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Research article

Cross-sectional study of camelpox virus in the western and southern regions of Kazakhstan in 2021-2022

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Abstract

Camelpox is a contagious disease of Old World camelids with limited prevalence data in Kazakhstan. This study determined camelpox virus (CMLV) prevalence and vaccination coverage in western and southern Kazakhstan in 2021-2022. Overall, 572 camels from 63 herds were sampled. Serum samples were tested for anti-CMLV antibodies by ELISA, blood and tissue samples were tested for CMLV DNA by PCR with sequencing of PCR-positive amplicons. The true adjusted animal-level seroprevalence in unvaccinated and vaccinated camels was 10.9% and 73.6%, respectively. All seropositive unvaccinated camels were from Atyrau oblast, where 30.8% (12/39; 95% CI: 17.0–47.6%) of unvaccinated herds contained at least one seropositive animal. CMLV DNA was detected in three (1.1%) unvaccinated seropositive camels from Atyrau oblast. Analysis of the complete C18L and ATI gene sequences revealed homology with the reference CMLV strain M-96 and high nucleotide identity (100% and 99.94%, respectively) with the CMLV strain isolated in Mangystau oblast in 2023. The findings indicate that a CMLV lineage – identical or closely related to strains documented in 1996 and again in 2023 in Mangystau oblast – was circulating in Atyrau oblast during 2021-2022. This suggests continued maintenance of the virus in western Kazakhstan and raises concerns about potential transboundary spread.

Keywords: Camelpox virus, Camel, Kazakhstan, Sequencing, Vaccination.

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INTRODUCTION

Camel breeding has had a special place in the agriculture of Kazakhstan since ancient times, as it meets the demand for meat, milk and wool in areas with desert and semi-desert climates. Nowadays, camel breeding is developing into a highly profitable industry in Kazakhstan (Akhmetsadykova et al., 2022). Camel operations can be found in all 17 oblasts of the country. On February 1, 2025, the total number of camels in the country amounted to 278,748 animals, with the largest concentration of camels in Mangystau, Kyzylorda, Atyrau, and Turkistan oblasts (Table 1) (Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan, 2025). The sustainable development of this sector requires effective measures to protect the camel population from infectious diseases, including camelpox, which have a significant economic impact (Wernery et al., 2014).

Camelpox is a highly contagious disease of Old World camelids (Dahiya et al., 2016) with rare cases of human infection (Khalafalla and Abdelazim, 2017). It is caused by camelpox virus (CMLV), which belongs to the family Poxviridae, genus Orthopoxvirus. The disease is endemic in almost all countries where camel husbandry is practiced (Dahiya et al., 2016) and is commonly spread through direct contact between infected and susceptible animals via inhalation or skin abrasions. The clinical manifestations of camelpox range from inapparent and mild localized infections to moderate and severe generalized infections. A generalized infection is characterized by swollen lymph nodes, fever, and papular or pustular eruptions on the skin and mucous membranes. The infection causes significant economic losses and poses a major threat to camelid households due to reduced milk production, abortions, and high mortality rates in young animals (Wernery et al., 2014).

In Kazakhstan, outbreaks of camelpox were regularly observed in the Mangystau and Atyrau oblasts in the 1930s, 1942-1943, and 1965-1969 (Roslyakov, A., 1969), and 1996 (Bulatov et al., 2010) (Figure 1A). In 2012, there was an outbreak of camel disease in Mangystau oblast, resulting in the death of animals and clinical signs resembling camelpox, although CMLV was not identified (News Agency in Kazakhstan, 2012). In 2019-2020, at least 70 animals contracted camelpox in Beineu district of Mangystau oblast (Maikhin et al., 2022). Camels are imported to Kazakhstan mainly from Turkmenistan (Knoll and Burger, 2012), where camelpox outbreaks occur regularly. The last outbreak in Turkmenistan was recorded in 2018, when at least five cases of human infection were reported in addition to infected camels (Fergana News, 2018).

Since the last outbreak in 2020, the camel population in Kazakhstan has increased annually (Table 1), which may increase the potential for rapid disease spread in the event of a future outbreak. Unfortunately, prevalence studies have been virtually absent in Kazakhstan, with the exception of Mangystau and Atyrau oblasts, although camelid husbandry is widespread in many other parts of the country (Bulatov et al., 2024). The following study was designed to provide data on the occurrence of camelpox and vaccination coverage in camels in the western and southern regions of Kazakhstan, which represent the primary centers of camel breeding in the country.

