BMC Veterinary Research

RESEARCH

Open Access

First study to describe the prevalence and epidemiology of African swine fever, classical swine fever, porcine reproductive and respiratory syndrome and swine flu in Kazakhstan

Yuliya V. Perfilyeva[1], Elina R. Maltseva[1], Yekaterina O. Ostapchuk[1], Andrey V. Zhigailov[1], Anna S. Nizkorodova[1], Alena S. Cherusheva[1], Dinara A. Naizabayeva[1], Zhanna A. Berdygulova[1], Akerke O. Bissenbay[1], Tatyana V. Kuznetsova[1], Saltanat A. Kuatbekova[1], Gulnara A. Ismagulova[1], Andrey M. Dmitrovskiy[1], Dong-Hun Lee[3], Seidigapbar M. Mamadaliyev[1], Yuriy A. Skiba[1] and Guillermo R. Risatti[2*]

Abstract

Background Kazakhstan, the ninth-largest country in the world, located in Central Asia and bordering China, Kyrgyzstan, Russia, Turkmenistan, and Uzbekistan, hosts a diverse population of domestic pigs across various environments, providing potential hosts for highly pathogenic viral diseases of swine. Here we monitored African Swine Fever Virus (ASFV), Classical Swine Fever Virus (CSFV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), and Swine Influenza Virus (SIV).

Results During the spring and fall of 2019, we sampled 1,459 domestic pigs in northern, central and eastern Kazakhstan. Samples were tested for antibodies by ELISA and for viral genomes by qPCR and RT-qPCR. No antibodies against ASFV or ASFV DNA were detected in sampled animals. Of the 84 farms sampled, 16.6% had at least one animal vaccinated against CSF. Seropositive pigs were found on a farm in Oskemen with no history of vaccination against CSFV. No CSFV RNA was detected in the blood of the sampled animals. Only 12.2% of the animals tested were vaccinated against PRRS with live-attenuated vaccines. The true animal-level seroprevalence of PRRS on unvaccinated farms was 16.6%. PRRSV RNA was detected in 17 unvaccinated animals in Pavlodar oblast on farms that were vaccinated against PRRS. The identified PRRSV-1 strains belonged to subtype 1 and clustered with the PRRS DV vaccine virus strain. A large proportion of the pigs had antibodies against SIV, with true animal-level seroprevalence of 35.9% and herd-level seroprevalence of 23.2%. Antibodies against the influenza A viruses of hemagglutinin subtypes H1 and H3 were found in the examined pigs. None of the animals were vaccinated against SIV. The variable 'commercial farming' showed an association with PRRSV and IAV seroprevalence. Of the unvaccinated farms, 9% were co-infected with PRRSV and SIV.

*Correspondence:
Guillermo R. Risatti
guillermo.risatti@uconn.edu
Full list of author information is available at the end of the article

Conclusions Results confirm the domestic pig population in Kazakhstan was not infected with ASFV but indicated exposure to PRRSV and SIV. This underscores the need for monitoring these infections in the region to manage their impact.

Keywords Kazakhstan, African Swine Fever, Classical Swine Fever, Porcine Reproductive and Respiratory Syndrome, Swine Influenza

Introduction

Pigs are an economically valuable livestock species in Kazakhstan, with a domestic swine population of around 800,000 animals as of December 2023, comprising indigenous and European breeds [1]. Pig farming is predominantly concentrated in North Kazakhstan, Kostanay, Pavlodar, and Karagandy regions (oblasts) [1]. Pigs are raised in diverse management systems, ranging from large-scale industrial farms to small family-owned farms, where biosecurity is low and herds can roam freely. Small family-owned farms house at least 50% of the domestic swine population [1]. Backyard pig farming increases the risk of contact with wild boars, which are natural reservoirs for a number of infectious diseases of domestic pigs [2–5]. The wild boar population in the country totals 35,140 animals, resulting in an average density of approximately 0.012 wild boars per square kilometer as of December 2023 [6]. Wildlife reservoirs, low-biosecurity farms, and uncontrolled animal movements across borders heighten the risk of introducing and sustaining highly pathogenic swine viral diseases [7–9]. Here we refer to highly pathogenic viral diseases of swine as diseases of swine caused by the African Swine Fever Virus (ASFV), Classical Swine Fever Virus (CSFV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), and Swine Influenza Virus (SIV).

ASFV and CSFV cause severe hemorrhagic fevers in domestic pigs and wild boars, which are often clinically indistinguishable [10–12]. ASFV is a large, double-stranded DNA virus and the only species in the family *Asfarviridae,* within the order *Asfuvirales* [13]. ASFV causes an exceptionally contagious disease in pigs, marked by mortality rates reaching up to 100% [14, 15]. Wild boars serve as the primary ASFV reservoir in Eurasia, spreading the virus through direct contact and carcasses [16, 17]. ASF has been reported in Africa, Europe, Asia, Oceania and the Americas (Caribbean) [18]. In October 2018, the World Organization for Animal Health recognized Kazakhstan as historically free of ASF [18]. However, the northern and eastern regions are at risk due to ASF outbreaks in neighboring Russia and China [18–20]. In 2019, during the period of animal sampling for this study, 79 ASF outbreaks in domestic pigs and 65 in wild boars occurred in Russia, including Volgogradskaya Oblast, which borders Kazakhstan, alongside 61 outbreaks in China [18, 20].

CSFV is a small RNA virus of the genus *Pestivirus,* within the family *Flaviviridae* [21]. In the acute and chronic forms of the disease, affected pigs show high fever, skin hemorrhages, anorexia, lethargy, conjunctivitis, respiratory signs, diarrhea and reproductive disorders [22]. Less virulent CSFV strains or less susceptible pigs suffer from decreased growth and productivity [23, 24]. CSF is widespread in Asia, including China, where CSFV expansion has been controlled via vaccination [25]. Live attenuated CSF vaccines are widely used and are highly effective in preventing the disease [26–28]. Kazakhstan also uses vaccination to control CSF, although not as part of a national control program [29]. Vaccination is not mandatory, and small-scale pig farms with free-ranging pigs often do not vaccinate against CSF. No outbreaks of CSF have been reported in Kazakhstan [30]. But due to the lack of surveillance, the actual disease prevalence remains unknown.

PRRSV is a member of the genus *Betaarterivirus,* within the family *Arteriviridae* and the order *Nidovirales* [31]. It is responsible for respiratory problems in pigs and reproductive complications in sows leading to stillbirths, mummification and increased pre-weaning mortality [32]. Genetic and antigenic assessments have revealed two distinct species, PRRSV-1 and PRRSV-2 [33, 34]. Both low- and high-pathogenic strains are present within each species [35, 36]. Highly pathogenic PRRSV (HP-PRRSV), which is originated from PRRSV-2, emerged in China in 2006, affecting millions of pigs [37]. Recently, HP-PRRSV-like lineages have been reported in the Xinjiang Uygur Autonomous Region of China, which borders southeastern and eastern Kazakhstan [38, 39]. Although both PRRSV-1 and PRRSV-2 have been isolated from pigs in northern and southern Kazakhstan, respectively, HP-PRRSV has not been reported in the country [40]. Several modified live vaccines against PRRS are commercially available in Kazakhstan, but similar to CSF, vaccination is not mandatory, and the extent of vaccination is unknown.

SIV is caused by influenza A viruses (IAV), mainly by subtypes H1 N2, H3 N2 and H1 N1, including the pandemic strain H1 N1pdm09 [41]. Infected pigs develop fever, loss of appetite, lethargy, coughing, and respiratory

distress, leading to high morbidity rates [42, 43]. Pigs are susceptible to avian, human, and swine IAVs [43]. In Central Asia, H3 N6, H4 N6, H1 N1, H5 N1, H5 N3, H11 N2, H13 N6, H16 N3, H10 N7, H3 N8, H13 N8, H5 N8 subtypes, as well as the new candidate subtype H19 have been detected in wild birds [44–48]. H1 N1 and H3 N2 IAVs have also been identified in domestic pigs in Kazakhstan, highlighting the need for systematic SIV surveillance [49]. Vaccination against SIV in pigs is neither encouraged nor mandated by the government, and despite the availability of some commercial vaccines, it remains unclear whether individual producers or companies are implementing SIV vaccination.

The Minister of Agriculture of Kazakhstan classifies ASF, CSF, and swine influenza as dangerous diseases, with state-funded prevention and eradication [29]. However, regular surveillance is not funded, leading to data gaps on their prevalence. Therefore, in this study, we assessed the prevalence of ASFV, CSFV, SIV, as well as PRRSV in Kazakhstan in high-risk areas by serological and molecular screening of domestic pigs**.**

Materials and methods

Sample size calculation and sample collection

Two-stage cluster design with simple random sampling was used for this study.

The minimum sample size was determined according to the following formula [50]:

$$n = Z_{\alpha}^{2}\mathrm{Pexp} \times \ (1 - \mathrm{Pexp})/\mathrm{d}.^{2}$$

,where: n= required minimum sample size; Pexp =expected prevalence; d= desired absolute precision; Z_{α}= normal distribution of the corresponding alpha value (type 1 error). Based on an expected infection prevalence of 50%, a confidence level of 95% and a maximum allowable error of 5%, the minimum sample size of 384 animals was determined. The obtained sample size was further adjusted for design effects (DE) to accommodate the clustering of observations: DE =1 +ρ (m-1), where ρ denotes the intra-cluster correlation coefficient and m indicates the cluster size (number of pigs sampled per herd) [50]. Using a ρ value of 0.2 [51] and an average herd size of 15 animals, the total sample size was estimated to be 1,459 animals.

As a result, in 2019, we randomly sampled 1,459 pigs from 92 herds across 84 farms in 28 provinces/cities in the central (Karagandy oblast), northern (Akmola, North Kazakhstan and Pavlodar oblasts) and eastern (Jetisu, Abai and East Kazakhstan oblasts) regions of Kazakhstan (Fig. 1). The average number of animals per sampled herd was 16. The blood was drawn by a trained veterinarian from either the jugular or the ear vein. The samples were transported at −20 °C in a mobile freezer to the Almaty Branch of the National Center for Biotechnology for further analysis. In total, 1,459 serum samples and 1,347 blood samples were collected. The serum was separated before freezing the samples.

