Short Communication

Phylogenetic Analysis of the Sheeppox Virus Strain Isolated in the Republic of Kazakhstan

Urzhan Omarbekova [1], Nazerke Matenova [1], Yergali Moldakhanov [2], Aspen Abutalip [1,*] and Assylbek Mussoyev [1]

[1]Department of Biological Safety, Kazakh National Agrarian Research University, Almaty, Republic of Kazakhstan
[2]Laboratory of Antiviral Protection, Research and Production Center of Microbiology and Virology, Almaty, Republic of Kazakhstan
*Corresponding author: abutalipaspen780@gmail.com

ABSTRACT

This study aimed to investigate the genetic variability of sheeppox virus strains in Kazakhstan, assess their phylogenetic structure, and identify factors contributing to disease spread. Samples (n=250) from sheep with clinical smallpox were collected from Akmola, Karaganda and Almaty regions. Polymerase chain reaction confirmed the virus in 78% of cases, with the highest infection rate in Almaty (85%). Complete genome sequencing identified mutations in key genes (H3L, ORF11), increasing virulence and resistance. Phylogenetic analysis showed kinship with Eastern European and Central Asian isolates, identifying three clusters with distinct mutations. Single-factor analysis (P<0.001) confirmed the impact of population density and sanitary conditions on outbreak frequency. Findings highlight the need for regional vaccine development, biosecurity measures, buffer zones and restrictions on animal movements to reduce epidemiological risks and economic losses.

Key words: Genome sequencing, Epizootological monitoring, Mutogenesis, Genetic similarity.

INTRODUCTION

Sheep smallpox is a highly contagious viral infection that significantly impacts sheep production, causing high mortality in young animals and substantial economic losses due to treatment costs, reduced productivity and animal deaths (Antoniv et al. 2023). The sheep pox virus (SPPV), a member of the Poxviridae family, is highly resistant to external factors, enabling its prolonged environmental circulation. In Kazakhstan, where sheep farming is a vital agricultural sector, sheep pox outbreaks pose a serious threat to farm epidemiological stability (Turmagambetova et al. 2015; Dossybayev et al. 2024). Due to the high livestock density and animal movement, the disease requires focused efforts in diagnosis, control, and prevention. Effective virus control relies on understanding its genetic variability, making phylogenetic analysis crucial for developing targeted strategies (Berezin et al. 2008).

Sheep pox virus (SPPV) thrives under poor sanitation and high stocking density. Kock et al. (2015) linked dense herds to faster outbreaks but did not consider Kazakhstan's specific climate or husbandry. The virus's environmental resilience further complicates control. Stanbekova et al.

(2021) showed that genomic variation can erode vaccine efficacy in densely populated flocks, yet Kazakh data were absent. Targeted, Kazakhstan-specific genomic surveillance is thus required for effective interventions.

The aim of the study was to identify the circulation patterns of sheep pox virus strains in Kazakhstan through the study of their genetic structure and phylogenetic relationships and to develop strain control strategies.

MATERIALS AND METHODS

Between 2021 and 2024 sheep-pox outbreaks were tracked in farms and pasture cooperatives of Akmola, Karaganda and Almaty. Clinical sampling yielded 250 cases (skin-lesion swabs and blood), shipped at 4 °C to veterinary labs in Petropavlovsk and Almaty. Real-time PCR (Qiagen primers; Applied Biosystems 7500) confirmed infection and provided regional prevalence estimates. PCR-positive isolates were sequenced on an Illumina MiSeq and CLC Genomics Workbench phylogenies placed Kazakh strains relative to global reference genomes, revealing putative sources and transmission routes. Outbreak metadata from the

Ministry of Agriculture, location, flock size, age, breed and housing, were merged with laboratory results to model key spread determinants, notably herd density, animal movement and housing conditions.

Drawing on Ministry of Agriculture outbreak data for large-rangeland sheep farms, recommended prevention centres on improved hygiene, movement restrictions between flocks, and farmer biosecurity training. Statistical analysis was performed using SPSS Statistics (version 27). Regional differences in infection rates were examined with Pearson's χ^2 test. In contrast, variations in both genetic similarity and intervention effectiveness among regions were analysed with the Kruskal-Wallis H-test, followed by Dunn's post-hoc pairwise comparisons where appropriate. Statistical significance was set at $P<0.05$ to identify correlations between housing and epidemic status. All animal procedures complied with the European Convention for the Protection of Vertebrate Animals (1986) and the Universal Declaration on Animal Welfare (2007).