Table 1 Number of camels reported in different oblasts of Kazakhstan in 2019-2025

Oblast	Number of camels (thousand animals)						
	2019	2020	2021	2022	2023	2024	2025
Abai	-	-	-	0.4	0.4	0.5	0.5
Akmola	0.1	0.1	0.1	0.1	0.1	0.6	0.2
Aktobe	17.8	17.6	18.5	20.2	20.0	21.2	25.6
Almaty	7.4	7.6	7.9	7.1	6.6	7.1	8.1
Atyrau	32.4	32.9	34.4	36	40.7	40.7	43.5
East Kaz.	0.6	0.6	0.6	0.2	2.5	2.6	2.7
Jetisu	-	-	-	1.5	1.8	2.0	2.3
Karaganda	1.4	1.5	1.7	1.2	1.2	1.3	1.4
Kostanay	0.2	0.2	0.2	0.3	0.3	0.3	0.2
Kyzylorda	47.9	51.6	54.1	58.6	56.5	59.5	65.8
Mangystau	67.3	71.1	80.8	81.2	86.4	89.3	94.8
North Kaz.	-	-	-	-	-	0.01	0.009
Pavlodar	0.1	0.1	0.1	0	0.03	0.1	0.2
Turkistan	30.4	34.3	35.0	36.8	37.5	39.7	43.1
Ulytau	-	-	-	0.7	0.7	0.7	0.6
West Kaz.	2.2	2.4	2.4	2.6	0.2	0.3	0.3
Zhambyl	6.9	7.2	7.5	7.5	7.1	7.4	8.3
Total	**214.8**	**227.2**	**243.**	**254.6**	**262.0**	**272.9**	**297,6**

Abai, Jetisu, and Ulytau oblasts were separated from East Kazakhstan, Almaty and Karaganda oblasts, correspondingly, on June 8th, 2022.
Data retrieved from: "Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan. Statistics of Agriculture, Forestry, Hunting and Fisheries. 2025. https://stat.gov.kz/ru/industries/business-statistics/stat-forrest-village-hunt-fish/publications/354920/ (accessed 13 November 2025).".

MATERIALS AND METHODS

Ethics Statements

This study was approved by the local ethics committee of the National Center for Biotechnology, Astana, Kazakhstan (approval #4/03.12.2021). 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.

Study area and sampling design

This cross-sectional study focused on determining the prevalence of camelpox at the individual animal level. According to a study by Zhigailov et al. that assessed the risk of CMLV infection (Zhigailov et al., 2022), the western and southern regions of Kazakhstan, which include Mangystau, Atyrau, Aktobe, Kyzylorda, Turkistan, Zhambyl, and Almaty oblasts, are particularly susceptible to camelpox. Therefore, the target population included the camel population in western and southern Kazakhstan. A two-stage sampling survey was designed. Initially, permission for sample collection was issued from the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of Kazakhstan. Assigned specialists visited local veterinarian centers to obtain lists of all registered camel operations in the area. Camel operations were randomly selected from the list and contacted prior to the visits. Selected camel operations were then visited if the owner was available and agreed to participate. Subsequently, in the second stage, a predetermined number of camels were selected from these camel operations. Given the free-ranging nature of the herds, the sampling process began with the animals in the closest proximity and moved outward to ensure a practical approach. All animals were eligible for sampling, with the exception of lactating or pregnant camels, as the owners did not consent to sampling these animals. To ensure a comprehensive assessment of herd health, camels were not excluded based on health status. Camels exhibiting signs of infection, including CMLV, were

also included, allowing for a broader evaluation of the CMLV prevalence within the population.

The total number of camels for this study was calculated using a formula for the estimation of proportion (Dohoo et al., 2014; Chouhan et al., 2022) by considering a 95% confidence level, 5% error and 30% seroprevalence as previously estimated by Bulatov et al. 2010 (Bulatov et al., 2010). This resulted in a minimum sample size of 323 camels. The obtained sample size was further adjusted for design effects (DE) to account for clustering of observations: DE = 1+ICC (n-1), where ICC denotes the intra-cluster correlation coefficient and n indicates the cluster size (number of camels sampled per herd) (Dohoo et al., 2014). Mathematically, the ICC is calculated as the between-cluster variability divided by the sum of the within-cluster and between-cluster variabilities. The study assumed an ICC of 0.1 for CMLV infection. This value is consistent with the ICC of less than 0.1 reported for multiple infections in domestic animals by Otte and Gumm (1997). An average herd size of 9 camels was used, based on official statistics stating that over 50% of the country's camels are concentrated in private backyards (Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan, 2025). Consequently, the total sample size was estimated to be 582 (from 65 camel operations), corresponding to approximately 65 camel operations. In the best effort to meet this required sample size, we sampled 572 camels from 63 camel operations (minimum cluster size =1, maximum cluster size = 67). For camel operations with fewer than 9 animals available, the shortfall was compensated by sampling additional animals from the next farm.