During sampling, information was collected on farm size, received vaccinations and clinical signs of the animals. Samples were obtained from pigs raised under varied management conditions, including animals raised in backyard households (27.3%; 399/1,459; 95% CI: 14.9–39.7%) and those raised on large commercial farms (72.6%; 1,060/1,459; 95% CI: 60.3–85.0%) (Supplementary Fig. 1). Of 1,459 animals sampled, 1,386 were apparently healthy, 26 had reproductive disorders, 40 had respiratory disorders, and 7 presented with skin rash or foamy mouth. Additionally, we collected gender information for 713 animals (499 females and 214 males) and age data for 1,380 animals. The study included animals across

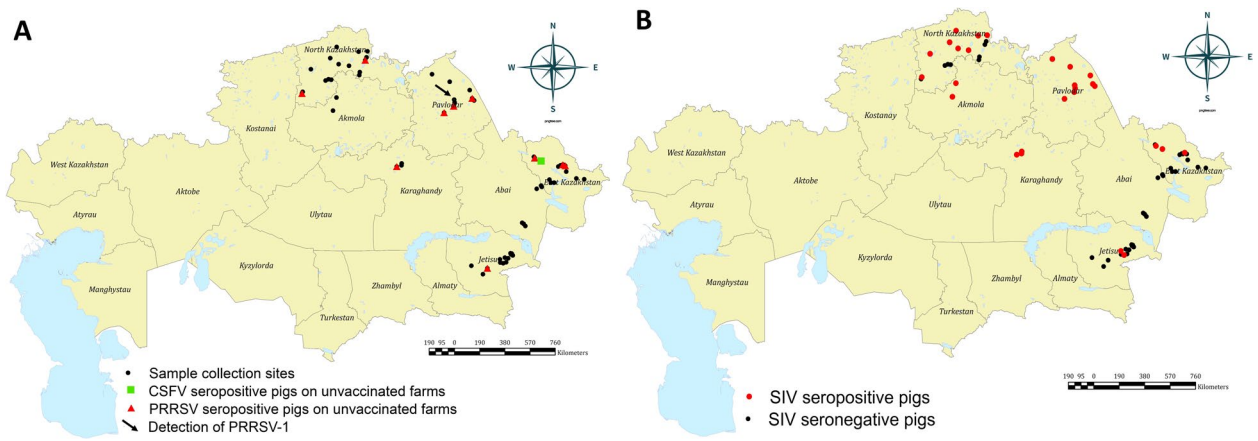

Fig. 1 Geographical distribution of CSFV and PRRSV seropositive animals on unvaccinated farms and sites of PRRSV-1 detection (**A**) and SIV seropositive animals (**B**)

various age groups, with a mean age of 6.9 months (ranging from less than 1 month to 36 months). Animals with recorded age data were included in the epidemiological risk factor analysis.

Detection of antibodies by ELISA

Commercially available ELISA kits were used to detect antibodies against PRRSV, SIV, ASFV and CSFV: PRRS Indirect ID Screen, Classical Swine Fever E2 Competition ID Screen, African Swine Fever Indirect Screening Test ID Screen, and Influenza A Antibody Competition Multi-Species ID Screen. Test procedures and interpretation of the results were performed according to the manufacturer's instructions. The diagnostic sensitivity (Se) and specificity (Sp) of the ELISA kits are shown in Supplementary Table 1. The Swinecheck® H3 N2 and H1 N1 ELISA kits (Biovet, USA) were further used for the detection of antibodies against the hemagglutinin subtypes H3 and H1 of IAV in serum samples that tested positive using the Influenza A Antibody Competition Multi-Species ID Screen (ID-Vet, France). The test procedures and interpretation of the results were performed according to the manufacturer's instructions. The Influenza A Antibody Competition Multi-Species ID Screen kit allowed for a "grey zone" of interpretation that includes inconclusive results (the competition percentage (S/N) $\leq$45%: positive; S/N $\geq$50%: negative, and S/N between 45 and 50%: doubtful). Similarly, the Classical Swine Fever E2 Competition ID Screen also allowed for a "grey zone" of interpretation (S/N $\leq$50%: positive; S/N >60%: negative and S/N between 50 and 60%: doubtful).

Detection of viral DNA and RNA in blood samples

All collected blood samples were pooled (up to 5 samples in one pool) for the detection of ASFV DNA and for the detection of CSFV and PRRSV RNA. In addition, we selected 127 samples from herds with a seroprevalence of more than 20% for PRRSV and CSFV and tested these in addition to the pooled samples. RNA and DNA were extracted from 200 µl of pooled or individual blood samples using the Trizol reagent (Thermofisher Scientific, USA) or DNeasy Blood and Tissue kit (Qiagen, Stanford, CA, USA), respectively, according to the manufacturers' instructions. The pair of primers "18S-sus-F" and "18S-sus-R" was employed to assess the integrity of the isolated RNA (Table 1).

Commercially available qPCR and RT-qPCR kits, African Swine Fever Virus DNA Test Kit and Classical Swine Fever Test Kit (Tetracore Inc., MD, USA), were used to detect ASFV and CSFV genomes, respectively, in nucleic acids extracted from blood samples using the QuantStudio 5 thermal cycler (Thermo Fisher Scientific, USA). A sample was considered positive if the cycle threshold (Ct) value was <45, in accordance with the manufacturer's recommendations.

For PRRSV, a One-Step RT-qPCR Kit w/ROX (Thermo Fisher Scientific, USA) was used for detection. The primers and TaqMan probes have been previously described [52–54]. DNA amplification and analysis of the results were carried out on the QuantStudio 5 thermal cycler (Thermo Fisher Scientific, USA). A sample was considered positive if the Ct value was <40, in accordance with the manufacturer's recommendations [52].

Table 1 Primers and probes used in the study

Name	Primer sequence (5′ → 3′)	Target	T_a [°C]	Product size, bp	Reference	Application
PRRSV-US-1 F	(5′)ATRATGRGCTGGCATTC	PRRSV ORF7 gene	60	77	[52]	Detection of US strains (type 2) of PRRSV by RT-qPCR
PRRSV-US-2 F	(5′)ATGATGRGCTGGCATTCT					
PRRSV-US-1R	(5′)ACACGGTCGCCCTAATTG					
PRRSV-US-1Pr	FAM-TGTGGTGAATGGCACTGATTGACA-BHQ1					
PRRSV-EU-2.1 F	(5′)GCACCACCTCACCCRRAC	PRRSV ORF7 gene	60	114		Detection of EU strains (type 1) of PRRSV by RT-qPCR
PRRSV-EU-2.1R	(5′)CAGTTCCTGCRCCYTGAT					
PRRSV-EU-2.1	FAM-CCTCTGYYTGCAATCGATCCAGAC-BHQ1					
PRRSV-HP-1 F	(5′)CCGCGTAGAACTGTGACAAC	PRRSV NSp2 gene	60	122	[53]	Typing of HP-PRRSV by qPCR
PRRSV-HP-1R	(5′)TCCAGGATGCCCATGTTCTG					
PRRSV-HP-1	FAM-ACGCACCAGGATGAGCCTCTG GAT-HQ1					
EU-F	(5′)TGAGGTGGGCTACAACCATT	PRRSV GP5 gene	60	703	[54]	Sequencing of GP5 gene of type I PRRSV
EU-R	(5′)AGGCTAGCACGAGCTTTTGT					
18S-sus-F	(5′)TGGAGCGATTTGTCTGGTTA	Swine 18S rRNA	60	200	[55]	Testing of RNA integrity by PCR
18S-sus-R	(5′)ACGCTGAGCCAGTCAGTGTA					

All primer and probe sequences used in the study are listed in Table 1. RNA isolated from the Porcilis® PRRSV vaccine (MSD Animal Health, USA) and LK-VNIIVViM CSF vaccine (Federal Research Center for Virology and Microbiology, Russia) was used as positive controls. Diagnostic Se and Sp of the used commercial kits and primers/probes are shown in Supplementary Table 1.

Sanger sequencing and phylogenetic analysis

Samples that were positive for PRRSV by RT-qPCR were further used for sequencing of amplicons. A conventional RT-PCR was used to amplify the PRRSV *GP5* gene using SuperScript™ III One-Step RT-PCR System with Platinum® Taq High Fidelity kit (Thermo Fisher Scientific, USA). Amplification was performed on the Mastercycler X50 s thermal cycler (Eppendorf, USA). PCR products were visualized in 1% TAE agarose gels. Amplicons were purified and subjected to Sanger sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing was performed on a 24-capillary genetic analyzer ABI 3500XL (Applied Biosystems, USA), using the 3500 Series Data Collection Software V.3.1 (Life Technologies, USA).

The analysis of raw data was performed with Sequencing Analyzer Software V.6.0 (Life Technologies, USA). The sequences for both directions (forward and reverse) were merged online (http://www.bioinformatics.nl/cgi-bin/emboss/merger). Multiple sequence alignments were performed with the MUSCLE algorithm [56]. The unrooted phylogenetic tree was generated by the Maximum Likelihood method using MEGA X software with a bootstrap of 1000 repetitions [57]. The"Find Best DNA/Protein Models"tool in MEGA X software was used to identify the most suitable substitution model for the sequences of the analyzed strains. Based on the Bayesian Information Criterion, the Kimura 2-parameter model was selected for subsequent analysis [58]. The tree with the highest log-likelihood is shown and bootstrap values above 20% are displayed. All positions with less than 95% site coverage were eliminated.

The GP5 sequences of the reference PRRSV isolates were obtained from the GenBank, NCBI database (https://www.ncbi.nlm.nih.gov/genbank/). Given the absence of sequences of Kazakhstani PRRSV-1 strains deposited in GenBank, we selected 30 PRRSV-1 strains (3 from the Netherlands, 5 from China, 4 from the United States, 1 from Spain, 1 from France, 1 from Germany, 1 from England, 1 from Denmark, 1 from Italy, 1 from Austria, 1 from Lithuania, 5 from Russia; 5 from Belarus) to represent the full range of genetic diversity and geographic locations of PRRSV-1, including sequences from Europe, Asia and North America. Three PRRSV-2 prototypes (2 from the USA, 1 from China) were used as out-groups in the phylogenetic analysis (Supplementary Table 2). A total of 15 of 17 sequences obtained in this study have been deposited in GenBank under the accession numbers PQ661611 – PQ661625 and PV250225, PV250226.

Statistical analysis

The data were recorded in Microsoft Excel 2013 and analyzed using the Epi Info 7.0 software [59] and STATA version 14 [60]. The apparent prevalence of infection at the animal level was determined by calculating the proportion of positive animals among all those tested. A herd prevalence was defined as the proportion of herds with at least one positive animal. To adjust animal-level prevalence for the clustering effect, the 95% Wald confidence interval (CI) was computed. The herd-level and animal-level prevalence was also adjusted for the diagnostic Se and Sp of the tests using the equation: $TP = (AP + Sp - 1)/(Se + Sp - 1)$, where TP is the true prevalence and AP is apparent prevalence [61].The herd-level Sp and Se were derived according to Dohoo et al. [50]. For herd prevalence, the 95% CI was computed using the Clopper-Pearson exact method based on the beta distribution. Herds sampled from the same location in fall and spring were regarded as different herds. Screening of potential risk factors related to highly pathogenic viral diseases of swine seropositivity was performed using an unadjusted random effects logistic regression model. The outcome variable was the “apparent” status. The herd was used as a random effect to account for the clustering of animals within herds. All the independent variables were further analyzed in an adjusted random effects logistic regression model.

Results

Exposure of pigs to African swine fever virus

Antibodies against ASFV were not detected in any of the tested animals ($n = 1,459$). Blood samples from 1,459 pigs also tested negative for ASFV DNA by qPCR.

Exposure of pigs to classical swine fever virus

CSFV vaccination with live-attenuated vaccines is used in Kazakhstan. Of the 1,459 animals sampled, 636 (43.6%; 95% CI: 26.8–60.4%) pigs had reliable records of vaccination against CSFV performed between February and May 2019. A total of 16.6% (14/84) of farms had at least one vaccinated animal with an average vaccination rate of 88.9%. The ELISA results showed that 72.8% (463/636; 95% CI: 52.4–93.1%) of immunized animals tested positive for antibodies to CSFV. Vaccination was applied in North Kazakhstan, East Kazakhstan, Karagandy, and Pavlodar oblasts. The rate of vaccination against CSFV was higher on commercial farms (55.8%: 591/1,060) as

compared to backyard farms (11.3%; 45/399). The apparent seroprevalence at the animal level in farms vaccinating against CSF was 69.1% (Table 2).