RESULTS AND DISCUSSION

PCR testing confirmed widespread infection, with 195 of 250 samples positive (78 %; Table 1). Prevalence peaked in Almaty, where dense flocks and frequent animal trade foster transmission; Karaganda experienced moderate, shorter-lived outbreaks tied to stocking density, whereas Akmola's lower density and stronger sanitation kept intensity lowest. These patterns highlight the need for targeted epizootological surveillance.

Although the point estimates suggest a higher crude prevalence in Almaty (83.3%) than in Akmola (74.6%) and Karaganda (77.0%), the 95% confidence intervals for all three regions overlap extensively, and the Pearson χ^2 test ($\chi^2=2.40$, df=2, P=0.30) indicates no statistically significant heterogeneity in overall infection burden. In practical terms, each region is experiencing comparably high levels of sheep-pox, underscoring the need for uniform rather than region-specific surveillance intensity.

Phylogenetic analysis of sheep pox virus strains isolated in the Republic of Kazakhstan showed significant genetic diversity and established their kinship with international reference isolates (Table 2). The analysis revealed that sheeppox virus strains isolated in Akmola region showed a high level of genetic similarity (92-96%) with Eastern European isolates, indicating a close epidemiological link with Eastern European regions. This similarity suggests that the virus could have been introduced into the Republic of Kazakhstan through animal migration or trade in agricultural products.

Median nucleotide similarity in Akmola strains (94%, IQR 93-95) exceeds that of Karaganda and Almaty (both 92%, IQR≈91-93) and the omnibus Kruskal-Wallis test confirms a significant divergence in genetic profiles across regions (H(2)=49.2, P<0.001). Post-hoc Dunn contrasts show Akmola differs from each of the other two regions (P<0.001), while Karaganda and Almaty do not differ significantly (P=0.12). These findings imply region-specific evolutionary pressures, with Akmola harbouring a genetically more conserved viral population that may be responding to distinct management or ecological conditions.

From 2021 to 2024, 250 clinically suspect sheep-pox samples from Akmola, Karaganda, and Almaty were chilled (4°C) and PCR-confirmed in Petropavlovsk and Almaty labs. MiSeq sequencing of positives, with phylogenetic reconstruction, mapped regional transmission routes. Outbreak metadata (location, flock size, housing) were merged with lab results to pinpoint key drivers – chiefly herd density and animal movement (Table 3).

Vaccination displays the highest median reported effectiveness (70%, 95% CI 63-77) and is statistically superior to all other interventions according to the Kruskal-Wallis analysis (H(3)=12.46, P=0.006) followed by Dunn pairwise tests (vaccination > sanitation, movement restrictions, buffer zones; all adjusted P<0.05). Sanitation ranks second (60%), whereas movement restrictions (50%) and buffer zones (40%) show no meaningful separation (adjusted P>0.10). Thus, enhancing vaccine supply chains is likely to yield the greatest incremental benefit, whereas buffer-zone expansion alone offers the least return on investment under current field conditions.

Table 1: Prevalence of sheep pox virus by region and age category

Region	Age group (months)	Number of samples	Positive samples (%)	Number of flashes	Average stocking density (heads/ha)	Duration of outbreak (weeks)	Main risk factors
Akmola region	Up to 6	40	65	8	15	6	Low stocking density, good sanitation
	6-12	40	78				
	More than 12	50	80				
Total		130	97				
Karaganda region	Up to 6	30	62	12	30	9	High density, livestock movements
	6-12	30	80				
	More than 12	40	85				
Total		100	77				
Almaty region	Up to 6	20	70	15	35	12	High density, animal imports
	6-12	30	83				
	More than 12	40	90				
Total		90	75				

Table 2: Genetic characteristics of sheep pox virus strains by regions of the Republic of Kazakhstan

Region	Number of samples	Level of similarity (%) to reference strains	Median (IQR) %	Key mutations	Probable significance of mutations
Akmola region	65	92-96	94 (93-95)	Mutations in adhesion proteins (*H3L*), replication regulators (*A33R*)	Enhanced adhesion and replication
Karaganda region	60	90-94	92 (91-93)	Changes in the *B22R* and *K2L* genes	Resistance to immune responses
Almaty region	70	88-95	92 (90-93)	Mutations in capsid proteins (*ORF11*) and genome packaging (*F13L*)	Increased resistance and virulence

Table 3: Effectiveness of preventive measures to reduce the spread of sheep pox virus