Sample collection

From October 2021 to December 2022, blood samples were collected from 572 camels aged 5 months to 21 years. Of these samples, 297 were collected from vaccinated animals and 275 from unvaccinated animals. An average sample size per camel farm was 9 for vaccinated animals and 7 for unvaccinated animals. Vaccination data were obtained from farm-owner records. These records were used for classifying the animals as either vaccinated or unvaccinated during the sampling procedure. The sampling sites were shown in Figure 1A. Blood was collected by trained veterinarians from the jugular vein in 5 ml vacutainer tubes containing K2EDTA or clot activator and transported to the laboratory at 4°C, where it was aliquoted and stored at -20°C until analyzed.

ELISA

Prior to the ELISA assay, serum samples were thawed and inactivated at 56°C for 20 minutes. Hemolytic samples were excluded from the analysis. Subsequently, a total of 486 serum samples, including 221 and 265 serum samples obtained from vaccinated and unvaccinated camels, respectively, were screened for antibodies against CMLV using a Camelpox Virus Antibody ELISA Kit (Abbexa Ltd., United Kingdom) according to the manufacturer's instructions. According to personal communication with the manufacturer Abbexa Ltd., the specificity (Sp) of the Camelpox Virus Antibody ELISA Kit is 98.5%. However, the sensitivity (Se) of the test has not been determined by the manufacturer. In the absence of this data, we assumed the test to have perfect Se. The intra-assay and inter-assay coefficients of variation (CVs) assessed by replicate testing of positive and negative control samples in our laboratory were 3.9% and 4.5%, indicating acceptable repeatability and reliability of the ELISA assay.

DNA extraction and PCR

Total DNA was extracted from blood samples and a skin lesion swab using the AmpliSens®RIBO-Sorb kit (InterLabService, Russia) according to the manufacturer's instructions. The quality and yield of the extracted DNA were assessed by spectrophotometric analysis. All samples were screened for CMLV DNA using two pairs of primers: the genus-specific primers OrtoPOX_ATI-F/OrtoPOX_ATI-R which target the A-type inclusion protein (ATI) gene (Monique

and Couacy-Hymann, 2022) and the species-specific primers CMLV-C18L-F/CMLV-C18L-R targeting the C18L gene encoding ankyrin repeat protein (Balamurugan, 2009) (Table 2). The reaction mixture and conditions were identical for both pairs of primers. The reaction mixture (25 µl) contained 10× standard reaction buffer (2.5 µl), 2.5 mM dNTPs (0.5 µl), 0.2 mM forward primer (0.5 µl), 0.2 mM reverse primer (0.5 µl), template DNA (2 µl), 0.625 U Hot Start Taq DNA polymerase (New England Biolabs, United States) (0.125 µl), and nuclease-free water. Reaction conditions were as follows: 95°C 8 min, [94°C 30 s, 58°C 30 s, 72°C 1 min] × 40, 72°C 5 min, 4°C 5 min. Distilled water was used as a negative control. An archived sample containing CMLV DNA was used as a positive control. PCR products were separated by electrophoresis in 1.5% agarose gel and visualized under UV light.

Table 2 Primers used in the study

Name	Primer sequence (5' → 3')	T_a (°C)	Product size (bp)	Reference
C18L gene				
CMLV-C18L-F	5'GCGTTAACGCGACGTCGTG	58	243	(Balamurugan et al., 2009)
CMLV-C18L-R	5'CCGGAGATATCATACTTTACTTTAG			
CMLV-C18L-1-F1	5'ATGGAAATCGTTAATCTTCTTATTG	58	618	This study
CMLV-C18L-618-R1	5'CTGGATGGCTGTATTGATGATCG			
CMLV-C18L-1131-R2	5'TTATTTAATGGAGTAATAAAGTATTTCACG	58	557	
***ATI* gene**				
OrtoPOX_ATI-F	5'GACAAATACAAGGAGGATCT	58	881	(Monique and Couacy-Hymann, 2022)
OrtoPOX_ATI-R	5'GTAGAACTTAACTTTTTTCTTTCTC			
CMLV-ATI-1-F1	5'ATGGAGCTCACGAACCTTATTG	58	1000	This study
CMLV-ATI-999-R1	5'GTACGCGGACACTTCCTCAT			
CMLV-ATI-575-F2	5'CCAGCGTTGATAGATACAGAAAC	58	809	
CMLV-1764-ATI-R2	5'CTCACGTAGTTACTAATAGTTGCCT			

