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Original article

Phylogenetic characteristics of the porcine reproductive and respiratory syndrome virus isolated from pigs in four regions of Kazakhstan

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) causes serious reproductive and respiratory effects in pig populations worldwide. The aim of this study was to determine the seroprevalence and phylogenetic relationships of field isolates of the PRRS virus (PRRSV) in pig populations in four regions of Kazakhstan. A representative sample of 323 pigs was selected to establish the seroprevalence of PRRSV in the study area. A serological test for detecting antibodies (ELISA) was performed, and the genetic characteristics of the virus were examined using the PCR method. Virus isolation from the blood sample, MARC-145, cell cultures were used. In total, 13.93% of the tested serum samples (n=45) revealed seropositivity against PRRSV antibodies. The genetic sequencing of the isolated strains indicated the widespread circulation of the North American genotype in the Almaty region. These results were confirmed by the cytopathic effects observed in cell cultures. Molecular analysis showed that the local strains have undergone significant genetic drift from the sequenced European strains and are phylogenetically more closely related to strains originating from North American. The results of genetic analyses indicate that the North American genotype has become established in Kazakhstan. This situation requires increased use of biosecurity systems and vaccines specific for this genotype. Our results highlight the importance of continuous monitoring and an active adaptive management system in controlling the spread of PRRS in regional pig populations.

Keywords: betaarterivirus suid 2, North American genotype, phylogenetic relationships, porcine reproductive and respiratory syndrome (PRRS), seroprevalence

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important infectious diseases in pigs. The PRRS virus (PRRSV) is responsible for reproductive failure, pneumonia, and increased susceptibility to secondary bacterial infections. The virus contains numerous strains characterized by significant differences in virulence and tropism, which is associated with a broad spectrum of clinical manifestations of the disease. The pathognomonic symptom of the disease is a characteristic blue discoloration of the piglet's skin, ears and vulva, which is why this disease is called "blue ear disease". This disease has a significant negative impact on the world's pig population, especially in European countries and the United States (Kim et al. 1993, Linhares et al. 2012, Nieuwenhuis et al. 2012).

The PRRSV was detected on the European and North American continents in the late 1980s. The virus is classified into two species, Betaarterivirus suid 1 (PRRSV-1, European) and Betaarterivirus suid 2 (PRRSV-2, North American). Both PRRSVs species show approximately 60% similarity at the nucleotide level (Adams et al. 2017).

Their structure is 70% identical (Renukaradhya et al. 2015). The PRRSV is a single-stranded positive-sense RNA virus of the family *Arteriviridae* and order *Nidovirales* (Wagner et al. 2010). The PRRSV is an enveloped virus, and its genome is 15.2 kb in length, with ~~ten~~ eleven open reading frames, including ORF1a, 1b, 2a, 2b, 3, 4, 5, 5a, 6, 7 and a short transframe (TF) ORF in the nsp2 region (Yim-Im et al. 2023).

Along with the entire genome, ORF5 is the most variable gene, which shows heterogeneity and translates glycoprotein. This gene is therefore responsible for stimulating the immune system to produce neutralizing antibodies against the PRRSV and for protecting piglets against heterologous strains through cross protection. ORF-6, known as the M gene, encodes PRRSV membrane proteins that are crucial to the structure and function of the virus. The M gene is responsible for inducing viral infection and evading the action of the immune system (Music et al. 2010). The M protein is a heterodimer with glycoprotein 5 (ORF-5), which is essential for infectivity (Snijder et al. 2003). A genetic analysis of virus structure revealed that substantial genetic divergence was observed between North American and European PRRSV1 (Meng et al. 1995).

The PRRSV infects pigs regardless of gender and age and can be transmitted both horizontally and vertically. Horizontal infection occurs through both direct and indirect contact. The prevention of this disease is mainly based on limiting the transmission of infection between herds (Clilverd et al. 2023). In this regard, analysis of PRRSV genetic diversity can be used to support epidemiological studies of a likely common source of infection or transmission between herds.

The PRRSV targets the cells of the porcine monocyte/macrophage line (Teifke et al. 2001), where CD163 is the main receptor for viral infection (Chen et al. 2014). The virus mainly replicates in differentiated porcine alveolar macrophages (PAMs) (Duan et al. 1997), but it has also been identified in macrophages located in tissues including the lymph nodes, thymus, spleen, Peyer's patches and liver (Pejsak et al. 1997).

