. In this case, it is important to understand the impact of strains isolated from wild pigs on the population of the virus isolated from domestic pigs in Ukrainian farms. An important element of monitoring the spread of genetic variants of the *Aujeszky's disease virus* is also understanding the threats to industrial pig production and assessing the potential effectiveness of vaccines. In our work, we aimed to conduct phylogenetic analysis of samples of *Aujeszky's disease virus*, isolated from affected pigs in Ukraine.

Material and methods

Pathological material. Two samples of internal organs of piglets were analysed from Dnipropetrovsk and Poltava regions (UA), which were infected with Aujeszky's disease in 2006 and 2020 respectively. The vital diagnosis of Aujeszky's disease in those piglets was managed by the serological ELISA-based testing for the presence of specific antibodies.

Pathological material from a dead piglet (fragments of the brain, lungs, kidneys, lymph nodes) was tested in 2006, which was obtained from the farm of Petrikivskyi district of Dnipropetrovsk region and obtained an isolate of the virus, which was named "Petrikovskiy-2006" (Sytyuk & Napnenko, 2006). The pathological material was tested in 2020 from an acute case of Aujeszky's disease in a piglet (fragments of the brain, lymph nodes), which was obtained from a farm in the Poltava region, the isolate was named "PRV_piglet_Ukraine_2020".

PRV antibody ELISAs. The study of the presence of specific humoral antibodies against Aujeszky's disease in the blood serum of pigs was carried out by enzyme-linked immunosorbent assay using commercial test systems Pseudorabies Virus gPI Antibody Test Kit, manufactured by IDEXX (USA).

DNA was isolated from pathological material (fragments of the brain, lungs, kidneys, lymph nodes) and was performed using the DNeasy blood and tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Further DNA was used for real-time PCR and classical PCR with electrophoretic detection of amplification products.

Detection of PRV genes gB (glycoproteins B) by real-time polymerase chain reactions. Real-time PCR was performed using oligonucleotide primers and a probe complementary (Table 1) to the gB gene sequence of the *Aujeszky's disease virus* (Ma et al., 2008).

Detection of PRV genes gE (glycoproteins E) by gel-based polymerase chain reactions. PCR was carried out to amplify the target fragments of the gE gene of PRV using specific primers complementary to this region of the gene (primers listed in Table 1). Detection and molecular weight confirmation of PCR products were performed by electrophoresis in 1.5% agarose gel containing 0.01% ethidium bromide in 1X TAE buffer. PCR products were purified from the gel using a commercial Cleanup-standard kit (EuroGen, Russia) according to the manufacturer's instructions.

Nucleotide sequencing of the PRV's gE gene fragment obtained by gel-based PCR was performed using an ABI 3130 genetic analyzer (Applied Biosystems, USA) and BigDye v.3.1 chain terminator kit (Applied Biosystems, USA) according to the manufacturer's instructions. Multiple nucleotide sequence alignment was performed using BioEdit software.

Phylogenetic analysis. For phylogenetic analysis, the studied DNA sequences were compared with the known gE gene sequences presented in the GenBank database (Table 2).

Phylogenetic trees were constructed based on the nucleotide sequences of the gE gene fragment using the method of minimal evolution. Bootstrap values were calculated for 500 repeats.

Table 1
Primers and probes used in the PRV's gene gB and gE screening by PCR

Gene	PCR type	Sequence	Amplicon size
gB	real-time	PRV-gB-F	5′-ACAAGTTCAAGGCCCACATCTAC-3′
		PRV-gB-R	5′-GTCYGTGAAGCGGTTCGTGAT-3′
		PRV-gB-probe	FAM-5′-ACGTCATCGTCACGACC-3′-BHQ1
		XIPC-F	5′-TTCGGCGTGTTATGCTAACTTC-3′
		XIPC-R	5′-GGGCTCCCGCTTGACAATA-3′
		XIPC-probe	Cy5–5'-CTCCGCAGA-TAO-AATCCAGGGTCATCG-3'-IAbRQSp
gE	gel-based	PRV-gE-F	5′-CCGCGGGCCGTGTTCTTTGT-3′
		PRV-gE-R	5′-GCGCCGGCGAGGTGAAGC-3′