Sequencing and phylogenetic analysis

For genotyping the detected CMLV strains, the complete C18L and ATI genes were sequenced by the Sanger method using the primer pairs CMLV-ATI-1-F1/CMLV-ATI-999-R1, CMLV-ATI-575-F2/CMLV-1764-ATI-R2, CMLV-C18L-1-F1/CMLV-C18L-618-R1, and CMLV-C18L-F/CMLV-C18L-1131-R2, which were developed for this study (Table 2). The primers designed for this study were generated using the National Center for Biotechnology Information (NCBI) Primer-BLAST.

Elution of DNA fragments from agarose gels was performed using a QIAquick gel extraction kit (Qiagen, USA). The purified DNA fragments were then sequenced in both directions using a BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) and an ABI 3500XL genetic analyzer (Applied Biosystems) by Sanger method. The concatenated sequences in FASTA format were uploaded to the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/), where they were compared with sequences in the online database. The sequences were aligned using the MUSCLE algorithm. The maximum likelihood algorithm was selected to generate the unrooted phylogenetic trees using MEGA 12 software (Kumar et al., 2024). Models with the lowest BIC scores (Bayesian Information Criterion) were considered to describe the substitution pattern the best. The bootstrap method with 1000 replicates was used to evaluate the reliability of the tree topologies. Support for internal nodes was evaluated using the nonparametric bootstrap method. Phylogenetic trees were constructed using complete genome sequences of CMLV strains from Kazakhstan, Iran, Israel, Morocco and United Arab Emirates available in the NCBI database. Cowpox virus (GenBank # AF482758.2) was used as an outgroup.

Statistical analysis

The apparent prevalence of camelpox antibodies at the animal level was determined by calculating the proportion of seropositive animals among all those

tested. Animal-level seroprevalence was adjusted for the different proportions of animals sampled from each cluster (camel operations) using the following equation (Cormack et al., 1991): adjusted Ap=(∑mi=1(wixpi))/(∑mi=1wi), where adjusted Ap is the adjusted apparent animal-level seroprevalence, wi is the sampling weight for cluster i, pi is the apparent animal-level seroprevalence for cluster i, m is the number of clusters sampled. To adjust for cluster sampling, the 95% confidence interval (CI) was computed using the Taylor series linearization method in the EpiInfo7 software (CDC). The animal-level seroprevalence was also adjusted for the diagnostic Se and Sp of the ELISA kit using the equation (Rogan et al., 1978): adjusted Tp=(adjusted Ap+Sp–1)/(Se+Sp–1), where adjusted Tp is the adjusted true animal-level seroprevalence. The apparent herd-level prevalence was defined as the proportion of herds with at least one positive animal among all herds tested. For within-herd seroprevalence, the 95% CI was computed using the Clopper-Pearson exact method based on the beta distribution.

The generalized estimating equation (GEE) model was employed to analyze the factors associated with CMLV infection at the animal level and the camel operation was included as random effect (Liang and Zeger, 1986). Odds ratios (OR) was assessed using the method of GEE with an “exchangeable” correlation matrix among observations within each herd. First, the univariable associations between animal-level seropositivity and independent variables (gender, age, farm size, as well as the camel density in a district) were explored. All camel operations were categorized into small farms (1-20 camels) and large farms (more than 20 camels). Data on camel density (animals/km2) at the district level were retrieved from the report of the Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan (2025) (Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan, 2025) (Figure 1B). Following the univariable analysis, those variables with $P<0.25$ were fitted into a multivariable model. Significance was determined at the level of $P<0.05$. The analysis was performed using the Stata 14 software (USA).