On unvaccinated farms, 5.1% (35/692; 95% CI: 0–14.5%) and 0.9% (6/692; 95% CI: 0–1.8%) tested positive and doubtful for antibodies to CSFV, respectively (Table 2). Repeated analyses of the conflicting samples produced consistent results, suggesting either low antibody concentrations or potential sample quality issues, both of which could lead to borderline optical density readings. All seropositive unvaccinated animals were found on one farm in Oskemen City, East Kazakhstan oblast (Fig. 1).

For direct CSFV detection, total RNA was extracted from 270 pools and 127 individual blood samples. Individual blood samples were selected from herds in which over 20% of the animals tested positive for CSFV antibodies by ELISA. Although a satisfactory quality of isolated RNA, none of the samples yielded a positive result for CSFV by RT-qPCR (data not shown).

Exposure of pigs to porcine reproductive and respiratory syndrome virus

Vaccine coverage against PRRSV is very limited among pig herds in Kazakhstan. Available records indicated that only 12.2% (178/1,459; 95% CI: 3.4–20.9%) of the pigs sampled were vaccinated with PRRSV live-attenuated vaccines (between February and May 2019). None of the animals raised in backyard households were vaccinated against PRRSV, while the rate of vaccination on commercial farms was 16.8% (178/1,060; 95% CI: 5.1–28.5%). Of 178 vaccinated pigs, 138 animals (77.5%; 95% CI: 52.1–100%) tested positive for IgG against PRRSV by ELISA. Taking into account the sensitivity and specificity of the ELISA test, the true prevalence of antibodies against PRRSV in vaccinated animals was calculated to be 77.6%. PRRS vaccination was carried out on six farms in Karagandy, Pavlodar and East Kazakhstan oblasts (7.1%; 6/84; 95% CI: 2.7–14.9%) with an average vaccination rate of 45.9% per farm. The apparent animal-level seroprevalence of PRRSV in vaccinated farms was 66.3% (Table 3).

The apparent seroprevalence of PRRSV in unvaccinated farms is shown in Table 3. The total true animal-level seroprevalence of PRRSV on farms that did not use PRRS vaccination was calculated to be 16.6% (95% CI: 1.4—34.2%). The seropositive unvaccinated pigs were distributed across 11 of 82 unvaccinated herds, resulting in a true herd-level seroprevalence of 12.0%. Seropositive animals on farms that did not use PRRS vaccination were found in nine of 28 provinces in the Jetisu, East-Kazakhstan, Karagandy, North Kazakhstan and Pavlodar oblasts. Figure 1 shows the geographical distribution of unvaccinated farms with seropositive animals. The unadjusted random effects model showed that the independent variable 'commercial farming', but not 'age over 6 months', or 'respiratory and reproductive disorders', was positively correlated with seropositivity for PRRSV on unvaccinated farms, however, the association disappeared in the adjusted model (Table 5).

For PRRSV direct detection, total RNA extracted from 270 pools and 127 individual blood samples was used for RT-qPCR analysis. The individual blood samples were selected from herds and over 20% of the animals tested positive for PRRSV antibodies by ELISA. PRRSV RNA was detected in six pools. We further split the positive pools and subjected the RNA extracted from individual blood samples to RT-qPCR testing. Of the 30 samples tested, only 17 yielded a positive result with a mean value of Ct = 28.8 ± 3.2. PRRSV RNA was detected in unvaccinated animals on two farms that applied vaccination against PRRSV with live-attenuated vaccines (Table 3), indicating an apparent PRRSV RNA prevalence of 3.4% (17/493) in animals on vaccinated farms. Considering the test's specificity, the total true animal-level prevalence of PRRSV RNA on vaccinated farms was estimated to be 0.9%. All PRRSV RNA-positive samples were collected in the Pavlodar province from eight seronegative and nine seropositive animals. None of the RT-qPCR-positive animals had any clinical signs.

To establish the genetic relationships of the identified PRRSV-1 strains, we constructed a phylogenetic tree using the gene sequences of 30 PRRSV-1 strains retrieved from the GenBank database (Fig. 2). As a result, all identified strains shared 99.2–100% homology and were assigned to subtype 1 (global). The obtained strains clustered with the vaccine strain DV. GP5 gene nucleotide homology of the 99.3–99.8% was shown for the PRRSV-1 strains detected in this study and vaccine strain DV. The GP5 homology between the samples analyzed here and the reference strain Lelystad Virus (GenBank no. NC 043487.1) ranged from 98.0% to 98.75%.

Exposure of pigs to influenza a virus

Vaccination of pigs against IAV is not commonly practiced in Kazakhstan. Neither of the pigs sampled had reliable records of vaccination against IAV. A total of 32 of 92 herds tested harbored pigs with antibodies against IAV with the apparent animal-level seroprevalence of 34.1%. Taking into account the ELISA test Sp and Se, the true animal-level seroprevalence was calculated at 35.9% (95% CI: 21.3—47.0%) and the true herd-level seroprevalence at 23.2%. Twenty-four animals (1.6%;95% CI: 0.3—2.9%) tested doubtful for antibodies to IAV. Doubtful samples were retested, yielding the same results. All these pigs were intermingling with animals that tested positive for antibodies to

Table 2 Apparent animal-level CSFV antibody prevalence in pigs in vaccinated and unvaccinated farms, Kazakhstan, 2019

Oblast	Province	No. of pigs sampled	No. of farms/herds sampled	Vaccinated farms				Unvaccinated farms			
				Total	Seropositive (%)	Serodoubtful (%)	Seronegative	Total	Seropositive (%)	Serodoubtful (%)	Seronegative
Akmola	Atbasar	50	1/1	-	-	-	-	50	-	-	50
	Sandyktau	25	1/2	-	-	-	-	25	-	-	25
	Total in the oblast	*75*	*2/3*	*0*	*0*	*0*	*0*	*75*	*0*	*0*	*75*
Jetisu	Aksu	16	1/1	-	-	-	-	16	-	-	16
	Alakol	19	4/4	-	-	-	-	19	-	-	19
	Sarkand	51	9/9	-	-	-	-	51	-	-	51
	Tekeli	5	1/1	-	-	-	-	5	-	-	5
	Karatal	10	2/2	-	-	-	-	10	-	-	10
	Total in the oblast	*101*	*17/17*	*0*	*0*	*0*	*0*	*101*	*0*	*0*	*101*
East Kazakhstan	Altai	52	10/10	-	-	-	-	52	-	-	52
	Oskemen city	88	2/2	-	-	-	-	88	35 (40.0%)	1 (1.0%)	52
	Ulan	67	2/2	57	45 (79.0%)	2 (4.0%)	10	10	-	-	10
	Katon-Karagay	16	5/5	-	-	-	-	16	-	-	16
	Total in the oblast	*223*	*19/19*	*57*	*45 (79.0%)*	*2 (4.0%)*	*10*	*166*	*35 (21.1%)*	*1 (0.6%)*	*130*
Abai	Kokpekti	38	11/11	-	-	-	-	38	-	2	36
	Urzhar	34	7/7	-	-	-	-	34	-	3	31
	Total in the oblast	*72*	*18/18*	*0*	*0*	*0*	*0*	*72*	-	*5 (6.9%)*	*67*
Karagandy	Abay	100	1/1	100	98 (98.0%)	0	2	-	-	-	-
	Bukhar-Zhyrau	197	2/4	197	147 (74.6%)	2 (1.0%)	48	-	-	-	-
	Total in the oblast	*297*	*3/5*	*297*	*245 (82.5%)*	*2 (0.7%)*	*50*	*0*	*0*	*0*	*0*
North Kazakhstan	Esil	19	1/1	19	10 (53.0%)	-	9	-	-	-	-
	Gabit Musirepov	37	2/2	-	-	-	-	37	-	-	37
	Magzhan Zhumabaev	40	4/4	-	-	-	-	40	-	-	40
	Shal Akyn	29	1/1	-	-	-	-	29	-	-	29
	Taiynsha	175	4/6	95	11 (12.0%)	2 (2.0%)	82	80	-	-	80
	Aiyrtau	50	3/3	13	-	-	12	37	-	-	37
	Kyzylzhar	20	1/1	-	-	-	-	20	-	-	20
	Total in the oblast	*370*	*16/18*	*127*	*21 (16.5%)*	*2 (1.6%)*	*104*	*243*	-	-	*243*

Table 2 (continued)

Oblast	Province	No. of pigs sampled	No. of farms/herds sampled	Vaccinated farms				Unvaccinated farms			
				Total	Seropositive (%)	Serodoubtful (%)	Seronegative	Total	Seropositive (%)	Serodoubtful (%)	Seronegative
Pavlodar	Pavlodar province and Pavlodar city	224	3/6	224	181 (80.8%)	13 (5.8%)	30	-	-	-	-
	Terenkol (Kashyr)	10	1/1	10	-	-	10	-	-	-	-
	Zhelezin	15	1/1	15	11 (74.0%)	4 (26.0%)	-	-	-	-	-
	Aksu	20	1/1	-	-	-	-	20	-	-	20
	Sharbakty	37	2/2	37	27 (73.0%)	-	10	-	-	-	-
	Uspen	15	1/1	-	-	-	-	15	-	-	15
	Total in the oblast	*321*	*9/12*	*286*	*219 (76.6%)*	*17 (5.9%)*	*50*	*35*	-	-	*35*
TOTAL		**1,459**	**84/92**	**767**	**530 (69.1%)**	**23 (2.9%)**	**214**	**692**	**35 (5.1%)**	**6 (0.9%)**	**651**

Table 3 Apparent animal-level PRRSV antibody prevalence in pigs in vaccinated and unvaccinated farms, Kazakhstan, 2019

Oblast	Province	Vaccinated farms				Unvaccinated farms			
		Total	Seropositive (%)	Seronegative	PCR-positive (%)	Total	Seropositive (%)	Seronegative	PCR-positive
Akmola	Atbasar	-	-		-	50	-	50	-
	Sandyktau	-	-		-	25	-	25	-
	Total in the oblast	*0*	*0*	*0*	*0*	*75*	*0*	*75*	*0*
Jetisu	Aksu	-	-		-	16	4 (25.0%)	12	-
	Alakol	-	-		-	19	-	19	-
	Sarkand	-	-		-	51	-	51	-
	Tekeli	-	-		-	5	-	5	-
	Karatal	-	-		-	10	-	10	-
	Total in the oblast	*0*	*0*	*0*	*0*	*101*	*4 (3.9%)*	*97*	*0*
East Kazakhstan	Altai	-	-		-	52	5 (9.6%)	47	-
	Oskemen city	88	67 (76.1%)	21	-	-	-	-	-
	Ulan	57	52 (91.2%)	5	-	10	10 (100%)	-	-
	Katon-Karagay	-	-	-	-	16	-	16	-
	Total in the oblast	*145*	*119 (82.1%)*	*26*	*0*	*78*	*15 (19.2%)*	*63*	-
Abai	Kokpekti	-	-	-	-	38	-	38	-
	Urzhar	-	-	-	-	34	-	34	-
	Total in the oblast	*0*	*0*	*0*	*0*	*72*	*0*	*72*	*0*
Karagandy	Abay	-	-	-	-	100	97 (97.0%)	3	-
	Bukhar-Zhyrau	167	86 (51.5%)	59	-	30	-	30	-
	Total in the oblast	*167*	*86 (51.5%)*	*59*	*0*	*130*	*97(74.6%)*	*33*	-
North Kazakhstan	Esil	-	-	-	-	19	-	19	-
	Gabit Musirepov	-	-	-	-	37	1 (2.7%)	36	-
	Magzhan Zhumabaev	-	-	-	-	40	1 (2.5%)	39	-
	Shal Akyn	-	-	-	-	29	-	29	-
	Taiynsha	-	-	-	-	175	-	175	-
	Aiyrtau	-	-	-	-	50	-	50	-
	Kyzylzhar	-	-	-	-	20	-	20	-
	Total in the oblast	*0*	*0*	*0*	*0*	*370*	*2 (0.5%)*	*368*	*0*
Pavlodar	Pavlodar province and Pavlodar city	181	122 (67.4%)	59	17 (9.4%)	43	3 (6.9%)	40	-
	Terenkol (Kashyr)	-	-	-	-	10	-	10	-
	Zhelezin	-	-	-	-	15	-	15	-
	Aksu	-	-	-	-	20	17 (85.0%)	3	-
	Sharbakty	-	-	-	-	37	23 (62.2%)	14	-
	Uspen	-	-	-	-	15	-	15	-
	Total in the oblast	*181*	*122 (67.4%)*	*59*	*17 (9.4%)*	*140*	*43 (30.7%)*	*97*	-
TOTAL		**493**	**327 (66.3%)**	**144**	**17 (3.6%)**	**966**	**161 (16.7%)**	**805**	**0**