Table 2
Isolates of PRV that are included in the phylogenetic investigation

No.	Number in GenBank	Genotype	Strain	Collection year	Host	Country
1	KM061380.1	2	ZJ01	2012	*Sus scrofa*	China
2	KM983048.1	2	TaiAn_SD_2013	2013	Swine	China
3	EU561349.1	2	HNJZ	2008	Swine	China
4	AY170318.1	2	Min-A	2002	Swine	China
5	AF171937.1	2	Ea	1999	Swine	China
6	AF207700.1	2	SH	1999	Swine	China
7	AY249861.1	2	Yangsan	2003	Swine	South Korea
8	FJ176390.1	2	P-PrV	2008	Swine	Malaysia
9	GQ926932.1	2	LXB6	2009	Swine	China
10	KT983811.1	1	Kolchis	2010	*Sus scrofa domesticus*	Greece
11	EU502923.1	1	NiA3	2008	Swine	Spain
12	JF797218.1	1	Kaplan	2011	*Sus scrofa*	Hungary
13	FJ605135.1	1	NS374	1971	*Sus scrofa*	Belgium
14	JF460026.1	1	CL/15	1988	Swine	Argentina
15	JF797219.1	1	Becker	2011	*Sus scrofa*	USA
16	FJ605133.1	1	75V19	2009	*Sus scrofa*	Belgium
17	FJ605134.1	1	89V87	1989	*Sus scrofa*	Belgium
18	FJ605132.1	1	00V72	2000	*Sus scrofa*	Belgium

Results

In the study of samples of biological material, the following result was obtained by real-time PCR: in samples number 1 and 3 the *Aujeszky's disease virus* genome was detected. According to the results of PCR study, DNA was successfully isolated from isolates "PRV_piglet_Ukraine_202020" and "Petrikivskyi-2006", and it could be used for amplification and following sequencing of gE gene fragment of *Aujeszky's disease virus*. The next step was the bioinformatic analysis of the gE gene fragment of the *Aujeszky's disease virus* in order to determine the phylogenetic relationships and homology of the nucleotide sequences of the analyzed virus isolates.

Fragments of the *Aujeszky's disease virus* genome corresponding to the C-terminal region of the gE gene 344 nucleotides long were selected for sequencing and further analysis. Results of sequencing of the "Petrikivskyi-2006" strain are shown below:

1 tgcgtgctgt gctcccggcg ccgggcggcc tcgcggccgt tccgggtgcc gacgcgggcg
61 gggacgcgca tgctctcgcc ggtgtacacc agcctgccca cgcacgagga ctactacgac
121 ggcgacgacg acgacgacga ggaggcgggc gacgcccgcc ggcggccctc ctcccccggc
181 ggggacagcg gctacgaggg gccgtacgtg agcctggacg ccgaggacga gttcagcagc
241 gacgaggacg acgggctgta cgtgcgcccc gaggaggcgc cccgctccgg cttcgacgtc
301 tggttccgcg atccggagaa accggaagtg acgaatgggc ccaa

Results of sequencing of strain "PRV_piglet_Ukraine_2020" are shown below:

1 tgcgtgctgt gctcccggcg ccgggcggcc tcgcggccg ttccgggtgc cgacgcgggcg
61 gggacgcgca tgctctcgcc ggtgtacacc agcctgccc acgcacgagg actactacgac
121 ggcgacgacg acgacg---a ggaggcgggc gacgcccgc cggcggccct cctcccccggc
181 ggggacagcg gctacgaggg gccgtacgtg agcctggac gccgaggacg agttcagcagc
241 gacgaggacg acgggctgta cgtgcgcccc gaggaggcg ccccgctccg gcttcgacgtc
301 tggttccgcg atccggagaa accggaagtg acgaatggg cccaa