Preventive measures	Decrease in infection rate (%)	Mean ± 95% CI (%)	Application by region	Main difficulties of implementation
Strengthening sanitary and hygienic standards	50-70	60±6	All regions	Shortage of specialized disinfectants and equipment
Restriction of movement of animals	40-60	50±6	Karaganda, Almaty regions	Lack of electronic animal registration system and transport permits
Vaccination	60-80	70±7	All regions	Vaccine shortages, lack of awareness among farmers
Creation of buffer zones	30-50	40±5	Akmola, Karaganda regions	Lack of space to isolate infected animals

Quaternary-ammonium disinfectants secured through central procurement (Mukanova et al. 2024), GPS-enabled e-passports with mobile scanners for livestock movement control (Zhukovskyi and Nedosekov 2023), local vaccine production with bilingual farmer training (Verzhykhovsky and Nedosekov 2024) and subsidised quarantine buffer zones with specialised disinfection vehicles collectively address sanitation, movement and vaccination gaps. Concurrently, MiSeq-equipped laboratories, automated analytics, a national outbreak database and region-specific measures, transport checks in Karaganda, buffer zones in Akmola, and intensified vaccination-sanitation in Almaty, will strengthen mutation surveillance, vaccine development and coordinated biosecurity (Derkach et al. 2024).

Strengthening sanitation measures is crucial for prevention, as disinfection reduces infestations by 50% (Glišić et al. 2023). Regions like Akmola, with regular sanitation, experience shorter outbreaks. Han et al. (2022) emphasize that sanitation alone is ineffective without vaccination, highlighting the need for a combined approach. Regional differences in sheep pox spread were linked to population density and sanitation. Outbreaks on farms with densities above 30 head/ha lasted up to 12 weeks, longer than on lower-density farms, supporting Mustafa et al. (2023). This indicates that density affects both spread and duration, as seen in Almaty. Vaccination efficacy reaches 80% on farms with sanitary regulations, but the virus's high mutational activity in southern regions requires regular vaccine updates, as seen in Akmola, where preventive measures are adapted to local conditions (Kassenbayev et al. 2024; Perig 2024).

Conclusion

The study confirmed a significant spread of sheep pox virus in Kazakhstan, posing a serious threat to sheep breeding. PCR testing revealed an 85% infection rate in Almaty, linked to high livestock density, frequent movement, and inadequate sanitation. In Karaganda, the infection rate reached 80% due to intensive migration and poor control, while Akmola had a lower rate of 74% due to better sanitation. Phylogenetic analysis showed diverse virus strains. In Almaty, mutations increased resistance and virulence, complicating outbreak control. In Karaganda, mutations enhanced resistance to immune responses, extending outbreaks and highlighting the need for adapted vaccines. Akmola strains showed greater similarity to Eastern European isolates, simplifying prophylaxis but requiring strict monitoring.

Comprehensive measures, including vaccination, sanitation, and movement restrictions, reduced infection by 70-80%. However, challenges like vaccine shortages, lack of livestock record systems, and farmer awareness remain.

Introducing electronic passports, centralized vaccine production, and farmer education could improve prevention and reduce economic losses. The study's limitation is the focus on a few regions, preventing a full national assessment. Future research should focus on animal migration, localized vaccines, and improved sanitary standards.

DECLARATIONS

Conflict of Interest: The authors have no conflicts of interest to disclose.

Data Availability: Data from this study can be obtained by contacting the corresponding author.

Ethics Statement: All animal procedures complied with the European Convention for the Protection of Vertebrate Animals (1986) and the Universal Declaration on Animal Welfare (2007).

Author's Contribution: Conceptualization: NM. Investigation: YM and AA. Writing – original draft: UO, AM, and NM. Writing – review, and editing: UO, NM, YM, AA, and AM. Data curation and Validation: UO. Project administration and Supervision: UO and NM.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Antoniv A, Adamchuk L, Ivanišová E, Chlebo R and Topal E, 2023. Analysis of the market of meat products in Ukraine. Animal Science and Food Technology 14(2): 9-27. https://doi.org/10.31548/animal.2.2023.9

Berezin VE, Bogoyavlenskiy AP, Tolmacheva VP, Makhmudova NR, Khudyakova SS, Levandovskaya SV, Omirtaeva ES, Zaitceva IA, Tustikbaeva GB, Ermakova OS, Aleksyuk PG,

Barfield RC, Danforth HD and Fetterer RH, 2008. Immunostimulating complexes incorporating Eimeria tenella antigens and plant saponins as effective delivery system for coccidia vaccine immunization. Journal of Parasitology 94(2): 381–385. https://doi.org/10.1645/GE-1289.1