The aim of this study was to determine the seroprevalence and phylogenetic relationships of PRRSV circulating among pigs in four regions of the Republic of Kazakhstan. This is the first study of the type conducted on such a large scale in Kazakhstan.

Materials and Methods

Study area

The study involved 2 000 pigs from 13 farms situated in four regions of Kazakhstan (4 farms in the Almaty region, 4 farms in the Karaganda region, 2 farms in the Akmola region, and 3 farms in the Kostanay region).

Sampling

The study was carried out to determine the epizootic situation with regard to the presence of PRRSV. For this purpose, the size of a representative sample in a population of 2 000 pigs was calculated using the following formula:

$$n = \frac{Z^2.P.(1-P)}{E^2}$$

where:

n = the sample size required to achieve reliable and valid results
Z = confidence interval adopted in the study (5%)
P = estimated proportion of the population with the analyzed characteristics
D = acceptable margin of error

With the use of the above formula, the sample size was calculated to be 323 pigs. The study population was not vaccinated against the PRRSV. Blood samples were collected for virological analyses from both severely sick and apparently healthy pigs aged 2-6 months. During the monitoring of farms in the studied regions, no sick animals were observed, a general sample was taken, and the samples were analyzed by PCR. Internal organs (lungs, liver, mediastinal lymph nodes) were

collected post mortem. The vacutainers were left at room temperature for 30-60 minutes to form a clot. The blood serum was drained into sterile cryotubes and placed in a Dewar vessel with liquid nitrogen. The collected tissues were placed in cryotubes.

Serological methods

The presence of PRRSV-specific antibodies in the blood serum of pigs was determined using ELISA kits (ID Screen® PRRS Indirect ELISA product code PRRSS-5P) according to the manufacturer`s instructions.

Virus isolation and characterization

Preparation of inoculum for virological analyses

For virological analyses, 1 g of the examined tissues (lungs, liver, mediastinal lymph nodes) was crushed with a homogenizer, adding 10 ml of saline or Hank's solution. The suspension was purified from coarse particles by centrifugation at low speed (3 000 rpm) for 30 minutes. The supernatant was placed in sterile tubes. Then to remove the virus from the cells, the suspension was the frozen and thawed three times at -40°C, then centrifuged again at 3 000 rpm for 30 minutes, and the supernatant was collected. The supernatant was passed through a 0.22 µm syringe filter. The filtrate was mixed with broad-spectrum antibiotics: penicillin – 200 IU/ml, streptomycin sulfate – 2 µg /ml, and gentamicin sulfate – 5 µg /ml.

Isolation of the virus in cell culture

The PRRSV was isolated using a monolayer culture of MARC-145 (Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna-Brescia-Italy, fetal monkey kidney cells), SPEV (Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna-Brescia-Italy, male Hampshire kidney cells), and PK-15 (Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna-Brescia – Italy, porcine kidney cells) and cell lines were cultured for 48-72 h. Before infection of the cell culture, the growth medium was drained, and the cell monolayer was washed with sterile Hank's solution. The cell cultures were exposed to 0.2 ml inoculum and then placed in an incubator for 1 h for adsorption. The volume of the supporting medium in the culture was adjusted to 7 ml, and was incubated at a temperature of 37°C. Cell culture flasks were monitored daily for 3-7 days using a light microscope. The presence of the virus in the tested samples was assessed based on the occurrence of cytopathic changes in the monolayer culture. Cell culture flasks with obvious cytopathic changes were frozen at -40°C until passage for the isolation and cultivation of the virus. The above procedure was in compliance with biological safety regulations, and class II biological safety cabinets (CBSC) were used.