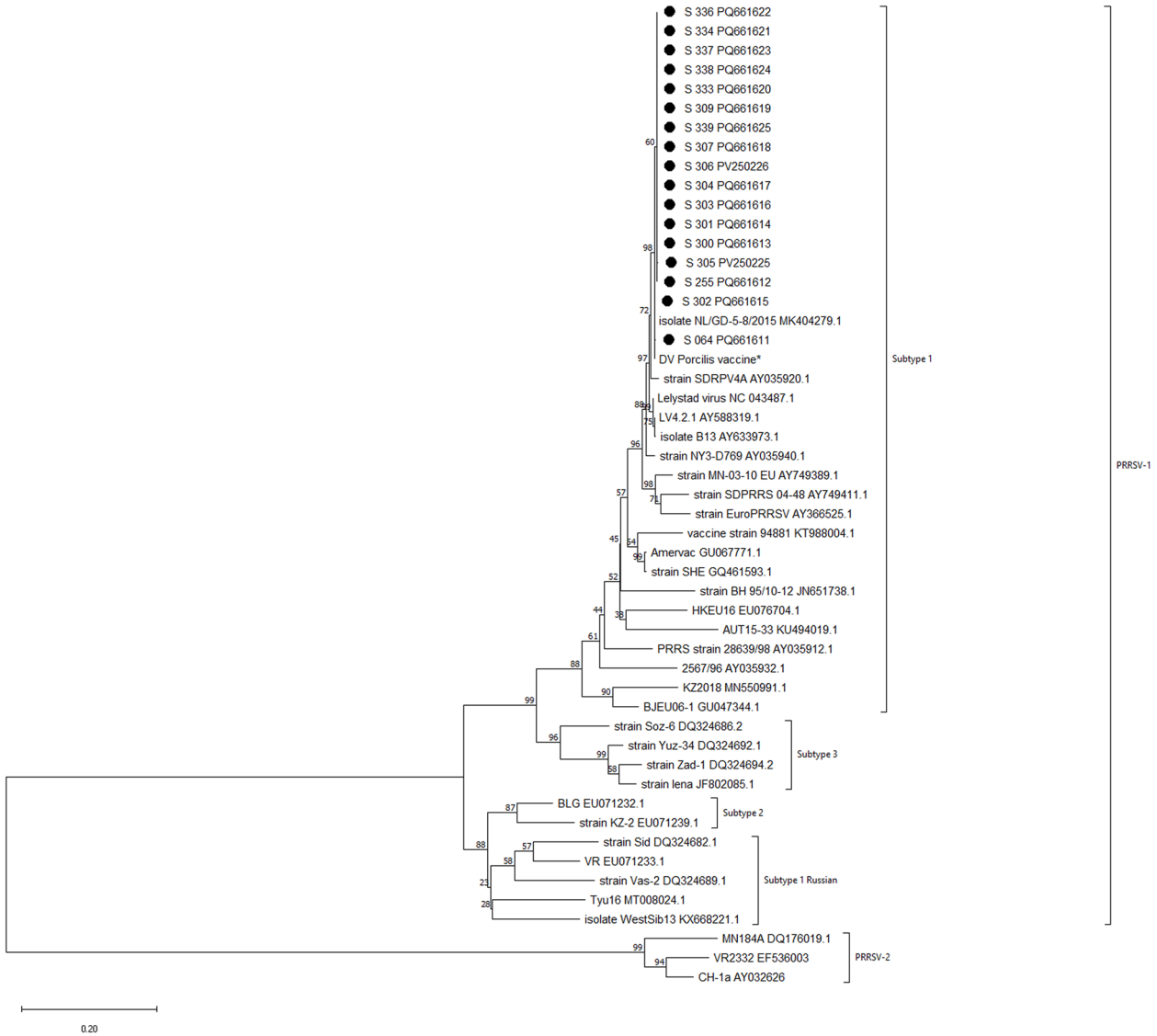

Fig. 2 Phylogenetic analysis based on Gp5 nucleotide sequences for PRRSV, detected in pig farms in Kazakhstan. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. The tree with the highest log likelihood (–5996.96) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+ G, parameter = 0.8658)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 50 nucleotide sequences. Evolutionary analyses were conducted in MEGA X. Bootstrap replication = 1000

IAV. This suggests that low antibody concentrations, falling just below the assay cut-off, were likely responsible for the doubtful results, possibly indicating early exposure or waning immunity. Only herds from Aksu, Alakol, Karatal and Tekeli in Jetisu oblast, Kokpetki and Urzhar in Abai oblast, Aiyrtau and Gabit Musirepov in North Kazakhstan and Katon-Karagay in East Kazakhstan oblast tested negative for IAV antibodies (Fig. 1). High apparent seroprevalence was observed in Akmola, Karagandy, and Pavlodar oblasts, with over half of the animals tested yielding positive results. The lowest apparent seroprevalence of 1.9% was detected in the Jetisu oblast (Table 4).

In both the unadjusted and adjusted random effects models, the independent predictor 'commercial farming', but not 'age over 6 months', and 'respiratory signs' was associated with IAV seroprevalence (Table 5).

Based on the remaining sample volume, a total of 386 of 498 seropositive samples were subjected to further serotyping. Antibodies specific for the H1 or H3 subtype of IAV were found in 13.0% (50/386; 95% CI: 8.5—25.8%) and 13.7% (53/386; 95% CI: 6.7—20.7%) of

Table 4 Apparent animal-level prevalence of Influenza A virus antibodies and RNA in domestic pigs, Kazakhstan, 2019

Oblast	Province	Serological screening			
		Total # of pigs	Seropositive (%)	Serodoubtful	Seronegative
Akmola	Atbasar	50	50 (100.0%)	-	-
	Sandyktau	25	1 (4.0%)	-	24
	Total in the oblast	*75*	*51 (68.0%)*	-	*24*
Jetisu	Aksu	16	-	-	16
	Alakol	19	-	-	19
	Sarkand	51	2 (3.9%)	-	49
	Tekeli	5	-	-	5
	Karatal	10	-	-	10
	Total in the oblast	*101*	*2 (1.9%)*	-	*99*
East Kazakhstan	Altai	52	2 (3.8%)	1 (1.9%)	49
	Oskemen city	88	33 (37.5%)	1 (1.1%)	54
	Ulan	67	4 (6.0%)	-	63
	Katon-Karagay	16	-	-	16
	Total in the oblast	*223*	*39 (17.5%)*	*2 (0.9%)*	*182*
Abai	Kokpekti	38	-	-	38
	Urzhar	34	-	-	34
	Total in the oblast	*72*	-	-	*72*
Karagandy	Abay	100	63 (63.0%)	1 (1%)	36
	Bukhar-Zhyrau	197	106 (53.8%)	5 (2.5%)	86
	Total in the oblast	*297*	*169 (56.9%)*	*6 (2.0%)*	*122*
North Kazakhstan	Esil	19	11 (57.9%)	2 (10.5%)	6
	Gabit Musirepov	37	2 (5.4%)	1 (2.7%)	34
	Magzhan Zhumabaev	40	17 (42.5%)	-	23
	Shal Akyn	29	6 (20.7%)	1 (3.4%)	22
	Taiynsha	175	6 (3.4%)	10 (0.6%)	159
	Aiyrtau	50	-	-	50
	Kyzylzhar	20	2 (10.0%)	-	18
	Total in the oblast	*370*	*44 (11.9%)*	*14 (3.8%)*	*312*
Pavlodar	Pavlodar province and Pavlodar city	224	149 (66.5%)	1 (0.4%)	74
	Terenkol (Kashyr)	10	2 (20.0%)	-	8
	Zhelezin	15	15 (100.0%)	-	-
	Aksu	20	15 (75.0%)	-	5
	Sharbakty	37	8 (21.6%)	-	29
	Uspen	15	4 (26.7%)	1 (6.7%)	10
	Total in the oblast	*321*	*193 (60.1%)*	*2 (0.5%)*	*126*
TOTAL		**1,459**	**498 (34.1%)**	**24 (1.6%)**	**937**

the samples tested, respectively. A fairly large proportion of samples tested double positive for antibodies against both H1 and H3 subtypes (22.0%; 85/386; 95% CI: 4.8—39.2%) (Table 6). Double seropositive animals were identified on eight farms, all of which also had single seropositive animals for both H1 and H3 subtypes.

Co-infections

Both farms in the city of Oskemen where CSFV-seropositive unvaccinated animals were identified were also infected with SIV. Seven of the 78 farms that did not apply PRRSV vaccination housed animals that were seropositive for both PRRSV and SIV, representing a co-infection rate of 9.0%. These co-infected farms were

Table 5 Associations of animal-level indicators with PRRSV and IAV seropositivity in pigs in Kazakhstan

Indicators	PRRSV seropositivity		IAV seropositivity	
	Unadjusted[a], Coef. (95%CI)	Adjusted[b], Coef. (95%CI)	Unadjusted[a], Coef. (95%CI)	Adjusted[b], Coef. (95%CI)
Age over 6 months	2.3 (0–4.9)	2.1 (−0.5 – 4.7)	0.01 (−0.5—0.5)	−0.02 (−0.5 – 0.5)
Commercial farming	6.3 (2.1–10.5)	4.2 (−1.2 – 9.7)	3.6 (1.8–5.5)	3.5 (1.5–5.4)
Respiratory (and reproductive for PRRSV) disorders	3.3 (0–7.9)	0	−0.12 (−1.2 – 0.94)	−0.2 (−1.3 – 0.8)

[a] Logistic regression coefficients from the unadjusted models estimates the crude relationship between the given independent and dependent variables

[b] Logistic regression coefficients from the adjusted models estimates the relationship between the given independent and dependent variables, adjusted for all of the other independent variables

Table 6 Apparent prevalence of antibodies to IAV subtypes H3 N2 and H1 N1 in pigs, Kazakhstan, 2019

Oblast	Total animals tested	No. seropositive animals			No. seronegative animals (%)
		H1 N1 (%)	H3 N2 (%)	Double positive	
Akmola	27	25 (92.6%)	0	1 (3.7%)	1 (3.7%)
Jetisu	2	0	0	0	2 (100%)
East Kazakhstan	26	5 (19.2%)	10 (38.5%)	4 (15.4%)	7 (26.9%)
Karagandy	158	12 (7.6%)	19 (12.0%)	43 (27.2%)	84 (53.2%)
North Kazakhstan	26	5 (19.2%)	0	0	21 (80.8%)
Pavlodar	147	3 (2.0%)	24 (16.3%)	37 (25.2%)	83 (56.5%)
Total	**386**	50 (13.0%)	53 (13.7%)	85 (22.0%)	198 (51.3%)

located in the Pavlodar, East-Kazakhstan, and Karagandy oblasts (Table 7). A total of 6 and 232 animals were found double seropositive for CSFV/SIV and PRRSV/SIV, respectively. Farms co-infected with all three pathogens – CSFV/PRRSV/SIV—were not identified.