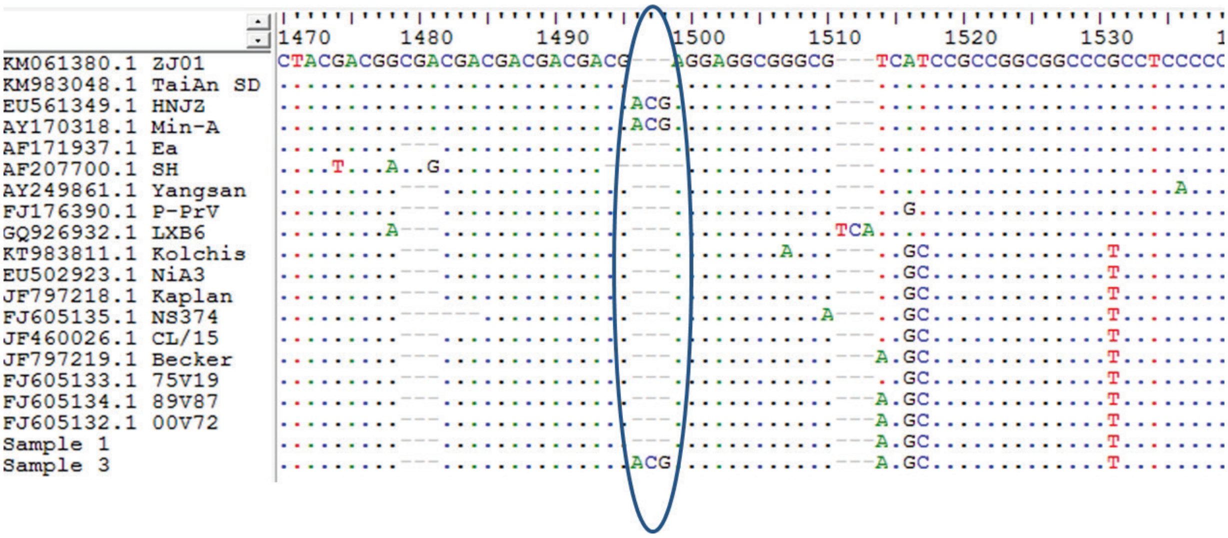
For phylogenetic analysis, the studied DNA sequences were analyzed with the known gE gene sequences presented in the GenBank database.

Based on the sequencing results, it was demonstrated that the nucleotide sequences of the analyzed samples were different from each other due to the presence of an ACG insert in the tandem repeats region in the "Petrikivskyi-2006" isolate (Fig. 1).

A comparative analysis of sequence data of *Aujeszky's disease virus* strains/isolates isolated in European and Asian countries and presented in the GenBank database showed that such an insertion is typical for Min-A and HNJZ strains (position 1487 in the gE gene) isolated in Asia.

We also performed nucleotide sequence homology analysis using the BLAST online service (http://blast.ncbi.nlm.nih.gov/Blast.cgi). This analysis demonstrated that the sequence of the gE gene fragment of the isolate "PRV_piglet_Ukraine_202020" is 100% identical to the sequences of strains 89V87 and 00V72 isolated in Belgium. The percent homology of the nucleotide sequence of the gE fragment of the "Petrikivskyi-2006" isolate with strains 89V87 and 00V72 was 99.1% (Table 3).

A detailed comparative analysis of the nucleotide sequence homology of *Aujeszky's disease virus* strains/isolates is presented in Table 3.