Electron Microscopy

The samples were purified and concentrated by ultracentrifugation. The virus-containing culture was clarified by centrifugation at 2 100 g for 15 minutes at 4°C (Eppendorf 5810R centrifuge). The clear viral suspension (5 ml) was further precipitated by ultracentrifugation at 192 000 g for one hour at 4°C. The resulting precipitate was resuspended in single phosphate buffered saline (Sigma) with pH 7.2 and a volume of 200 µl. For electron microscopy, a drop of the test material was placed in the well of a Teflon plate, a support grid with a formvar substrate sprayed with carbon was the applied to the virus drop. The sample mesh was transferred for 1-2 minutes to a drop of 2% aqueous solution of phosphoric-tungstic acid (pH 6.8), and then for 5 minutes to a drop of phosphoric-tungstic acid solution with pH 7.0. After contrasting and removing the excess contrast agent with filter paper, the preparation was dried in air and considered ready for examination under an electron microscope. The samples were examined in a transmission electron microscope, JEM-100 CX II, JEOL (Japan), at an accelerating electron voltage of 80 kV and a magnification of 20 000 to 40 000 times. Negative plates photographed and developed at a certain magnification were used to study the morphometric characteristics of PRRSV. Virion morphometry was performed directly on a photonegative using a magnifying glass with a 0.1 mm graduation.

Molecular analyses

Isolation of viral RNA

RNA was isolated from purified and concentrated virus-containing material using Trizol reagent (Invitrogen), according to the manufacturer's instructions. The primers for the M gene (PRRS_F:, 5`- GAGTTTCA GCGGAACAATGG-3`, PRRS_R: 5` GCCGTTGAC CGTAGTGGAG-3`, product size – 491 bp) were used for reverse transcription PCR using SuperScript III One-Step RT-PCR with the Platinum Taq enzyme kit, AccuPrime High-Fidelity (Invitrogen), according to the manufacturer's instructions. The PCR was performed in a thermal cycler, GeneAmp PCR System 9700 (Applied Biosystems, USA) under the following conditions: 50°C for 30 min for the RT enzyme, initial denaturation at 94°C for 15 min, then 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 40 s, and extension

at 72°C for 40 s; final extension at 72°C for 7 min. The PCR was stopped at 4°C until the sample removal and the PCR product was visualized by gel electrophoresis at 120 V, 400 mA for 20 min in 1% agarose gel prepared in 1X TAE buffer with the 100 bp marker.

The viral gene segment was extracted from agarose gel using a QIA quick gel extraction kit (250) (Qiagen), according to the manufacturer's instructions.

Genome sequencing and phylogenetic analysis

The samples were prepared for sequencing using a BigDye Terminator v3. 1 Cycle Sequencing kit (Applied Biosystems). The sequencing was performed on an automatic 16-capillary sequencer, 3130xl genetic analyzer (Applied Biosystems/Hitachi). The analysis and the assembly of the nucleotide sequences were performed using the Sequencher 4.0 program.

For comparative analysis of the nucleotide sequences, the nucleotide sequences of the M gene of PRRSV was extracted from the NCBI (National Center for Biotechnology Information) website (http://www.ncbi.nlm.nih.gov/genbank/), then aligned using ClustalW with a bootstrap value of 1000 in BioEdit v. 7.2.5 and saved as a FASTA file. The alignment file was used in MEGA XI for a phylogenetic analysis of the sequences, which was performed using a neighbor joining tree with the bootstrap method, a bootstrap value of 1000, and Kimura's two-parameter model with substitutions to include transitions + transversions.

Ethical approval

The research project was approved by the Local Ethics Committee of The Research Institute of Biological Safety Problems in Kazakhstan (No. 1/2017).

Statistical analysis

The results were processed statistically using the SPSS v. 20 statistical program. The PCR and ELISA results were analyzed using descriptive statistics and statistical tools to determine the prevalence of PRRS cases in the study area. Prior to statistical calculations, two hypotheses were formulated. The null hypothesis postulated that the proportions of positive and negative test results were independent of farm, and the alternative hypothesis postulated that the proportions of positive and negative test results varied significantly among farms. The data were normally distributed. The expected frequencies were calculated using the following equation:

$$Eij = \sum_{row} * \sum_{column} / \sum_{total}$$

where: Eij – expected frequency
$\sum_{row}$ – row total
$\sum_{column}$ – column total
$\sum_{total}$ – total

The research hypotheses were validated using the Chi-squared test:

$$\chi^2 = \Sigma \frac{(Oi–Ei)}{(Ei)}$$

where: χ^2 – chi-square
Oi – observed value
Ei – expected value

The results were regarded as statistically significant at $p \leq 0.05$.

Results

The study showed that out of 323 serum samples analyzed by ELISA, 45 (13.93%) tested positive for the presence of anti-PRRSV antibodies. All seropositive samples were collected from the same herd in the Almaty region (Table 1).