Discussion

ASFV, CSFV, and PRRSV are significant threats to pigs and pig industries worldwide, while IAV represents a major risk not only to pigs and birds but also to human populations [62–65].

Following the successful eradication of ASF in Europe by the late twentieth century, ASF reappeared in Georgia in 2007 and spread throughout Eurasia with the highly virulent genotype II [66–71]. Although genotype II continues to be prevalent in Asia and Russia, variants with lower virulence have since emerged, including moderate virulence strains in Armenia (2011), Russia (2013–2018) and China (2020–2021), sons [72–76]. Kazakhstan shares extensive borders with both the Russian Federation and China and maintains significant economic ties with these countries, heightening the risk of ASF introduction. The introduction of ASF remains possible through several routes, including wild boar movements, illegal animal transportation, unauthorized meat importation, and human-mediated spread via contaminated equipment, clothing, or vehicles [77–80]. Our molecular screening results showed that at the time of sampling no animals were infected with ASFV. The interpretation of serological data is more nuanced. Pigs infected with highly virulent strains often die before producing a significant antibody response, making serology less informative in such cases. However, in regions where lower-virulence ASFV strains circulate, serological testing is essential for identifying recovered or infected animals [81]. Since no seropositive pigs were found in the surveyed areas and no ASF outbreaks or mass pig and wild boar deaths have been reported in Kazakhstan [18], our findings suggest that the circulation of highly virulent or low- to moderate-virulence ASFV strains among domestic pigs in these regions in 2019 was highly unlikely.

Outbreaks of CSF have not been officially reported in Kazakhstan [82]. Moreover, as of September 13, 2024, the World Assembly of Delegates reinstated Kazakhstan's status as a'country free of CSF' [83]. Molecular screening conducted in this study found no evidence of active CSFV infection in pigs in 2019. For comparison, rates of infection with CSFV in China were found to be 12.3% in 2019–2021 [84]. Serological analysis showed that seropositive animals were primarily found on farms utilizing CSFV-modified live vaccines. Unvaccinated seropositive animals were only identified on a single farm in

Table 7 Proportion of co-infections among unvaccinated swine farms

Oblast	Province	Co-infection (No. of positive farms/No. of unvaccinated farms)	
		CSF/SIV	PRRSV/SIV
Akmola	Atbasar	0/1	0/1
	Sandyktau	0/1	0/1
	Total in the oblast	*0/2*	*0/2*
Jetisu	Aksu	0/1	0/1
	Alakol	0/4	0/4
	Sarkand	0/9	0/9
	Tekeli	0/1	0/1
	Karatal	0/2	0/2
	Total in the oblast	*0/17*	*0/17*
East Kazakhstan	Altai	0/10	0/10
	Oskemen city	2/2	-
	Ulan	0/1	1/1
	Katon-Karagay	0/5	0/5
	Total in the oblast	*2/18*	*1/16*
Abai	Kokpekti	0/11	0/11
	Urzhar	0/7	0/7
	Total in the oblast	*0/18*	*0/18*
Karagandy	Abay	-	1/1
	Bukhar-Zhyrau	-	1/2
	Total in the oblast	*0/0*	*2/3*
North Kazakhstan	Esil	-	0/1
	Gabit Musirepov	0/2	0/2
	Magzhan Zhumabaev	0/4	0/4
	Shal Akyn	0/1	0/1
	Taiynsha	0/3	0/4
	Aiyrtau	0/2	0/3
	Kyzylzhar	0/1	0/1
	Total in the oblast	*0/13*	*0/13*
Pavlodar	Pavlodar district and Pavlodar city	-	2/3
	Terenkol (Kashyr)	-	0/1
	Zhelezin	-	0/1
	Aksu	0/1	1/1
	Sharbakty	-	1/2
	Uspen	0/1	0/1
	Total in the oblast	*0/2*	*4/9*
TOTAL		**2/70 (3.0%)**	**7/78 (9.0%)**

Oskemen city, which had no history of vaccination. This raises concerns about the possible circulation of wild strains of CSFV in the eastern region of Kazakhstan, an area in close proximity to China, where CSF still remains endemic [85, 86]. To substantiate this speculation, the identification of field strains of CSFV is imperative. Another possible explanation for the seropositive animals in Oskemen city is the cross-reactivity of antibodies to CSFV with other pestiviruses, such as border disease virus (BDV) and bovine viral diarrhea virus (BVDV) [87, 88]. Although BDV has not been reported in Kazakhstan, BVDV has been documented in the country [89].

PRRSV antibodies were detected in animals on both vaccinated and unvaccinated farms. Vaccination was implemented on six commercial farms, where the seroprevalence was significantly higher at 66.3%, compared to 16.6% on unvaccinated farms. Among the 493 animals on the vaccinated farms, reliable vaccination

records were available for only 178 animals, yet 327 were seropositive. This suggests that the high seroprevalence on vaccinated farms likely resulted from exposure to vaccine-derived strains of PRRSV rather than natural infection. Supporting this hypothesis, RNA of PRRSV-1 of European origin was detected in 17 animals on two vaccinated farms. Phylogenetic analysis of the GP5 gene revealed that the PRRSV-1 strains identified in this study were closely related to the subtype 1 (global) strains of PRRSV-1 and clustered with the DV isolate from the Porcilis PRRS vaccine (MSD Animal Health, Netherlands). This live-attenuated vaccine is used in Kazakhstan. None of the RT-qPCR-positive pigs had records of vaccination, suggesting that the strain was transmitted from vaccinated to unvaccinated animals within the herds. Efficient transmission of PRRSV vaccine strains to contact pigs has been demonstrated in previous studies. Eclercy et al. (2021) investigated the transmission and evolution of the DV vaccine strain, reporting that all contact pigs became infected, with viremia levels varying by vaccine batch [90]. Reversion to a virulent state has been documented for other PRRSV vaccines as well [91–99]. It is noteworthy that 20 of the 40 animals with respiratory signs sampled in this study, although not testing positive for PRRSV, originated from herds in which PRRSV RT-qPCR-positive animals were identified. These clinical signs may be associated with PRRSV-1 infection occurring outside the viremic window or may reflect other respiratory infections, potentially facilitated by the immunosuppressive effects of PRRSV.

PRRSV-seropositive animals were also detected on unvaccinated farms in northern, eastern, and central Kazakhstan, suggesting exposure to either vaccine or field strains of the virus. Both PRRSV-1 and PRRSV-2 are known to circulate in neighboring countries. In Russia, PRRSV-1 subtypes 1 ("Russian subtype") and 2 are predominant, with occasional reports of PRRSV-2 [100–103]. In China, both PRRSV-1 and PRRSV-2 are prevalent [104]. Although Kazakhstan does not import breeding animals from China, it does source them from Russia, which increases the risk of PRRSV strains being introduced by infected pigs [105, 106]. The PRRSV strains identified in this study showed low homology with Russian PRRSV-1 subtype 1 isolates, ranging from 77.7% (Tyu16 strain) to 80.4% (VR strain), and with subtype 2 isolates, ranging from 79.5% (KZ-2) to 81.8% (BLG strain). This genetic divergence suggests that PRRSV introduction from Russia could pose challenges, even for vaccinated animals in Kazakhstan, as PRRSV-modified live vaccines provide only partial protection against heterologous strains [107–109].

PRRSV seroprevalence among animals on unvaccinated farms was associated with commercial farming as opposed to backyard farms in an adjusted random effects model. These findings align with studies linking larger herd sizes to a higher risk of PRRSV infection [110, 111]. Large-scale farms, characterized by high pig densities and frequent animal imports, create conditions that facilitate the introduction and transmission of the virus. In contrast, small-scale and backyard farms, typically family-owned and focused on local consumption, import fewer animals, reducing the risk of disease introduction. However, after adjusting for the variables 'age over 6 months' and 'respiratory and reproductive disorders', the association between farm size and PRRSV seropositivity was no longer statistically significant, suggesting that other risk factors may be confounding the observed relationship.

The high SIV antibody prevalence (> 50%) in northern and central Kazakhstan indicates substantial IAV exposure. Detection of both H1 and H3 antibodies suggests co-circulation of both subtypes in these regions. The obtained serological results are in line with the molecular screening findings reported by Lukmanova et al. (2024). The authors detected RNA of H3 N2 and H1 N1 IAV in pig samples collected in 2019 in northern and central Kazakhstan [49]. Similarly, H1 N1 and H3 N2 subtypes are the prevalent SIVs in the neighbouring China with average seropositive frequencies of 31.1% and 28.6%, respectively [112]. A later study demonstrated a total SIV seroprevalence of 48.8% in unvaccinated pigs in China [113]. However, a considerably lower seroprevalence of less than 10% was detected in pigs in Russia, as shown by the hemagglutination inhibition assay [114].

Interestingly, 22% of the animals tested were double seropositive for antibodies against both H1 and H3 subtypes of IAV. These animals were found on farms where single seropositive animals for both H1 and H3 subtypes were also detected, suggesting the simultaneous occurrence of both subtypes within the same farm. Previous studies have documented co-infections with H1 N1 and H3 N2 in pigs under natural, non-experimental conditions as well as in humans [115, 116]. Such co-infections in pig populations have been shown to promote the reassortment of IAV strains, leading to the rapid emergence of viruses with novel genetic properties [115]. Also, in our study, about half of the animals that tested positive for IAV antibodies could not be serotyped. The most likely explanation is that the ELISA kits used for serotyping H1 and H3 antibodies were manufactured in the USA and may have a higher sensitivity toward American SIV strains and low effectiveness in detecting antibodies against Eurasian strains. Secondly, the presence of other IAVs than H1 N1 and H3 N2 reassortants could account for the

untyped samples. Since the identification of IAV subtypes primarily relies on serological reactions to the hemagglutinin subtype and, to a lesser extent, to the neuraminidase subtype [117], we used the Swinecheck® H3 N2 and H1 N1 ELISA kits (Biovet, USA) for serotyping according to the hemagglutinin subtype. However, the Swinecheck® H1 N1 ELISA kit (Biovet, USA) may have higher sensitivity for detecting antibodies against seasonal H1 N1 and lower sensitivity for identifying antibodies against H1 N2 or the distinct pandemic H1 N1pdm09 IAV. Notably, the circulation of H1 N1pdm09 has previously been documented in pigs, dogs, and humans in Kazakhstan [118–121].