RESULTS

Molecular screening of camels for CMLV and genotyping

We collected blood samples from 572 camels. One of the sampled animals presented with skin lesions suggestive of CMLV infection, and an additional swab sample was obtained from this camel. All blood samples and the lesion swab were screened for CMLV DNA by PCR using two pairs of primers (Tables 3 and 4). Three samples - two blood samples and the tissue swab - tested positive for CMLV DNA using both primer pairs, resulting in an overall infection rate of 1.1% (95% CI: 0.1–1.5%). All three PCR-positive samples were collected from unvaccinated camels in Atyrau oblast (2.3%). Two samples were collected from camels on a large farm near Atyrau city – one from a 9 and one from a 5-year-old- and an additional sample was obtained from an 8-year-old female camel in a private household in Ortakshil village, Makhambet district (Figure 1A, Table 4).

Of the three PCR-positive samples, only the lesion swab yielded sufficient amount of DNA for further sequencing. The detected strain showed 100% nucleotide homology with the reference CMLV strain M-96, isolated in Mangystau oblast of Kazakhstan in 1996, for both the ATI and C18L genes, and clustered with reference strains from Iran and Morocco (Figure 2 and 3). The detected strain also demonstrated 100% and 99.94% nucleotide identity in the C18L and ATI genes, respectively, with the CMLV strain isolated by Bulatov et al. in 2023 in Mangystau oblast.

Table 3 Results of serological and molecular screening of vaccinated camels for CMLV infection in western and southern Kazakhstan according to sampling sites

Oblast	District/City	No. seropositive/No. examined herds (%)	No. seropositive/No. examined animals (%)	No. PCR positive/No. examined animals (%)
Aktobe	Shalkar	1/1 (100)	17/19 (89.5)	0/52 (0)
Almaty	Ile	1/1 (100)	33/33 (100)	0/46 (0)
Atyrau	Kyzylkoga	10/15 (66.7)	19/26 (73.1)	0/26 (0)
Zhambyl	Baizak	1/1 (100)	7/7 (100)	0/9 (0)
	Sarysu	1/1 (100)	20/20 (100)	0/20 (0)
	Talas	1/1 (100)	19/19 (100)	0/20 (0)
Turkistan	Kentau	1/1 (100)	5/5 (100)	0/5 (0)
	Otyrar	1/1 (100)	49/65 (75.4)	0/91 (0)
	Sauran	1/1 (100)	19/19 (100)	0/20 (0)
	Turkistan city	1/1 (100)	8/8 (100)	0/8 (0)
TOTAL		19/24 (79.2)	196/221 (88.7)	0/297 (0)

Table 4 Results of serological and molecular screening of unvaccinated camels for CMLV infection in western and southern Kazakhstan according to sampling sites

Oblast	District/City	No. seropositive/No. examined herds (%)	No. seropositive/No. examined animals (%)	No. PCR positive/No. examined animals (%)
Atyrau	Atyrau city	1/1 (100)	21/39 (53.9)	2/40 (5)
	Makat	3/3 (100)	8/17 (47.1)	0/20 (0)
	Makhambet	8/8 (100)	63/68 (92.7)	1/69 (1.5)
Kyzylorda	Aral	0/3 (0)	0/6 (0)	0/6 (0)
	Karmakshy	0/1 (0)	0/20 (0)	0/20 (0)
	Shieli	0/1 (0)	0/20 (0)	0/20 (0)
Mangystau	Karakiya	0/7 (0)	0/20 (0)	0/20 (0)
	Mangystau	0/6 (0)	0/16 (0)	0/20 (0)
	Munaily	0/4 (0)	0/20 (0)	0/20 (0)
	Tupkaragan	0/1 (0)	0/20 (0)	0/20 (0)
	Beyneu	0/4 (0)	0/19 (0)	0/20 (0)
TOTAL		12/39 (30.8)	92/265 (34.7)	3/275 (1.1)

CMLV antibody seroprevalence

Of 572 serum samples collected, 486 were analyzed by ELISA. The apparent individual seroprevalence among the vaccinated camels was 88.7% (196/221), from which, taking into account the test accuracy and cluster sampling, the true adjusted animal prevalence among the vaccinated camels was estimated to be 73.6%. Of the 24 herds of vaccinated camels, 19 had at least one seropositive animal, corresponding to an apparent herd-level seroprevalence of 79.2%. All vaccinated animals sampled in Almaty and Zhambyl oblasts were seropositive, whereas apparent seroprevalence among vaccinated animals in Aktobe, Turkistan, and Atyrau oblasts was around 80% (Table 3).