Of the 45 samples collected from seropositive animals, 25 showed cytopathic effects exerted by the virus on cell lines (Table 2).

The virus exerted cytopathic effects on the MARC-145 cell line at passages 2 and 3. Positive results were found in samples from piglets aged 0 to 5 months and from one boar (Fig. 1).

Electron microscopy of PRRSV particles isolated on the MARC-145 cell line from tissue homogenates originating from seropositive animals showed virions with spherical particles with a diameter of 45-70 nm. They consisted of an icosahedral nucleocapsid with a diameter of 25-35 nm and a lipoprotein shell with 12-15 nm long protrusions on the surface (Fig. 2).

Total RNA was extracted and confirmed by RT-PCR for ORF-6 (M protein) in 25 isolates. The PCR products of two samples were sequenced using a BigDye 3.1 terminator cycle sequencing kit (Applied Biosystems) on an automatic sequencer, 3130xl genetic analyzer (Applied Biosystems). The 491 bp nucleotide sequences obtained in the study were compared with the reference sequences provided by NCBI (http://www.ncbi.nlm.nih.gov/genbank/) using the Sequencher 4.0 data processing and analysis program.

A phylogenetic analysis of genomic sequence data performed in BioEdit and Mega XI confirmed that the obtained sequences belonged to the PRRSV. The sequences were trimmed from the 5' and 3' ends to contain only the M gene of the tested isolate and aligned to the reference sequences of known species of PRRSV-1 and PRRSV-2 strains using ClustalW in BioEdit.

A phylogenetic tree constructed using Mega XI

Table 1. Enzyme immunoassay of samples collected to detect porcine reproductive and respiratory syndrome virus (PRRSV)-specific antibodies in pig farms in Kazakhstan.

Region	Name of the Farm	Number of samples taken	Results of enzyme immunoassay
Almaty region	"Bayseyit" Farm	45	Positive
	"Karaoi" Limited Liability Company	32	Negative
	"Li" Individual Entrepreneur	27	Negative
	"Bacon" Limited Liability Company	29	Negative
Akmola region	"Astana Holding" Limited Liability Company	27	Negative
	"Agrarnoe" Limited Liability Company	19	Negative
Kostanay region	"Vladimirovskoe" Agro-industrial Limited Liability Company	18	Negative
	"Amanzhol Akro" Limited Liability Company	22	Negative
	"Beryozka Agro" Farm	19	Negative
Karaganda region	"Medeo" Limited Liability Company	19	Negative
	"Astro-Agro" Limited Liability Company	20	Negative
	"Volsky" Limited Liability Company	19	Negative
	"Nechaev" Individual Entrepreneur	27	Negative

Explanation: an optical density value of 0.4 or greater was considered a positive result

showed clear clustering with the North American group, which means that the genotype circulating in the Almaty region belongs to the North American type (Fig. 3). The isolated field strains showed <0.00% divergence from North American strains and >0.3% divergence from European strains (Table 3).

Discussion

The PRRSV is an important viral pathogen of pigs that is currently circulating in many pig-producing countries. In the Republic of Kazakhstan, pig production is limited to a few regions. However, there is a demand for pork on the market. Therefore, effective control of infectious diseases in pigs, especially those with such a devastating potential as PRRS, is an important consideration.

In this study, a representative sample of 323 pigs from different farms located in four regions of Kazakhstan was analyzed. During monitoring in Kazakhstan, an outbreak of PRRS in pigs was observed on the Baiseyit farm in the Almaty region, where 45 positive cases of the disease were detected, which accounts for 13.93% of all positive cases in the study area. A study by Zhou et al. (2022) in southern China showed a 24% seroprevalence of PRRSV (Zhou et al. 2022). Another study demonstrated that the prevalence of PRRSV in China ranged from 17.5% to 53.3% (Li et al. 2024). A study by Pejsak et al. (1997), conducted in Poland, also revealed a high seroprevalence of PRRSV in the pig population. The cited authors also found that most animals with PRRSV antibodies exhibited clinical signs of the disease, and subclinical cases were rare (Pejsak et al. 1997). No cases of the disease were observed in the other regions of Kazakhstan. The occurrence of the disease on the Baiseyit farm was due to poor biosecurity measures and poor sanitary practices. The remaining farms followed strict biosecurity measures in accordance with veterinary regulations, which probably contributed to the absence of positive cases of the disease.