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                    1470      1480      1490      1500      1510      1520      1530      1
KM061380.1 ZJ01     CTACGACGGCGACGACGACGACGACG---AGGAGGCGGGCG---TCATCCGCCGGCGGCCCGCCTCCCCC
KM983048.1 TaiAn SD ..........................---............---..........................
EU561349.1 HNJZ     ..........................ACG............---..........................
AY170318.1 Min-A    ..........................ACG............---..........................
AF171937.1 Ea       .........---..............---............---..........................
AF207700.1 SH       ....T...A..G............------...........---..........................
AY249861.1 Yangsan  .........---..............---............---......................A...
FJ176390.1 P-PrV    .........---..............---............---..G.......................
GQ926932.1 LXB6     ........A---..............---............TCA..........................
KT983811.1 Kolchis  .........---..............---........A...---..GC.............T........
EU502923.1 NiA3     .........---..............---............---..GC.............T........
JF797218.1 Kaplan   .........---..............---............---..GC.............T........
FJ605135.1 NS374    .........------...........---...........A---..GC.............T........
JF460026.1 CL/15    .........---..............---............---..GC.............T........
JF797219.1 Becker   .........---..............---............---A.GC.............T........
FJ605133.1 75V19    .........---..............---............---..GC.............T........
FJ605134.1 89V87    .........---..............---............---A.GC.............T........
FJ605132.1 00V72    .........---..............---............---A.GC.............T........
Sample 1            .........---..............---............---A.GC.............T........
Sample 3            .........---..............ACG............---A.GC.............T........
```

Fig. 1. Comparison of the nucleotide sequences of the analyzed samples with the sequences of *Aujeszky's disease virus* strains/isolates isolated in European and Asian countries presented in the GenBank database; the ACG insertion in the tandem repeat region in the "Petrikivskyi-2006" isolate and the Min-A and HNJZ strains are marked with a blue oval

Table 3

Matrix of determination nucleotide sequence homology of the gE gene fragment of *Aujeszky's disease virus* of different strains/isolates (maximum homology 1.000)

No.	Strain/isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	ZJ01	–	0.994	0.976	0.982	0.985	0.965	0.982	0.973	0.976	0.953	0.953	0.956	0.941	0.953	0.953	0.956	0.956	0.956	0.956	0.948
2	TaiAn_SD_2013	0.994	–	0.971	0.976	0.979	0.959	0.976	0.968	0.971	0.947	0.947	0.950	0.936	0.947	0.947	0.950	0.950	0.950	0.950	0.942
3	HNJZ	0.976	0.971	–	0.994	0.968	0.953	0.971	0.962	0.960	0.942	0.942	0.945	0.930	0.942	0.942	0.945	0.945	0.945	0.945	0.953
4	Min-A	0.982	0.976	0.994	–	0.974	0.959	0.976	0.968	0.965	0.948	0.948	0.951	0.936	0.948	0.948	0.951	0.951	0.951	0.951	0.959
5	Ea	0.985	0.979	0.968	0.974	–	0.959	0.991	0.982	0.985	0.961	0.961	0.964	0.950	0.961	0.961	0.964	0.964	0.964	0.964	0.956
6	SH	0.965	0.959	0.953	0.959	0.959	–	0.962	0.953	0.956	0.944	0.944	0.947	0.933	0.944	0.944	0.947	0.947	0.947	0.947	0.939
7	Yangsan	0.982	0.976	0.971	0.976	0.991	0.962	–	0.985	0.982	0.964	0.964	0.967	0.953	0.964	0.964	0.967	0.967	0.967	0.967	0.959
8	P-PrV	0.973	0.968	0.962	0.968	0.982	0.953	0.985	–	0.973	0.961	0.961	0.964	0.950	0.961	0.961	0.964	0.964	0.964	0.964	0.956
9	LXB6	0.976	0.971	0.960	0.965	0.985	0.956	0.982	0.973	–	0.953	0.953	0.956	0.941	0.953	0.953	0.956	0.956	0.956	0.956	0.948
10	Kolchis	0.953	0.947	0.942	0.948	0.961	0.944	0.964	0.961	0.953	–	0.988	0.997	0.976	0.988	0.988	0.991	0.991	0.991	0.991	0.982
11	NiA3	0.953	0.947	0.942	0.948	0.961	0.944	0.964	0.961	0.953	0.988	–	0.991	0.988	1.000	0.994	0.997	0.991	0.991	0.991	0.982
12	Kaplan	0.956	0.950	0.945	0.951	0.964	0.947	0.967	0.964	0.956	0.997	0.991	–	0.979	0.991	0.988	0.994	0.994	0.994	0.994	0.985
13	NS374	0.941	0.936	0.930	0.936	0.950	0.933	0.953	0.950	0.941	0.976	0.988	0.979	–	0.988	0.982	0.985	0.979	0.979	0.979	0.970
14	CL/15	0.953	0.947	0.942	0.948	0.961	0.944	0.964	0.961	0.953	0.988	1.000	0.991	0.988	–	0.994	0.997	0.991	0.991	0.991	0.982
15	Becker	0.953	0.947	0.942	0.948	0.961	0.944	0.964	0.961	0.953	0.988	0.994	0.988	0.982	0.994	–	0.997	0.997	0.997	0.997	0.988