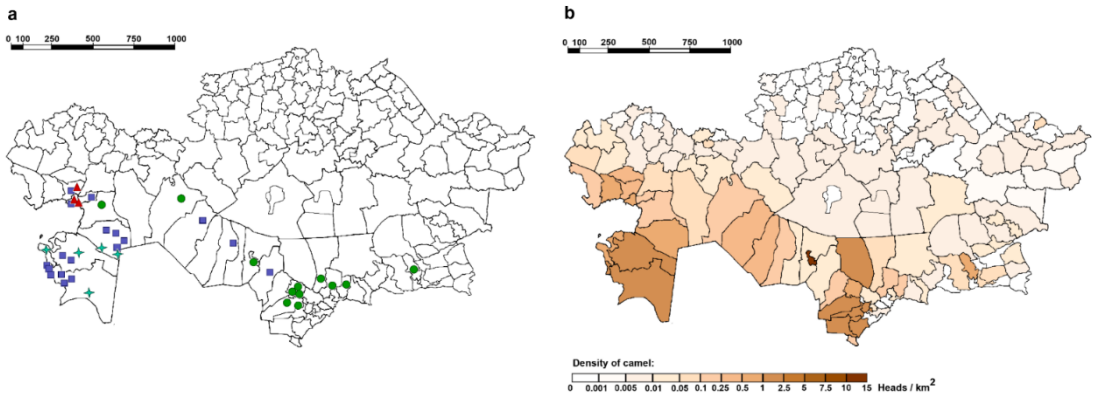


Figure 1 Sample collection sites in Kazakhstan, 2021-2022. (A) Circles indicate sites where vaccinated camelids were sampled. Quadrants indicate sites where unvaccinated camels were sampled. Triangles indicate locations where PCR-positive samples were detected. Stars indicate locations of camelpox outbreaks in 1996 and 2020. (B) Map of Kazakhstan showing data on camel density in 2022 (Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan, 2023).

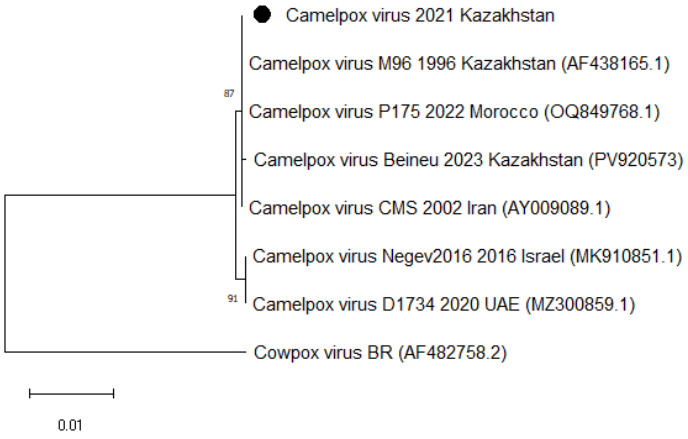


Figure 2 Phylogenetic tree based on the ATI gene of the CMLV isolate obtained in this study (marked by •) and representative CMLV strains. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-2664.16) is shown. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). Bootstrap values (1,000 replicates) are shown at the nodes.

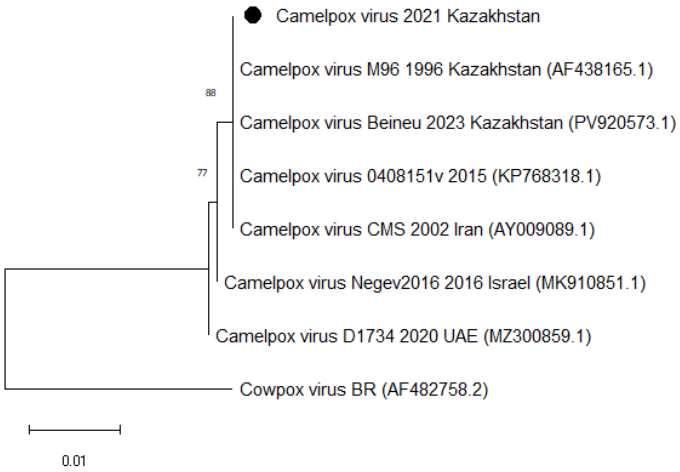


Figure 3 Phylogenetic tree based on the C18L gene of the CMLV isolate obtained in this study (marked by •) and representative CMLV strain. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-1849.94) is shown. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). Bootstrap values (1,000 replicates) are shown at the nodes.