Molecular analysis of the virus isolated in cell culture showed that the nucleotide sequence of the ORF6 PRSV was characteristic of the North American type. The highest viral growth rate was obtained on the MARC-145 cell line where cytopathic effects were noted at passages 2 and 3.

In the present study, the best results were achieved in culture on MARC-145; however, not all researchers agree that this cell line is most suitable for PRRSV isolation. In a study by Yim-im et al. (2022), 1.4% of PRRSV 145 cell cultures had nonmatching viral sequences between clinical samples and isolates when grown on MARC-2. Such a phenomenon was not observed when PRRSV-2 was isolated in other cell lines, e.g. ZMAC, and was considered to be typical only of North American strains (Yim-im et al. 2022). With regard to cell culture analysis, it is recommended that vaccination campaigns should focus primarily on the elimination of North American strains from the Almaty region, as the observed cell culture lesions were caused by North American strains, whereas European strains caused negligible lesions. Other studies have also shown that MARC-145 cells tend to exhibit cytopathic activity earlier than other cell lines such as SPEV and PK-15 (Linhares et al. 2011). This observation supports our

Table 2. Presence of the porcine reproductive and respiratory syndrome virus (PRRSV) in monolayer cell cultures of blood samples collected from serologically PRRSV-positive pigs in Kazakh pig farms.

Age	№	Cell culture and passage number		
	1	MARC-145		
		I	II	III
Piglet, 4 months old	2	-	p\dc	+
Piglet, 5 months old	3	-	p\dc	+
Sow	4	-	-	-
Piglet, 21 days old	5	-	-	-
Piglet, 28 days old	6	-	-	-
Piglet, 38 days old	7	-	p\dc	+
Boar	8	-	p\dc	+
Boar	9	-	p\dc	p\dc
Piglet, 3 months old	10	-	p\dc	+
Piglet, 5 months old	11	-	p\dc	+
Piglet, 1.5 months old	12	p\dc	+	+
Piglet, 1 month old	13	p\dc	+	+
Piglet, 2 months old	14	+	+	+
Piglet, 3 months old	15	+	+	+
Piglet, 40 days old	16	-	-	-
Piglets, 3-4 months old	17	p\dc	+	+
	18	p\dc	+	+
Piglets, 4-6 months old	19	p\dc	+	+
	20	p\dc	+	+
	21	-	-	-
Stillborn piglets	22	-	p\dc	+
Piglet, 1 month old	23	+	+	+
Piglet, 2 months old	24	p\dc	+	+
Piglets, 2.5 months old	25	+	+	+
Sows	26	-	-	-
	27	-	-	-
	28	-	-	-
Piglet, 2 months old	29	p\dc	+	+
Piglet, 3 months old	30	p\dc	+	+
Piglet, 1.5 months old	31	-	p\dc	+
Piglet, 3 months old	32	p\dc	+	+
Piglet, 2 months old	33	+	+	+
Piglet, 2.5 months old	34	-	p\dc	p\dc
Sows	35	-	-	-
Piglets, 4-6 months old	42	-	-	-
	43	-	p\dc	+
	44	-	-	-
	45	-	p\dc	+

MARC-145 – monolayer cell culture, "–" – absence of PRRSV or destructive changes in monolayer cell culture, "+" – presence of PRRSV in monolayer cell culture, p\dc" – presence of destructive changes in monolayer cell culture.