16	75V19	0.956	0.950	0.945	0.951	0.964	0.947	0.967	0.964	0.956	0.991	0.997	0.994	0.985	0.997	0.997	–	0.994	0.994	0.994	0.985
17	89V87	0.956	0.950	0.945	0.951	0.964	0.947	0.967	0.964	0.956	0.991	0.991	0.994	0.979	0.991	0.997	0.994	–	1.000	1.000	0.991
18	00V72	0.956	0.950	0.945	0.951	0.964	0.947	0.967	0.964	0.956	0.991	0.991	0.994	0.979	0.991	0.997	0.994	1.000	–	1.000	0.991
19	PRV_piglet_Ukraine_2020	0.956	0.950	0.945	0.951	0.964	0.947	0.967	0.967	0.956	0.991	0.994	0.994	0.979	0.991	0.997	0.994	1.000	1.000	–	0.991
20	Petrikivskyi-2006	0.948	0.942	0.953	0.959	0.956	0.939	0.939	0.956	0.948	0.982	0.982	0.985	0.970	0.982	0.988	0.985	0.991	0.991	0.991	–

In order to clarify the belonging of the analyzed samples to a particular gene group (genotype, genetic cluster), a phylogenetic dendrogram reflecting the phylogenetic relationships between *Aujeszky's disease virus* strains/isolates was constructed. It was demonstrated that the analyzed isolates belong to genetic cluster 1, which combines European strains/isolates and is most genetically close to strains 89V87 and 00V72. Genetic cluster 2 includes strains/isolates isolated in Asian countries (Fig. 2).

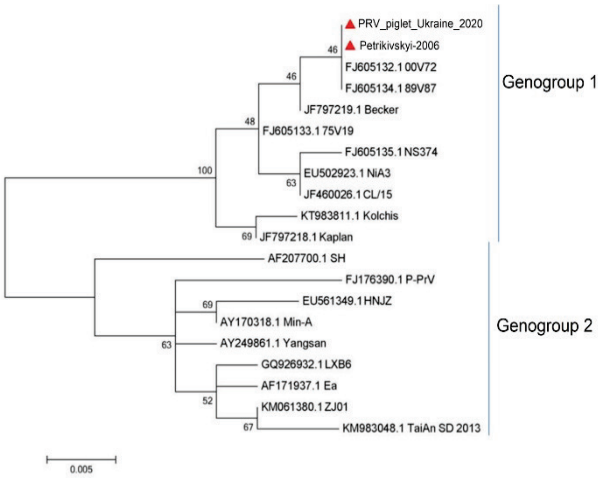

Fig. 2. Phylogenetic dendrogram based on the sequences of the gE gene fragment of the *Aujeszky's disease virus*: a minimum evolution algorithm (bootstrap = 500) was used for the construction; the studied isolates are marked with a red triangle; the sequences of the strains/isolates used for comparison were obtained from the Genbank international database (identification numbers are indicated)

Discussion

This study presents the first phylogenetic analysis of field strains of *Aujeszky's disease virus* isolated from diseased domestic pigs in Ukraine. Virus samples were collected at various times in the central region of the country. The detection of diseased animals confirms the possibility of outbreaks in industrial pig farms, despite the widespread use of vaccination. However, the occurrence of outbreaks is not associated with critical phylogenetic differences between field and vaccine strains.

Our analysis of two strains of the *Aujeszky's disease virus* isolated in Ukraine in Dnipropetrovsk and Poltava regions shows that they belong to genogroup I and are related to European strains of the virus. These isolates do not belong to "variants" and it is likely that the use of vaccines from the Bartha-K61 strain will provide sufficient immune protection in susceptible animals. In contrast to PRV strains of the European group, Asian strains are generally quite different phylogenetically, in terms of the characteristics of the gE, gB and gC genes. Chinese researchers explain the low effectiveness of the Bartha-K61 vaccine in preventing PRV outbreaks on pig farms by the weak genetic links of field PRV strains associated with the outbreak (Wang et al., 2015). Some researchers point out that genomic changes in gB contribute to the increased virulence of new PRV variants (Yu et al., 2017). The results of the study of nucleotide sequence divergence for the gE gene were 1.7% in the cluster where the PRV strains associated with the outbreak were localized and 2.3% with other clusters in the phylogenetic tree, respectively. Similar studies were also conducted (Luo et al., 2014), where they also pointed out significant differences between field strains and the vaccine Bartha-K61 in China, as revealed by molecular genetic studies.

Phylogenetic analysis of PRV strains by Belgian researchers among wild boars and domestic pigs revealed some differences between the European strains themselves (isolates from domestic pigs were grouped into class A, while isolates from wild pigs were grouped into class B (Verpoest et al., 2014). Italian researchers, based on the molecular and phylogenetic analysis of PRV strains, divide the strains into PRV clusters (Moreno et al., 2015). After all, the differences relate to the source of origin (wild or domestic pigs). Nevertheless, all strains were assigned to the European PRV group. However, the researchers point out that the isolates had identical sequences within both populations. In this case, a difference was found between the strains in the gE gene fragment, they differ from each other by the presence/absence of an ACG insertion in the tandem repeat region, which may indicate the circulation of several variants of *Aujeszky's disease virus* in Ukraine. Studies