A total of 92 of 265 serum samples obtained from unvaccinated camels tested positive for CMLV antibodies by ELISA, corresponding to an apparent animal-level seroprevalence of 34.7% (Table 4). After adjustment to oversampling in some herds and test performance, the true adjusted seroprevalence among unvaccinated animals was calculated to be 10.9%. Seropositive unvaccinated animals were not detected in Kyzylorda and Mangystau oblast, while 92 of 124 sampled animals in Atyrau oblast had antibodies to CMLV, resulting in an apparent seroprevalence of 74.2% and a true adjusted animal-level seroprevalence of 67.5% (Table 4). Seropositive animals were found in farms in the vicinity of Atyrau city and within the Makat and Makhambet districts. Of note, all animals from the camel herd in Ortakshil village of Makhambet district, where one of the PCR-positive samples was found, tested positive for CMLV antibodies (100%; 20/20; 95% CI: 83.2-100%). Apparent individual-level seroprevalence in the herd in Atyrau city, where two other PCR-positive samples were found, was 53.8% (21/39; 95% CI: 37.2-69.9%). All PCR-positive samples were found in seropositive animals. At least one seropositive animal was found in 12 of 39 herds tested (30.8%; 95% CI: 17.0–47.6%).

Risk factor analysis

The variables examined included gender and age of animals, farm size and camel density in a district. Univariable and multivariable associations between animal-level CMLV seropositivity and these variables are presented in Table 5. None of the variables explored showed a statistically significant association with CMLV seropositivity in the GEE model. However, although not statistically significant, we found a strong association between animal-level CMLV seropositivity and camel density greater than 0.3 animals/km2 (Table 5).

VETERINARY INTEGRATIVE SCIENCES

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Table 5 Univariate and multivariate analysis of independent variables associated with animal-level CMLV seropositivity in unvaccinated camels in Kazakhstan.

Variable (category)	Univariable GEE model		Multivariable GEE model	
	P value	Odds ratio (95% confidence interval)	*P* value	Odds ratio (95% confidence interval
Gender (female)	0.31	1.1 (0.9-1.2)	-	-
Age (age ≥10 years)	0.07	1.5 (0.9-2.3)	0.31	1.0 (0.9-1.1)
Farm size (>20 animals)	0.54	1.5 (0.4-1.1)	-	-
Camel density in a district (> 0.3 animals/km^2):	0.15	3.4 (0.6-17.6)	0.11	3.9 (0.1-0.5)

Wald chi^2 = 3.40, Prob > chi^2 = 0.18

DISCUSSION

The epidemiology of camelpox has not been extensively investigated. Most studies have focused on the clinical course of the infection and the isolation and identification of the virus. Recent systematic reviews of viral diseases in animal populations have emphasized the critical need for comprehensive epidemiological studies to understand disease patterns and inform control strategies (Tito et al., 2026). Given the economic importance of the disease, we conducted this study to investigate the prevalence of CMLV infection and vaccine coverage in the western and southern regions of Kazakhstan.

The true overall adjusted seroprevalence among unvaccinated animals in this study reached 10.9%, closely aligning with the CMLV animal-level seroprevalence in Libya (9.8%) (Azwai et al., 1996) and Saudi Arabia (9.14%) (Housawi, 2007). The herd-level seroprevalence of 30.8% observed in this study indicates a notable level of exposure of the herds within the tested regions to CMLV. All seropositive unvaccinated animals were found in the Atyrau oblast, resulting in the apparent unadjusted seroprevalence of 74.2%, which is comparable to the seroprevalence observed

in unvaccinated camels in Sudan following a CMLV outbreak (72.5%) (Khalafalla et al., 1998). Consistently, all three PCR-positive animals identified in this study were also from Atyrau oblast, where the CMLV infection rate reached 2.3%. These results together with a relatively high animal-level prevalence of antibodies to CMLV found in unvaccinated camels in Atyrau oblast indicate circulation of CMLV in the region in 2021-2022. These findings are supported by a recent study by Bulatov et al. (2024), which also confirmed the circulation of CMLV in western Kazakhstan later in 2023 – 2024, specifically in the Atyrau and Mangystau oblasts. The study detected CMLV DNA in camel blood at a notably higher prevalence of 5.2% across six districts of Atyrau oblast, suggesting a trend toward increasing viral activity and broader persistence of the pathogen in the oblast compared with the 2021-2022 period. Sequencing of the complete C18L and ATI genes in our study revealed 100% and 99.94% nucleotide identity, respectively, with the CMLV strain isolated by Bulatov et al. in 2023 in the Beineu district of Mangystau oblast. The strain detected in our study also showed 100% homology in both genes with the reference strain M-96, which was isolated during a severe camelpox outbreak in Mangystau oblast in 1996 that affected approximately 8,000 camels and resulted in 43 camel deaths (Bulatov et al., 2025). Together, these findings suggest that an identical or a closely related CMLV lineage has been circulating across neighboring regions of western Kazakhstan for decades, indicating ongoing viral maintenance and potential transboundary movement within local camel populations.