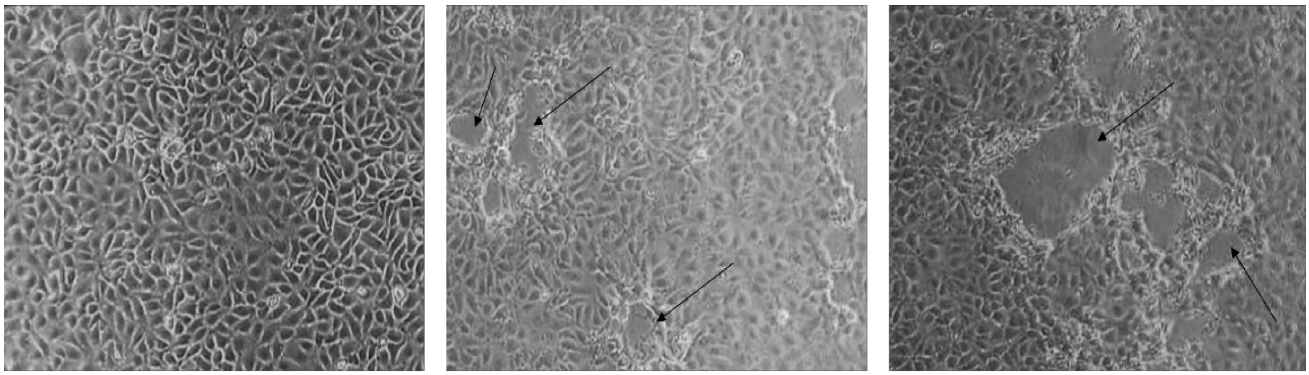

Fig. 1. Viral culture on the MARC-145 cell line. A – control MARC-145 cells in a cell culture flask, B – cytopathic effects observed after 1 minute of exposure to the porcine reproductive and respiratory syndrome virus PRRSV in MARC-145 cell culture on days 2-3, C – cytopathic effects caused by the PRRSV (black arrows) in MARC-145 cell culture on days 4-5.

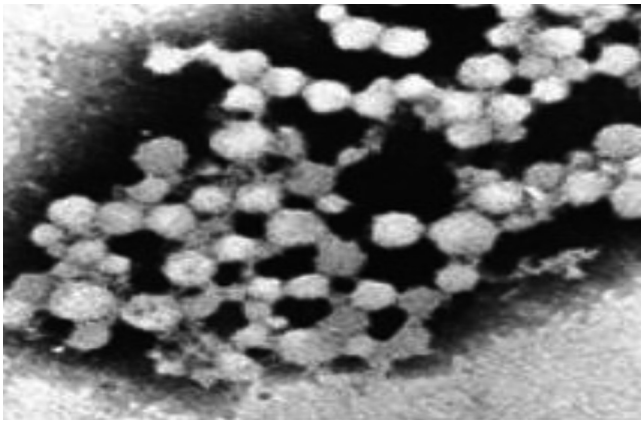

Fig. 2. Electron microscopy of the porcine reproductive and respiratory syndrome virus (PRRSV): Electron microscopy of the cultured isolate showing spherical particles (45-70 nm in diameter), an icosahedral nucleocapsid (25-35 nm in diameter), and a lipoprotein shell with 12-15 nm long protrusions on the surface (x100,1000)

findings of optimizing PRRSV culture and achieving faster cytopathic changes in MARC-145 cells, highlighting the reliability and utility of the MARC-145 cell line for PRRSV research aimed at vaccine development and PRRS diagnostics.

The results of the genetic analysis performed in this study showed a close relationship between the Almaty field strain and North American PRRSV strains (PRRSV-2). These results are consistent with the results of previous studies on the genetic diversity of PRRSV. The study by Shi et al. (2010) showed significant genetic differences between PRRSV-1 and PRRSV-2 strains. A comparison of the Almaty strain with the North American strains suggests possible routes of transmission and emphasizes the importance of understanding global PRRSV movement patterns. Similar phylogenetic analyses performed by Murtaugh et al. (1995) have demonstrated distinct clustering of strains based on geographical origin. The present findings underline the need to reorganize PRRSV control strategies in light of the detection of the North American PRRSV genotype.

Previous studies by Mengeling et al. (1995) and Zimmerman et al. (2019) have shown that PRRSV genotypes can have a significant impact on vaccine effectiveness. This emphasizes the need to use region-specific vaccines based on the genotypic characteristics of the circulating virus.

The present findings are consistent with the recommendations of Holtkamp et al. (2013), who suggested that enhancing surveillance in areas with higher seroprevalence is essential. Implementing targeted vaccination strategies that account for the genetic makeup of circulating PRRSV strains can improve disease management. These findings emphasize the need for continuous monitoring and genetic characterization to dynamically adapt control measures in response to evolving virus genotypes. Similar suggestions have also been made by Rossow et al. (1996) and Nathues et al. (2017).

In addition, the results of the current study clearly demonstrated the need for strict biosecurity practices. Of the 16 pig farms included in the study, the PRRSV was detected in only one farm where biosecurity rules were not followed.