H1 N1 IAV is uncommon in wild birds in Central Asia, with H3 N8 and H13 N6 being the dominant IAV subtypes in Kazakhstan, while the triple reassortant H3 N2 remains unreported in birds in the region [44, 46, 48, 122]. Epidemiological data obtained in this study showed higher odds of IAV infection in pigs raised indoors on commercial farms compared to backyard farms, where contact with wild birds is more probable. This suggests that the infection on commercial farms was introduced either through the purchase of infected pigs or by infected humans rather than wild birds. Accordingly, the 2018–2019 influenza season in Kazakhstan was dominated by H3 N2 and H1 N1 IAV subtypes circulating in humans [118]. These findings suggest that improved biosecurity measures, such as limiting farm access to essential personnel, vaccinating farm workers and their families against seasonal influenza, enforcing strict hygiene practices, and quarantining new animals, may help reduce SIV viral zoonotic transfer on farms in Kazakhstan [123].

All age groups of animals appeared to have the same risk of IAV infection, as this study found no association between age and IAV seroprevalence. This finding contrasts with other studies that indicate a higher risk of infection in younger pigs [124–127]. Respiratory and reproductive clinical signs were not positively associated with IAV or PRRSV antibodies in this study. While the reasons for this remain unclear, possible explanations include undetectable antibody levels in early infection stages or the involvement of other pathogens.

The analysis identified nine unvaccinated farms with animals seropositive for CSFV/SIV or PRRSV/SIV, highlighting the significance of multi-pathogen co-infections in pig herds. Co-infections, particularly with PRRSV and SIV, amplify respiratory pathology and are widespread in Europe and China, contributing to respiratory disease complexes [128–134]. These infections are challenging to diagnose due to overlapping clinical signs [134], risking further spread. Improved cross-pathogen diagnostics and surveillance are needed in the studied regions.

The study has a number of limitations. Transporting blood samples at −20 °C likely reduced viral RNA detection sensitivity. The use of blood samples for PRRSV RNA testing limited detection to animals with viremia, whereas nasal swabs or oral fluids would have been preferable. Molecular screening for IAV RNA was not conducted, leaving serological findings unsupported by direct virus detection. Additionally, uncertainty about the sensitivity and specificity of the Swinecheck® ELISA kits hindered accurate estimation of antibody prevalence. Further research is needed to analyze circulating viral genotypes and risk factors in domestic pigs and wild boar in the country.

Conclusions

The main conclusions obtained in this study can be summarized in the following points: 1) ASFV was not detected, nor was there evidence of exposure to moderate virulace ASFV strains, in pigs from central, northern, and eastern Kazakhstan in 2019; 2) vaccine strains of PRRSV are circulating in domestic pigs, highlighting the need for better management of modified live vaccines; 3) CSF remains a significant threat in Kazakhstan due to the absence of a national vaccination program, with low vaccination rates among pigs; 4) poor farm biosecurity has led to significant SIV exposure, particularly in the northern and central regions, emphasizing the need for improved biosecurity measures to prevent bidirectional IAV transmission.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04784-y.

Supplementary Material 1. Supplementary Figure 1 Different conditions for rearing pigs in Kazakhstan. Top left –pigs in a private backyard, bottom left – free-range pigs, right – pigs on a large commercial farm.

Supplementary Material 2. Supplementary Table 1. Details of ELISA kits and qPCR/RT-qPCR used in this study.

Supplementary Material 3. Supplementary Table 2. The PRRSV-1 and PRRSV-2 GP5 reference sequences.

Acknowledgements

We are grateful to the veterinarians who facilitated the farm visits. We are thankful for the farm managers/owners who agreed to participate in the study. We also extend our gratitude to Esri for providing ArcMap 5.0, which was instrumental in creating the maps in this work.

Declaration of Generative AI and AI-assisted technologies in the writing process:

We are grateful to the veterinarians who facilitated the farm visits. We are thankful for the farm managers/owners who agreed to participate in the study. We also extend our gratitude to Esri for providing ArcMap 5.0, which was instrumental in creating the maps in this work.

Authors' contributions

Y.V.P. interpreted data, performed the statistical analysis and wrote the manuscript; E.R.M. participated in funding acquisition, conceptualized the study, collected samples, revised the final version of the manuscript; A.V.Z., A.S.C.,

D.A.N., Z.A.B. designed the study, collected samples and performed experimental work; Y.O.O. edited the final version of the manuscript and performed experimental work; A.O.B., T.K., S.A.K., G.A.I. collected samples and performed experimental work; S.M.M., Y.A.S., A.M.D. conceptualized and designed the study, participated in funding acquisition; A.M.D. and D.H.L. conceptualized the study; G.R.R. designed the study and drafted the manuscript. All authors read and approved the final manuscript.

Funding
This research was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan (program number BR24992948) and the US Defense Threat Reduction Agency (Project KZ-35_Cooperative Biological Research "Monitoring of Highly Pathogenic Swine Fever viruses in Kazakhstan").

Data availability
The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the local institutional ethics committee of the National Center for Biotechnology, Astana, Kazakhstan. The owners/managers of the participating farms have given verbal informed consent for their participation in the study. All experiments were performed in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Almaty Branch of the National Center for Biotechnology, 14 Zhahanger St., Almaty 050054, Kazakhstan. [2]Department of Pathobiology and Veterinary Science, Connecticut Veterinary Medical Diagnostic Laboratory, College of Agriculture, Health and Natural Resources, University of Connecticut, 61 N. Eagleville Road, Unit 3089, Storrs, CT 06269-3089, USA. [3]Wildlife/One Health Lab, College of Veterinary Medicine, Konkuk University, Seoul, South Korea.

Received: 25 June 2024 Accepted: 23 April 2025
Published online: 28 May 2025

References

1. Bureau of National Statistics A for SP and R of the R of Kazakhstan. Key metrics for livestock development in the Republic of Kazakhstan. . Accessed 22 Nov 2024.
2. Meng XJ, Lindsay DS. Wild boars as sources for infectious diseases in livestock and humans. Biological Sciences: Philosophical Transactions of the Royal Society B; 2009. p. 364.
3. Kamakawa A, Thu HTV, Yamada S. Epidemiological survey of viral diseases of pigs in the Mekong delta of Vietnam between 1999 and 2003. Vet Microbiol. 2006;118(1–2):47–56.
4. Ribbens S, Dewulf J, Koenen F, Laevens H, De Kruif A. Transmission of classical swine fever. A review. Vet Quarterly. 2004;26(4):146–55.
5. Moennig V. The control of classical swine fever in wild boar. Front Microbiol. 2015;6:1211.
6. Forestry and Wildlife Committee of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan. Assessed December 2023.
7. Brown VR, Miller RS, Pepin KM, Carlisle KM, Cook MA, Vanicek CF, et al. African swine fever at the wildlife-livestock interface: challenges for management and outbreak response within invasive wild pigs in the United States. Front Vet Sci. 2024;11:1348123.
8. Martínez-López B, Ivorra B, Ramos AM, Fernández-Carrión E, Alexandrov T, Sánchez-Vizcaíno JM. Evaluation of the risk of classical swine fever (CSF) spread from backyard pigs to other domestic pigs by using the spatial stochastic disease spread model Be-FAST: The example of Bulgaria. Vet Microbiol. 2013;165(1–2):79–85.
9. Beltran-Alcrudo D, Falco JR, Raizman E, Dietze K. Transboundary spread of pig diseases: The role of international trade and travel. BMC Vet Res. 2019;15(1):64.
10. Sánchez-Vizcaíno JM, Mur L, Gomez-Villamandos JC, Carrasco L. An update on the epidemiology and pathology of African swine fever. J Comp Pathol. 2015;152(1):9–21.
11. Schulz K, Staubach C, Blome S. African and classical swine fever: Similarities, differences and epidemiological consequences. Vet Res. 2017;48(1):84.
12. Hu L, Lin XY, Yang ZX, Yao XP, Li GL, Peng SZ, et al. A multiplex PCR for simultaneous detection of classical swine fever virus, African swine fever virus, highly pathogenic porcine reproductive and respiratory syndrome virus, porcine reproductive and respiratory syndrome virus and pseudorabies in swines. Pol J Vet Sci. 2015;18.
13. Alonso C, Borca M, Dixon L, Revilla Y, Rodriguez F, Escribano JM. ICTV virus taxonomy profile: Asfarviridae. J Gen Virol. 2018;99(5):613–4.
14. Salguero FJ. Comparative pathology and pathogenesis of African swine fever infection in swine. Front Veter Sci. 2020;7:282.
15. Ward MP, Tian K, Nowotny N. African Swine Fever, the forgotten pandemic. Transboundary and Emerging Diseases. 2021;68.
16. Juszkiewicz M, Walczak M, Woźniakowski G, Podgórska K. African Swine Fever: Transmission, Spread, and Control through Biosecurity and Disinfection, Including Polish Trends. Viruses. 2023;15(11):2275.
17. Denstedt E, Porco A, Hwang J, Nga NTT, Ngoc PTB, Chea S, et al. Detection of African swine fever virus in free-ranging wild boar in Southeast Asia. Transbound Emerg Dis. 2021;68(5):2669–75.
18. World Organization for Animal Health. African swine fever.
19. CABI. African swine fever. CABI Compendium. 2022.
20. Federal Service for Veterinary and Phytosanitary Surveillance of the Russian Federation. African swine fever. . Accessed 30 Nov 2024.
21. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, et al. ICTV virus taxonomy profile: Flaviviridae. J Gen Virol. 2017;98(1):2–3.
22. Blome S, Staubach C, Henke J, Carlson J, Beer M. Classical swine fever—an updated review. Viruses. 2017;9(4):86.
23. Hinojosa Y, Liniger M, García-Nicolás O, Gerber M, Rajaratnam A, Muñoz-González S, et al. Evolutionary-Related High- and Low-Virulent Classical Swine Fever Virus Isolates Reveal Viral Determinants of Virulence. Viruses. 2024;16(1):147.
24. Chander V, Nandi S, Ravishankar C, Upmanyu V, Verma R. Classical swine fever in pigs: Recent developments and future perspectives. Anim Health Res Rev. 2014;15(1):87–101.
25. Luo Y, Li S, Sun Y, Qiu HJ. Classical swine fever in China: A minireview. Vet Microbiol. 2014;172(1–2):1–6.
26. Blome S, Meindl-Böhmer A, Loeffen W, Thuer B, Moennig V. Assessment of classical swine fever diagnostics and vaccine performance. OIE Revue Scientifique et Technique. 2006;25(3):1025–38.
27. Greiser-Wilke I, Moennig V. Vaccination against classical swine fever virus: limitations and new strategies. Anim Health Res Rev. 2004;5(2):223–6.
28. Coronado L, Perera CL, Rios L, Frías MT, Pérez LJ. A critical review about different vaccines against classical swine fever virus and their repercussions in endemic regions. Vaccines. 2021;9(2):154.
29. Information and legal system of regulatory legal acts of the Republic of Kazakhstan "Adilet." Decree of the Minister of Agriculture of the Republic of Kazakhstan "On approval of regulatory legal acts in the field of veterinary medicine" is available at No. 7–1/559 of 30 Oct 2014. Accessed 13 Nov 2024.
30. CABI Digital Library. Classical swine fever distribution map. Accessed 13 Nov 2024.
31. Brinton MA, Gulyaeva AA, Balasuriya UBR, Dunowska M, Faaberg KS, Goldberg T, et al. ICTV virus taxonomy profile: Arteriviridae 2021. J Gen Virol. 2021;102(8):001632.
32. Kimman TG, Cornelissen LA, Moormann RJ, Rebel JMJ, Stockhofe-Zurwieden N. Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine. 2009;27(28):3704–18.
33. Kuhn JH, Lauck M, Bailey AL, Shchetinin AM, Vishnevskaya TV, Bào Y, et al. Reorganization and expansion of the nidoviral family Arteriviridae. Arch Virol. 2016;161:755–68.