According to official sources (Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan, 2023),

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state-sponsored vaccination of camels against CMLV is carried out only in the western region of Kazakhstan (Aktobe, Atyrau, and Mangystau oblasts) and covers only about 36% of the camel population in the region, including 25% in Atyrau oblast. At the same time, farmers in Kazakhstan also vaccinate their camels privately. In this study, 45.5% of sampled animals had reliable records of vaccination. The adjusted animal-level seroprevalence among vaccinated animals was estimated to be 73.6%, suggesting that the vaccination-associated seroprevalence in the analyzed regions is relatively high.

Another aspect of this study was the identification of risk factors for CMLV infection in camel populations in Kazakhstan. Risk factor analysis has become increasingly important for understanding viral disease transmission patterns in livestock populations, as demonstrated in recent studies of emerging diseases (Nubgan and Al-Saadi, 2023). Several factors were explored, including age and gender of the animals, farm size, and camel density. In contrast to previously published studies (Kriz, 1982; Khalafalla and Mohamed, 1996; Abou et al., 2012; Israeli et al., 2019), we found no association between the gender or age of animals, nor the farm size, and CMLV seroprevalence. This suggests that animals of any ages and gender have a similar likelihood of CMLV infection, regardless of farm size. Although not statistically significant, we found a strong association between animal-level CMLV seroprevalence and camel density in the districts. Higher camel density was associated with higher odds of CMLV infection. This finding may be attributed to the contagious nature and contact transmission of CMLV, which spreads among animals within the same herd or between herds when camels congregate for grazing and watering. Therefore, a higher density of camels in an area may favor transmission of the infection.

This study has several limitations. The sample selection process introduces potential biases. Only camel operations with consenting owners were included. Owners willing to participate may have better-managed herds or fewer health issues, leading to an underestimation of infection prevalence. Because information on privately administered vaccination was not centrally recorded and could only be obtained at the farm level during field visits, it was not feasible to systematically include both vaccinated and unvaccinated herds within each oblast. ELISA sensitivity was assumed to be 100%, which may lead to an underestimation of true seroprevalence if actual sensitivity is lower. Further adjustments would be necessary in such a case. Because the serological assay applied in this study cannot distinguish between vaccine-induced and infection-induced antibodies, vaccination status was recorded based on herd owner information. This limits attribution of antibody origin in vaccinated animals and may lead to further underestimation of seroprevalence attributable to natural infection.

CONCLUSIONS

In summary, this study explored CMLV prevalence in western and southern regions, areas of Kazakhstan characterized by a high concentration of camel operations. Despite high vaccine-associated seroprevalence, PCR-positive cases in unvaccinated herds and elevated seroprevalence among unvaccinated animals indicate active virus circulation in western Kazakhstan in 2021–2022. Genotyping of the detected virus demonstrated its close genetic relationship to historical and recently identified strains circulating in Mangystau and Atyrau oblasts, suggesting long-term regional persistence of an identical or closely related CMLV lineage. Given these findings, strengthening vaccination monitoring and disease control measures in the region is crucial to mitigating further transmission.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yekaterina O. Ostapchuk: Investigation, Formal analysis, Writing – Original Draft. **Akzhigit S. Mashzhan, Sofiya A. Kan, Anzhelika V. Lushova, Nurshat Abdolla, Saltanat A. Kuatbekova:** Investigation. Yuliya V. Perfilyeva: Data curation, Writing – Review & Editing.
Andrey V. Zhigailov: Investigation, Conceptualization.
Sofiya A. Kan, Anzhelika V. Lushova: Visualization.
Dinara A. Naizabayeva, Maxat A. Berdikulov: Resources.
Seidigapbar M. Mamadaliyev: Funding acquisition, Project administration.

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