with PRV strains conducted in Italy during 1984–2010 using UL44 (gC) and US8 (gE) gene sequencing showed differences in strains isolated from different animal species, and also revealed the presence of "old" strains that have been circulating in animal populations for several decades (Sozzi et al., 2014). It has been shown that the gC gene is closely related to older strains of porcine PRV and the gE gene is similar to recently isolated strains. Finally, the studies still emphasize the differences between strains isolated from wild and domestic pigs, but they are not critical, i.e., they are within the same European group. Brazilian researchers (Fonseca et al., 2010) showed that a group of strains close to the standard PRV Shope strain and the Bartha vaccine strain circulate in their country, but analysis of the gE and gC genes of other Brazilian PRV isolates showed that they belonged to cluster B and differed from the virus isolated in other countries. Serbian researchers (Csabai et al., 2019), comparing the genetic composition of the isolated PRV-MdBio strain with the European virulent Kaplan strain and the Chinese reference strain Ea, found that the most common point mutations preceded conserved and silent mutations, as indicated by other authors (Tombácz et al., 2009; Tombácz et al., 2017). The authors also noted genetic differences between European and Chinese PRV strains. European researchers (Müller et al., 2010; Müller et al., 2011) studied PRV isolates from Germany, France, Spain, Italy, Slovakia and Hungary for the period 1993–2008 by sequencing. Gene sequence analysis revealed a significant division of European isolates into a clade containing isolates from North Rhine-Westphalia, Rhineland-Palatinate (Germany), France and Spain (clade B), and a more variable clade including isolates from Brandenburg, Baden-Württemberg, Saxony, Saxony-Anhalt (Germany), Slovakia, Hungary, Italy and France (clade A). Italian researchers (Abbate et al., 2021) also point to the fact that the PRV virus circulating in the country belongs to the European type, but the analysis of the viral glycoprotein G genes indicates not only the spread of the virus in wild boar populations in Sicily, but also provides evidence of direct interspecies transmission of this pathogen. Phylogenetic analysis using gG sequences showed that PRV strains isolated from dogs can be grouped with strains associated with wild boars and domestic pigs, but transmission can also occur from other animal populations. A small number of gG sequences deposited in GenBank are divided into different clades, which suggests that their geographical origin is independent of the host species, the latter being an important characteristic of gC proteins of all PRV strains from Europe/America or Asia (Deblanc et al., 2019). This suggests that the phylogenetic analysis of PRV and its available nucleotide sequences from Europe emphasizes the distinctiveness of isolates from wild boars and confirms the results of a possible multiple introduction from domestic pigs to wild boars. In addition, the genetic identity of PRV isolates from wild and domestic pigs indicates that virus transmission occurred in both directions (Müller et al., 2010).

During the evolutionary changes in PRV, insertions or deletions are found in the gD, gE, gI, and PK genes. In addition, sequence alignment mostly demonstrates that insertions in gD and gE are unique molecular characteristics of newly emerging PRV strains in China (Fan et al., 2016). The authors also noted that in studies of isolated field *Aujeszky's disease viruses* in China, the differences were primarily related to the gE gene fragment. However, they pointed out that insertions or deletions were also observed in the gD, gI, and PK genes compared to other PRV isolates from around the world. Sequence analysis showed that the insertions in gD and gE are unique molecular characteristics of the newly emerging strains, which is also supported by our findings. A group of Chinese scientists (Hu et al., 2021) sequenced 54 genomes of new PRV variants isolated in China during 2012–2017 and used phylogenetic analysis to show that Chinese and European/American strains belonged to two different genotypes (geographic clustering) and evolved independently of each other. The authors also conclude that the classic vaccine strain Bartha-K61 may contribute to the formation of new PRV variants as a result of the interaction between virulent and vaccine strains. The PRV strains isolated by