34. Rimayanti R, Khairullah AR, Lestari TD, Hernawati T, Mulyati S, Utama S, et al. Porcine reproductive and respiratory syndrome developments: An in-depth review of recent findings. Open Veterinary Journal. 2024;14:2138–52.
35. Dortmans JCFM, Buter GJ, Dijkman R, Houben M, Duinhof TF. Molecular characterization of type 1 porcine reproductive and respiratory syndrome viruses (PRRSV) isolated in the Netherlands from 2014 to 2016. PLoS One. 2018;14.
36. Risser J, Ackerman M, Evelsizer R, Wu S, Kwon B, Hammer JM. Porcine reproductive and respiratory syndrome virus genetic variability a management and diagnostic dilemma. Virol J. 2021;18:1–2.
37. Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, et al. Emergence of Fatal PRRSV Variants: Unparalleled Outbreaks of Atypical PRRS in China and Molecular Dissection of the Unique Hallmark. PLoS One. 2007;2(6):e526.
38. Li J, Gong W, Mao L, Pan X, Wu Q, Guo Y, et al. Molecular epizootiology of porcine reproductive and respiratory syndrome virus in the Xinjiang Uygur Autonomous Region of China. Front Microbiol. 2024;15(15):1419499.
39. Li C, Zhao J, Li W, Xu H, Gong B, Sun Q, et al. Prevalence and genetic evolution of porcine reproductive and respiratory syndrome virus in commercial fattening pig farms in China. Porcine Health Manag. 2024;10(1):5.
40. Tabynov K, Sansyzbay A, Tulemissova Z, Tabynov K, Dhakal S, Samoltyrova A, et al. Inactivated porcine reproductive and respiratory syndrome virus vaccine adjuvanted with Montanide™ Gel 01 ST elicits virus-specific cross-protective inter-genotypic response in piglets. Vet Microbiol. 2016;192:81–9.
41. Chauhan RP, Gordon ML. A systematic review analyzing the prevalence and circulation of influenza viruses in swine population worldwide. Pathogens. 2020;9(5):355.
42. Cheung J, Bui AN, Younas S, Edwards KM, Nguyen HQ, Pham NT, et al. Long-term epidemiology and evolution of swine influenza viruses Vietnam. Emerg Infect Dis. 2023;29(7):1397.
43. Petro-Turnquist E, Pekarek MJ, Weaver EA. Swine influenza A virus: challenges and novel vaccine strategies. Front Cell Infect Microbiol. 2024;14:1336013.
44. Marchenko VY, Alekseev AY, Sharshov KA, Petrov VN, Silko NY, Susloparov IM, et al. Ecology of influenza virus in wild bird populations in central Asia. Avian Dis. 2012;56(1):234–7.
45. Fereidouni S, Starick E, Karamendin K, Di Genova C, Scott SD, Khan Y, et al. Genetic characterization of a new candidate hemagglutinin subtype of influenza A viruses. Emerg Microbes Infect. 2023;12(2):2225645.
46. Kydyrmanov A, Sayatov M, Karamendin K, Zhumatov K, Asanova S, Daulbayeva K, et al. Monitoring of influenza A viruses in wild bird populations in Kazakhstan in 2002–2009. Arch Virol. 2017;162:147–55.
47. Amirgazin A, Shevtsov A, Karibayev T, Berdikulov M, Kozhakhmetova T, Syzdykova L, et al. Highly pathogenic avian influenza virus of the A/H5N8 subtype, clade 2.3.4.4b, caused outbreaks in Kazakhstan in 2020. PeerJ. 2022;10:e13038.
48. Sultankulova KT, Dzhekebekov KK, Orynbayev MB, Burashev YD, Melisbek AM, Barmak SM, et al. Evidence for flock transmission of individual subtypes and strains of avian influenza viruses: A monitoring study of wild birds in Kazakhstan. Virus Res. 2022;320:198898.
49. Lukmanova G, Klivleyeva N, Glebova T, Ongarbayeva N, Shamenova M, Saktaganov N, et al. Influenza A virus surveillance in domestic pigs in Kazakhstan 2018–2021. Ciencia Rural. 2024;54(10):e20230403.
50. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research – Third printing of the Second Edition | Ian Dohoo | Wayne Martin | Henrik Stryhn. 2009.
51. Otte MJ, Gumm ID. Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. Prev Vet Med. 1997;31.
52. Kleiboeker SB, Schommer SK, Lee SM, Watkins S, Chittick W, Polson D. Simultaneous detection of North American and European porcine reproductive and respiratory syndrome virus using real-time quantitative reverse transcriptase-PCR. J Vet Diagn Invest. 2005;17(2):165–70.
53. Wernike K, Hoffmann B, Dauber M, Lange E, Schirrmeier H, Beer M. Detection and typing of highly pathogenic porcine reproductive and respiratory syndrome virus by multiplex real-time RT-PCR. PLoS One. 2012;7(6):e38251.
54. Gerber PF, O'Neill K, Owolodun O, Wang C, Harmon K, Zhang J, et al. J Clin Microbiol. 2013;51(2):547–56.
55. Li Q, Domig KJ, Ettle T, Windisch W, Mair C, Schedle K. Evaluation of potential reference genes for relative quantification by RT- qPCR in different porcine tissues derived from feeding studies. Int J Mol Sci. 2011;12(3):1727–34.
56. Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5):1792–7.
57. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(1):1547–9.
58. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111–20.
59. Dean AG, Arner TG, Sunki G, Friedman R, Lantinga M, Sangam S, et al. Epi Info™, a database and statistics program for public health professionals. 2011.
60. StataCorp. Stata Statistical Software:Release 14. Stata Statistical Software. 2015.
61. Rogan WJ, Gladen B. Estimating prevalence from the results of a screening test. Am J Epidemiol. 1978;107(1):71–6.
62. Pileri E, Mateu E. Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination. Vet Res. 2016;47(1):108.
63. Casal J, Tago D, Pineda P, Tabakovski B, Santos I, Benigno C, et al. Evaluation of the economic impact of classical and African swine fever epidemics using OutCosT, a new spreadsheet-based tool. Transbound Emerg Dis. 2022;69(5):e2474-84.
64. Haden CB, Painter T, Fangman T, Holtkamp D. Assessing production parameters and economic impact of swine influenza, PRRS and Mycoplasma hyopneumoniae on finishing pigs in a large production system. Proceedings of the 2012 American Association of Swine Veterinarians Annual Meeting. 2012.
65. Roberts NJ, Krilov LR. The continued threat of influenza a viruses. Viruses. 2022;14(5):883.
66. Blome S, Franzke K, Beer M. African swine fever – A review of current knowledge. Virus Res. 2020;287:198099.
67. Rowlands RJ, Michaud V, Heath L, Hutchings G, Oura C, Vosloo W, et al. African swine fever virus isolate, Georgia, 2007. Emerg Infect Dis. 2008;14(12):1870.
68. Ruedas-Torres I, Thi to Nga B, Salguero FJ. Pathogenicity and virulence of African swine fever virus. Virulence. 2024;15.
69. Sánchez-Cordón PJ, Montoya M, Reis AL, Dixon LK. African swine fever: A re-emerging viral disease threatening the global pig industry. Vet J. 2018;233:41–8.
70. Zhou L, Yu EYW, Wang S, Sun C. African swine fever epidemic in China. Veterinary Record. 2019;184.
71. Chapman DAG, Darby AC, da Silva M, Upton C, Radford AD, Dixon LK. Genomic analysis of highly virulent Georgia 2007/1 isolate of African swine fever virus. Emerg Infect Dis. 2011;17(4):599.
72. Chen S, Wang T, Luo R, Lu Z, Lan J, Sun Y, et al. Genetic variations of african swine fever virus: major challenges and prospects. Viruses. 2024;16(6):913.
73. Sargsyan MA, Voskanyan HE, Karalova EM, Hakobyan LH, Karalyan ZA. Third wave of African swine fever infection in Armenia: Virus demonstrates the reduction of pathogenicity. Vet World. 2018;11(1):5.
74. Pershin A, Shevchenko I, Igolkin A, Zhukov I, Mazloum A, Aronova E, et al. A long-term study of the biological properties of ASF virus isolates originating from various regions of the russian federation in 2013–2018. Vet Sci. 2019;6(4):99.
75. Sun E, Zhang Z, Wang Z, He X, Zhang X, Wang L, et al. Emergence and prevalence of naturally occurring lower virulent African swine fever viruses in domestic pigs in China in 2020. Sci China Life Sci. 2021;64:752–65.
76. Sun E, Huang L, Zhang X, Zhang J, Shen D, Zhang Z, et al. Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection. Emerg Microbes Infect. 2021;10(1):2183–93.
77. Goicolea T, Cisneros-Araújo P, Vega CA, Sánchez-Vizcaíno JM, Mateo-Sánchez Mc, Bosch J. Landscape connectivity for predicting the spread of ASF in the European wild boar population. Sci Rep. 2024;14.