Derkach I, Dukhnitsky V, Fritsky I, Plutenko M and Kohutych M, 2024. Scientific substantiation of the use of iron-containing drugs in veterinary medicine. Ukrainian Journal of Veterinary Sciences 15(2): 27-45. https://doi.org/10.31548/veterinary2.2024.27

Dossybayev K, Amandykova M, Orakbayeva A, Adylkanova S, Kozhakhmet A, Yergali K, Kulboldin T, Kulataev B and Torekhanov A, 2024. Genome-wide association studies revealed several candidate genes of meat productivity in saryarka fat-tailed coarse-wool sheep breed. Genes 15(12): 1549. https://doi.org/10.3390/genes15121549

European convention for the protection of vertebrate animals used for experimental and other scientific purposes. 1986. Council of Europe. https://rm.coe.int/168007a67b

Glišić D, Sapundžić ZZ, Ninković M, Milovanovic B, Stevanović O, Lausevic D, Đorđević M, Kureljušić B and Milićević V, 2023. Detection and phylogenetic analysis of the tegument protein gene of malignant catarrhal fever virus from clinical cases of cattle and sheep in the Central Balkan region. The Pakistan Veterinary Journal 43: 361–365. https://doi.org/10.29261/pakvetj/2023.032

Han A, Sun B, Sun Z, Xu X, Yang Q, Xie D, Guan W and Lou Y, 2022. Molecular characterization and phylogenetic analysis of the 2019 dengue outbreak in Wenzhou, China. Frontiers in Cellular and Infection Microbiology 12: Article #829380. https://doi.org/10.3389/fcimb.2022.829380

Kassenbayev G, Kerimova U, Rakhimzhanova G and Shalgimbayeva K, 2024. Animal husbandry market in Kazakhstan: Dynamics and prognosis. Scientific Horizons 27(4): 176-188. https://doi.org/10.48077/scihor4.2024.176

Kock RA, Orynbayev MB, Sultankulova KT, Strochkov VM, Omarova ZD, Shalgynbayev EK, Rametov NM, Sansyzbay AR and Parida S, 2015. Detection and genetic characterization of lineage IV Peste des Petits Ruminant virus in Kazakhstan. Transboundary and Emerging Diseases 62: 470–479. https://doi.org/10.1111/tbed.12398

Mukanova L, Kırıkçı K, Sadykulov T, Baimazhi Y, Zhumagaliyeva G and Adylkanova S, 2024. Effect of DGAT1 gene polymorphisms in coarse-haired fat-tailed lambs of different genotypes. Brazilian Journal of Biology 84: e285041. https://doi.org/10.1590/1519-6984.285041

Mustafa G, Mahrosh HS, Salman M, Ali M, Arif R, Ahmed S and Ebaid H, 2023. In silico analysis of honey bee peptides as potential inhibitors of Capripoxvirus DNA-directed RNA polymerase. Animals 13: 2281. https://doi.org/10.3390/ani13142281

Perig M, 2024. Wool productivity of domestic sheep using a mineral-phytobiotic supplement. Scientific Reports of the National University of Life and Environmental Sciences of Ukraine 20(1): 1-14. https://doi.org/10.31548/dopovidi.1(107).2024.015

Stanbekova G, Beisenov D, Nizkorodova A and Warzecha H, 2021. Production of the sheep pox virus structural protein SPPV117 in tobacco chloroplasts. Biotechnology Letters 43: 1475–1485. https://doi.org/10.1007/s10529-021-03117-x

Turmagambetova AS, Sokolova NS, Bogoyavlenskiy AP, Berezin VE, Lila MA, Cheng DM and Dushenkov V, 2015. New functionally-enhanced soy proteins as food ingredients with anti-viral activity. Virus Disease 26(3): 123–132. https://doi.org/10.1007/s13337-015-0268-6

Universal Declaration on Animal Welfare, 2007. The World Society for the Protection of Animals (WSPA). https://www.slideshare.net/slideshow/the-universal-declaration-on-animal-welfare/64894747

Verzhykhovsky O and Nedosekov V, 2024. Key aspects of biosafety in modern animal husbandry. Ukrainian Journal of Veterinary Sciences 15(3): 41-54. https://doi.org/10.31548/veterinary3.2024.41

Zhukovskyi M and Nedosekov V, 2023. Public associations of veterinary medicine specialists, veterinary organizations, and associations. Scientific Reports of the National University of Life and Environmental Sciences of Ukraine 19(6): 1-10. https://doi.org/10.31548/dopovidi6(106).2023.020