Chinese scientists turned out to be extremely related and genetically close to classical Chinese strains (e.g., strains Ea, Fa, SC). The RDP analysis conducted by the authors revealed 23 recombinations in new variants of the pathogen, which confirms the evolutionary nature of any recombinations. It is the phylogenetic and selection analyses of gB, gC, and gE that reveal an increase in PRV genetic diversity in China since 2011 and the identification of several sites of adaptive mutations in gC and gB (He et al., 2019).

Japanese researchers (Minamiguchi et al., 2019) also noted that the PRV strains they isolated in Japan differed in genetic profiles from the European commercial vaccine strains Bartha and Begonia and were grouped as an Asian type of PRV based on the characteristics of full nucleotide sequences and phylogenetic analysis. Chinese researchers (Huang et al., 2022) used third-generation sequencing technologies in their study of PRV. They sequenced the complete genome of the epidemic strain FJ and studied the characteristics and differences compared to the classic Chinese strain and strains from other countries. The authors assigned the PRV FJ strain to the genotype II branch, and it showed a close evolutionary relationship with the epidemic PRV variants isolated in China after 2011. The gB, gC, gD, gH, gL, gM, gN, TK, gI, and PK genes of the FJ strain were assigned to the same branch as other Chinese epidemic mutants; their gG genes were assigned to the same branch as the classical Chinese strains Fa and Ea; and the gE genes were assigned to a new, relatively independent branch. As in our studies, the gE gene still remains the critical point of research. Such studies allow scientists to detect "fresh" molecular epidemiological changes in PRV and ultimately use this information to develop new, more effective vaccines against the disease. Molecular epidemiologists also insist on continuous molecular monitoring of strains (Zhai et al., 2019) by sequencing the main glycoproteins (gB, gC, gD and gE) to study the evolutionary characteristics of PRV and develop vaccines against altered PRV variants. Phylogenetic studies of PRV strains conducted by Chinese scientists have shown not only that the virus is now legitimately divided into 2 separate clusters, with Chinese strains having genotype II and PRV isolated in other countries (European and American countries) belonging to genotype I. They point out that such genetic differences between the two genotypes may have arisen due to long independent evolution, which to some extent explains the low effectiveness of the Bartha strain vaccine in protecting pigs infected with PRV genotype II (or Asian genotype). In addition, these authors note that recombinations between field PRV strains and strains similar to the vaccine (from the Bartha strain) have led to the emergence of this independent genotype (Ye et al., 2015, 2016). All this information once again emphasizes the need for continuous molecular monitoring of PRV strains circulating in Ukraine. Such research should not be limited to domestic pigs, but should also include strains from wild pigs and other animal species. The researchers also point out that human and animal migration contributes to the spread and variation of different viruses, high replication rates of several virulent strains have the potential to increase the level of dsDNA virus replacement, and positive selection in regions of intensive vaccination may also produce appropriate levels of protein replacement (Firth et al., 2010).

Conclusion

Phylogenetic analysis showed that the PRV isolates we isolated in Central Ukraine grouped into an independent branch together with other strains isolated in our country and in European countries in recent years, and that they show a closer genetic relationship with each other. These isolates are new variants with unique molecular features, but they still belong to the isolates of the genetic group that unites European strains and isolates. The nucleotide sequences of the gE gene fragment of the analyzed samples differ from each other by the presence/absence of an ACG insertion in the tandem repeat region.

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The authors declare that there is no competing of interest.

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