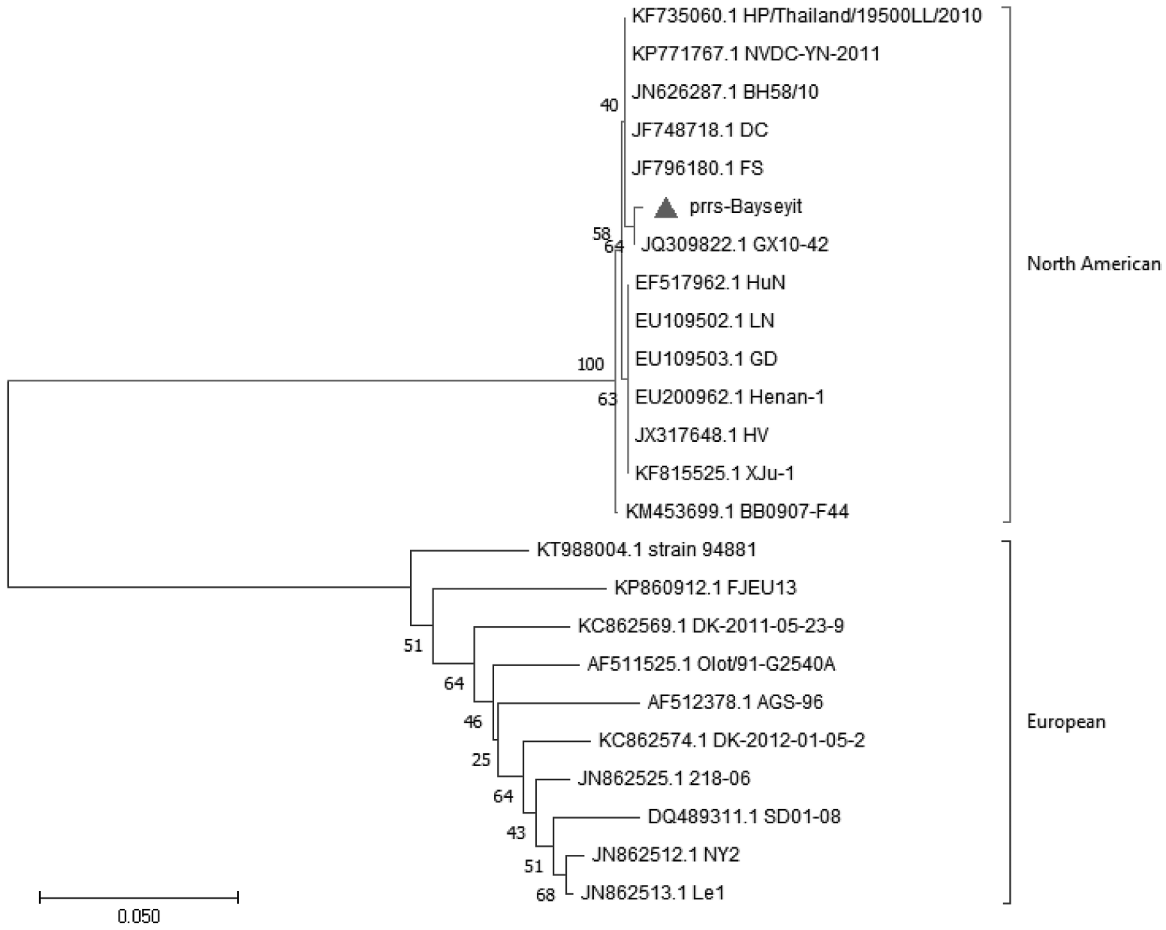

Fig. 3. A phylogenetic tree of the nucleotide sequences of the PRRSV M gene, constructed by the nearest neighbor method using the MEGA XI program (bootstrap = 1000), showing the clustering of field strains with North American strains.

Conclusion

The results of the present study are largely consistent with the findings of other authors, thereby strengthening the global understanding of the epidemiology of PRRS and highlighting the need for local control strategies. The identification of the North American PRRSV genotype in the Almaty region presents both a challenge and an opportunity to refine PRRS management practices in Kazakhstan. Ongoing research, surveillance, and the development of genotype-specific vaccines and biosecurity protocols will be essential in mitigating the impact of PRRS on swine health and productivity.