78. Hsu CH, Schambow R, Montenegro M, Miclat-Sonaco R, Perez A. Factors affecting the spread, diagnosis, and control of African swine fever in the Philippines. Pathogens. 2023;12(8):1068.
79. Mauroy A, Depoorter P, Saegerman C, Cay B, De Regge N, Filippitzi ME, et al. Semi-quantitative risk assessment by expert elicitation of potential introduction routes of African swine fever from wild reservoir to domestic pig industry and subsequent spread during the Belgian outbreak (2018–2019). Transbound Emerg Dis. 2021;68(5):2761–73.
80. Cheng J, Ward MP. Risk factors for the spread of African Swine Fever in China: A systematic review of Chinese-language literature. Transbound Emerg Dis. 2022;69(5):e1289-98.
81. Gaudreault NN, Madden DW, Wilson WC, Trujillo JD, Richt JA. African Swine Fever Virus: An Emerging DNA Arbovirus. Front Vet Sci. 2020;7:215.
82. Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan. Accessed 5 Dec 2024.
83. World Organisation for Animal Health. Classical swine fever. Assessed Nov 30, 2024.
84. Tang Q, Ge L, Tan S, Zhang H, Yang Y, Zhang L, et al. Epidemiological survey of four reproductive disorder associated viruses of sows in Hunan province during 2019–2021. Vet Sci. 2022;9(8):425.
85. Lu X, Ward MP. Spatiotemporal analysis of reported classical swine fever outbreaks in China (2005–2018) and the influence of weather. Transbound Emerg Dis. 2022;69(5):e3183-95.
86. Zhou B. Classical swine fever in China - An update minireview. Front Vet Sci. 2019;6:187.
87. Tao J, Liao J, Wang Y, Zhang X, Wang J, Zhu G. Bovine viral diarrhea virus (BVDV) infections in pigs. Vet Microb. 2013;165(3–4):185–9.
88. Dastjerdi A, Strong R, La Rocca SA, Wessels M, Wessels J, Whitaker K, et al. Investigation into an outbreak of Border disease virus in pigs in England. Transboundary and Emerging Diseases. 2022;69.
89. Zhigailov AV, Perfilyeva YV, Ostapchuk YO, Kan SA, Lushova AV, Kuligin AV, et al. Molecular and serological survey of bovine viral diarrhea virus infection in cattle in Kazakhstan. Res Vet Sci. 2023;162:104965.
90. Eclercy J, Renson P, Hirchaud E, Andraud M, Beven V, Paboeuf F, et al. Phenotypic and genetic evolutions of a porcine reproductive and respiratory syndrome modified live vaccine after limited passages in pigs. Vaccines (Basel). 2021;9(4):392.
91. Grebennikova TV, Clouser DF, Vorwald AC, Musienko MI, Mengeling WL, Lager KM, et al. Genomic characterization of virulent, attenuated, and revertant passages of a North American porcine reproductive and respiratory syndrome virus strain. Virology. 2004;321(2):383–90.
92. Khatun A, Shabir N, Seo B-J, Kim B-S, Yoon K-J, Kim W-I. The Attenuation phenotype of a ribavirin-resistant porcine reproductive and respiratory syndrome virus is maintained during sequential passages in pigs. J Virol. 2016;90(9):4454–68.
93. Eclercy J, Larcher T, Andraud M, Renson P, Bernard C, Bigault L, et al. PCV2 co-infection does not impact PRRSV MLV1 safety but enhances virulence of a PRRSV MLV1-like strain in infected SPF pigs. Vet Microbiol. 2020;244:108656.
94. Bøtner A, Strandbygaard B, Sørensen KJ, Have P, Madsen KG, Madsen ES, et al. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. Vet Rec. 1997;141(19):497–9.
95. Nielsen HS, Oleksiewicz MB, Forsberg R, Stadejek T, Bøtner A, Storgaard T. Reversion of a live porcine reproductive and respiratory syndrome virus vaccine investigated by parallel mutations. J Gen Virol. 2001;82(6):1263–72.
96. Woo-Taek Oh, Van-Giap Nguyen, Hyung-Joon Moon, Jee-Hoon Lee, Hye-Kwon Kim, Seong-Jun Park, et al. Investigation on Infectious Agents of Aborted Pig Fetuses and Its Correlation with PRRSV MLV Vaccine. J Agric Sci Technol A. 2017;7.
97. Zhou L, Ge X, Yang H. Porcine reproductive and respiratory syndrome modified live virus vaccine: A "leaky" vaccine with debatable efficacy and safety. Vaccines. 2021;9(4):362.
98. Charerntantanakul W. Porcine reproductive and respiratory syndrome virus vaccines: Immunogenicity, efficacy and safety aspects. World J Virol. 2012;1(1):23.
99. Zhou L, Yang B, Xu L, Jin H, Ge X, Guo X, et al. Efficacy evaluation of three modified-live virus vaccines against a strain of porcine reproductive and respiratory syndrome virus NADC30-like. Vet Microbiol. 2017;207:108–16.
100. Raev S, Yuzhakov A, Bulgakov A, Kostina L, Gerasianinov A, Verkhovsky O, et al. An outbreak of a respiratory disorder at a russian swine farm associated with the co-circulation of PRRSV1 and PRRSV2. Viruses. 2020;12(10):1169.
101. Yuzhakov AG, Raev SA, Skrylev AN, Mishin AM, Grebennikova TV, Verkhovsky OA, et al. Genetic and pathogenic characterization of a Russian subtype 2 PRRSV-1 isolate. Vet Microbiol. 2017;211:22–8.
102. Stadejek T, Oleksiewicz MB, Potapchuk D, Podgórska K. Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in eastern Europe support the definition of new genetic subtypes. J Gen Virol. 2006;87(7):1835–41.
103. Stadejek T, Stankevicius A, Murtaugh MP, Oleksiewicz MB. Molecular evolution of PRRSV in Europe: Current state of play. Vet Microbiol. 2013;165(1–2):21–8.
104. Sun Q, Xu H, An T, Cai X, Tian Z, Zhang H. Recent progress in studies of porcine reproductive and respiratory syndrome virus 1 in China. Viruses. 2023;15(7):1528.
105. Carlsson U, Wallgren P, Renström LHM, Lindberg A, Eriksson H, Thorén P, et al. Emergence of porcine reproductive and respiratory syndrome in Sweden: Detection, response and eradication. Transbound Emerg Dis. 2009;56(4):121–31.
106. Mortensen S, Stryhn H, Sogaard R, Boklund A, Stärk KDC, Christensen J, et al. Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus. Prev Vet Med. 2002;53(1–2):83–101.
107. Martelli P, Gozio S, Ferrari L, Rosina S, De Angelis E, Quintavalla C, et al. Efficacy of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: Clinical protection and cell-mediated immunity. Vaccine. 2009;27(28):3788–99.
108. Kiss I, Sámi L, Kecskeméti S, Hanada K. Genetic variation of the prevailing porcine respiratory and reproductive syndrome viruses occurring on a pig farm upon vaccination. Arch Virol. 2006;151:2269–76.
109. Scortti M, Prieto C, Simarro I, Castro JM. Reproductive performance of gilts following vaccination and subsequent heterologous challenge with European strains of porcine reproductive and respiratory syndrome virus. Theriogenology. 2006;66(8):1884–93.
110. Firkins LD, Weigel RM. A retrospective study of risk factors for porcine reproductive and respiratory syndrome virus infection and clinical disease in swine herds in Illinois during the early years of the pandemic. J Swine Health Prod. 2004;12(1):23–8.
111. Zhao P, Wang C, Cao W, Fang R, Zhao J. Risk factors and spatial-temporal analysis of porcine reproductive and respiratory syndrome SEROPREVALENCE in China before and after african swine fever outbreak. Front Vet Sci. 2022;9:929596.
112. Liu W, Wei MT, Tong Y, Tang F, Zhang L, Fang L, et al. Seroprevalence and genetic characteristics of five subtypes of influenza A viruses in the Chinese pig population: A pooled data analysis. Vet J. 2011;187(2):200–6.
113. Zhao Y, Han L, Chen T, Sang H, Ding G, Li Y, et al. Serological Surveillance of the H1N1 and H3N2 Swine Influenza A Virus in Chinese Swine between 2016 and 2021. Biomed Res Int. 2022;2022(1):5833769.
114. Danilenko DM, Komissarov AB, Fadeev AV, Bakaev MI, Ivanova AA, Petrova PA, et al. Antigenic and Genetic Characterization of Swine Influenza Viruses Identified in the European Region of Russia, 2014–2020. Front Microbiol. 2021;12:662028.
115. Abe H, Mine J, Parchariyanon S, Takemae N, Boonpornprasert P, Ubonyaem N, et al. Co-infection of influenza A viruses of swine contributes to effective shuffling of gene segments in a naturally reared pig. Virology. 2015;484:203–12.
116. Liu W, De Li Z, Tang F, Wei MT, Tong YG, Zhang L, et al. Mixed infections of pandemic H1N1 and seasonal H3N2 viruses in 1 outbreak. Clin Infect Dis. 2010;50(10):1359–65.
117. Chen X, Liu S, Goraya MU, Maarouf M, Huang S, Chen JL. Host immune response to influenza A virus infection. Front Immunol. 2018;9:320.
118. Klivleyeva NG, Ongarbayeva NS, Baimukhametova AM, Saktaganov NT, Lukmanova GV, Glebova TI, et al. Detection of influenza virus and pathogens of acute respiratory viral infections in population of Kazakhstan during 2018–2019 epidemic season. Russian J Infect Immun. 2021;11:137–47.
119. Shamenova MG, Glebova TI, Klivleyeva NG, Baiseiit SB, Baimukhametova AM, Saktaganov NT, et al. Serological studies of influenza infection

among population in southern region of Kazakhstan during the 2018–2021 epidemic season. J Pak Med Assoc. 2023;73(4):804–7.
120. Klivleyeva N, Lukmanova G, Glebova T, Shamenova M, Ongarbayeva N, Saktaganov N, et al. Spread of pathogens causing respiratory viral diseases before and during CoVID-19 Pandemic in Kazakhstan. Indian J Microbiol. 2023;63(1):129–38.
121. Glebova TI, Klivleyeva NG, Baimukhametova AM, Saktaganov NT, Lukmanova GV, Ongarbayeva NS, et al. Circulation of influenza viruses in the epidemic season of 2018–2019 among people residing in northern and western Kazakhstan. Infektsionnye Bolezni. 2021;19(2):70–5.
122. Munster VJ, Baas C, Lexmond P, Waldenström J, Wallensten A, Fransson T, et al. Spatial, temporal, and species variation in prevalence of influenza a viruses in wild migratory birds. PLoS Pathog. 2007;3(5):e61.
123. Salvesen HA, Whitelaw CBA. Current and prospective control strategies of influenza A virus in swine. Porcine Health Management. 2021;7:1–7.
124. Candotti P, Foni E, Leotti G, Joisel F, Longo S, Nodari SR. Serological prevalence for swine influenza virus in pigs between 3 and 15 weeks of age in italian farms: evaluation of a maternal antibody decay curve.
125. Markowska-Daniel I, Stankevicius A. Seroprevalence of antibodies against swine influenza virus in pigs of different age. Bull Vet Instit Pulawy. 2005;49(3):7.
126. Loeffen WLA, Nodelijk G, Heinen PP, Van Leengoed LAMG, Hunneman WA, Verheijden JHM. Estimating the incidence of influenza-virus infections in Dutch weaned piglets using blood samples from a cross-sectional study. Vet Microbiol. 2003;91(4):295–308.
127. Takemae N, Parchariyanon S, Ruttanapumma R, Hiromoto Y, Hayashi T, Uchida Y, et al. Swine influenza virus infection in different age groups of pigs in farrow-to-finish farms in Thailand. Virol J. 2011;8:1–1.
128. Bougon J, Deblanc C, Renson P, Quéguiner S, Gorin S, Mahé S, et al. Successive inoculations of pigs with porcine reproductive and respiratory syndrome virus 1 (PRRSV-1) and swine H1N2 influenza virus suggest a mutual interference between the two viral infections. Viruses. 2021;13(11):2169.
129. Zhao D, Yang B, Yuan X, Shen C, Zhang D, Shi X, et al. advanced research in porcine reproductive and respiratory syndrome virus co-infection with other pathogens in Swine. Front Vet Sci. 2021;8:699561.
130. Van Reeth K, Nauwynck H, Pensaert M. Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: A clinical and virological study. Vet Microbiol. 1996;48(3–4):325–35.
131. Choi YK, Goyal SM, Joo HS. Retrospective analysis of etiologic agents associated with respiratory diseases in pigs. Can Vet J. 2003;44(9):735.
132. Shi X, Liu X, Wang Q, Das A, Ma G, Xu L, et al. A multiplex real-time PCR panel assay for simultaneous detection and differentiation of 12 common swine viruses. J Virol Methods. 2016;236:258–65.
133. Jiménez LFM, Nieto GR, Alfonso VV, Correa JJ. Association of swine influenza H1N1 pandemic virus (SIV-H1N1p) with porcine respiratory disease complex in sows from commercial pig farms in Colombia. Virol Sin. 2014;29:242–9.
134. Chen Y, Luo S, Tan J, Zhang L, Qiu S, Hao Z, et al. Establishment and application of multiplex real-time PCR for simultaneous detection of four viruses associated with porcine reproductive failure. Front Microbiol. 2023;14:1092273.

Publisher's Note