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Table 3. Genetic divergence of the isolated PRRSV field strains (in raw) from the reference strains from Nord American and European strains (in column).

		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII
I	PRRS-Bayseyit [1]																							
II	AF511525.1_Olot/91-G2540A [2]	0.303																						
III	AF512378.1_AGS-96 [2]	0.317	0.060																					
IV	DQ489311.1_SD01-08 [1]	0.323	0.057	0.073																				
V	EF517962.1_HuN [1]	0.007	0.303	0.319	0.319																			
VI	EU109502.1_LN [1]	0.007	0.303	0.319	0.319	0.000																		
VII	EU109503.1_GD [1]	0.007	0.303	0.319	0.319	0.000	0.000																	
VIII	EU200962.1_Henan-1 [1]	0.007	0.303	0.319	0.319	0.000	0.000	0.000																
IX	JF796180.1_FS [1]	0.005	0.303	0.317	0.319	0.002	0.002	0.002	0.002															
X	JF748718.1_DC [1]	0.005	0.303	0.317	0.319	0.002	0.002	0.002	0.002	0.000														
XI	JN626287.1_BH58/10 [1]	0.005	0.303	0.317	0.319	0.002	0.002	0.002	0.002	0.000	0.000													
XII	JQ309822.1_GX10-42 [1]	0.002	0.300	0.314	0.321	0.005	0.005	0.005	0.005	0.002	0.002	0.002												
XIII	JN862512.1_NY2 [2]	0.312	0.039	0.049	0.030	0.307	0.307	0.307	0.307	0.307	0.307	0.307	0.310											
XIV	JN862513.1_Le1 [2]	0.307	0.037	0.050	0.028	0.303	0.303	0.303	0.303	0.303	0.303	0.303	0.305	0.007										
XV	JN862525.1_218-06 [2]	0.305	0.039	0.057	0.039	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.303	0.016	0.017									
XVI	JX317648.1_HV [1]	0.007	0.303	0.319	0.319	0.000	0.000	0.000	0.000	0.002	0.002	0.002	0.005	0.307	0.303	0.300								
XVII	KC862569.1_DK-2011-05-23-9 [2]	0.303	0.051	0.072	0.062	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.300	0.039	0.042	0.044	0.298							
XVIII	KC862574.1_DK-2012-01-05-2 [2]	0.310	0.050	0.060	0.046	0.306	0.305	0.305	0.305	0.305	0.305	0.305	0.307	0.021	0.023	0.034	0.305	0.055						
XIX	KF815525.1_XJu-1 [1]	0.007	0.303	0.319	0.319	0.000	0.000	0.000	0.000	0.002	0.002	0.002	0.005	0.307	0.303	0.300	0.000	0.298	0.305					
XX	KF735060.1_HP/Thailand/ 19500LL/ 2010 [1]	0.005	0.303	0.317	0.319	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.002	0.307	0.303	0.300	0.002	0.298	0.305	0.002				
XXI	KM453699, 1_BB0907-F44 [1]	0.007	0.300	0.314	0.317	0.005	0.005	0.005	0.004	0.002	0.002	0.002	0.005	0.305	0.300	0.298	0.005	0.296	0.303	0.005	0.002			
XXII	KP860912.1_FJEU13 [2]	0.312	0.080	0.094	0.083	0.307	0.308	0.307	0.307	0.307	0.307	0.307	0.310	0.071	0.071	0.073	0.307	0.083	0.071	0.307	0.307	0.305		
XXIII	KP771767.1_NVDC-YN-2011 [1]	0.005	0.303	0.317	0.319	0.002	0.002	0.002	0.002	0.000	0.000	0.000	0.002	0.307	0.303	0.300	0.002	0.298	0.305	0.002	0.000	0.002	0.307	
XXIV	KT988004.1_strain_94881[2]	0.294	0.060	0.080	0.067	0.287	0.287	0.287	0.287	0.289	0.289	0.289	0.291	0.055	0.055	0.053	0.287	0.074	0.064	0.287	0.289	0.287	0.085	0.289

PRRSV – porcine reproductive and respiratory syndrome virus

The given values indicate the genetic divergence of the isolated PRRSV field strains and the lower the value, the greater the divergence. Superscript letters indicate: [1] North American strains; [2] European strains.

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