

## Original Article

## Seroprevalence of Antibodies of Crimean-Congo Hemorrhagic Fever Virus in Cattle in the Turkestan Region of Kazakhstan



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## ABSTRACT

**Background:** Crimean-Congo hemorrhagic fever (CCHF) is endemic in southern Kazakhstan, yet data on livestock exposure remain scarce. Because cattle can amplify virus-infected ticks, accurate prevalence estimates are essential for risk-based control.

**Objectives:** This study aimed to determine the seroprevalence of CCHF virus (CCHFV) antibodies in cattle across the Turkestan region and to identify demographic and spatial factors associated with exposure.

**Methods:** In 2024, a cross-sectional survey generated 840 bovine serum samples (420 in spring and 420 in autumn) from 14 districts of the Turkestan Region, Kazakhstan. Sampling was performed using a random approach with stratification: 42 rural settlements were included (3 rural districts per district, and 10 heads of cattle from each district). Animals were selected from multiple owners in each village, ensuring the data were representative for assessing the cattle population in the region. CCHFV antibodies were detected using a commercial double-antigen enzyme-linked immunosorbent assay (ELISA), which enhances specificity and reduces the risk of cross-reactivity with related viruses. Spatial variation in seroprevalence was visualized in ArcGIS 10.8, while statistical analysis, comprising descriptive summaries,  $\chi^2$  test, and multivariable logistic regression, was conducted in R software, version 4.1.0. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. Results with  $P=0.05$  were deemed significant.

**Results:** The overall seroprevalence was 36.2%, with a significant range of district-level seroprevalence from 10% to 76.6%. The study identified higher seropositivity in adult cattle (43.42%) than in juveniles (11.86%), and females had slightly higher rates than males (38.32% vs 29.99%). Logistic regression analysis showed that age was a significant risk factor, with adults having 4.06 times higher odds of seropositivity (OR=4.06; 95% CI, 2.62%, 6.29%) compared to juveniles.

**Conclusion:** The findings confirm active, heterogeneous circulation of CCHFV among cattle in the Turkestan region, with older animals and districts practicing communal grazing at greatest risk. Incorporating livestock serosurveillance into routine tick control programs and focusing interventions on identified hotspots could curtail zoonotic spill-over to humans.

**Keywords:** Antibodies, Cattle, Crimean-Congo hemorrhagic fever (CCHF), Kazakhstan, Ticks

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## Introduction

Criméan–Congo hemorrhagic fever (CCHF) is a severe tick-borne zoonotic disease with clinical manifestations in humans ranging from mild febrile illness to hemorrhagic syndrome, multi-organ failure, and death. The case fatality rate among hospitalized patients can exceed 30% (Ricco et al., 2023; Spengler et al., 2016; Hawman & Feldmann, 2023). The causative agent, CCHF virus (CCHFV), is a negative-strand RNA virus belonging to the genus *Orthobunyavirus* (family *Nairoviridae*, order *Bunyvirales*) (Tariq et al., 2023). Transmission is closely linked to the ecology of *Hyalomma* ticks, which serve as both vectors and reservoirs of the virus (Oygar et al., 2023). Humans are typically infected through the bite of infected ticks, direct contact with blood or tissues of viremic livestock, or nosocomial transmission. Occupational groups such as farmers, veterinarians, abattoir workers, and health-care staff remain at the highest risk (Tahir et al., 2024; Ahmed et al., 2021; Kasi et al., 2020).

CCHF is widely distributed across Africa, the Middle East, Eastern Europe, and Asia, with outbreaks reported in multiple countries since its initial identification in Crimea and the Belgian Congo (Atim et al., 2023; Fe-reidouni et al., 2023; Francesca et al., 2025). In Central Asia, endemic zones include Turkmenistan, Uzbekistan, Tajikistan, southern Russia, and Kazakhstan, as well as parts of southern Europe such as Turkey and the Balkans (Li et al., 2020; Ivanov & Kutsin, 2015; Kostyukova, 2021). In Kazakhstan, CCHF was first identified in 1948 in the Turkestan region, initially described as “Central Asian fever” (Temirbekov et al., 1980; Nurmakhanov et al., 2017; Nurmakhanov, 2024). Since then, natural foci have been documented in Turkestan, Kyzylorda, and Zhambyl provinces, as well as Shymkent City, with *Hyalomma asiaticum*, *Hyalomma anatolicum*, *Hyalomma scupense*, *Hyalomma marginatum*, and *Dermacentor niveus* identified as the predominant vectors (Turliev & Usatayeva, 2019; Nurmakhanov, 2013; Head et al., 2020). More recent serological and molecular studies have revealed CCHFV activity in western and northern parts of the country, regions previously considered non-endemic (Gazezova et al., 2025; Grazhdanov, 2012; Abuova et al., 2024).

The Turkestan region is of particular concern due to its favorable ecological conditions for tick survival and extensive livestock farming, which facilitates close human–animal–tick interactions. Despite recurrent human cases and outbreaks, comprehensive data on livestock

seroprevalence across all districts of the region remain limited. This study aimed to assess the seroprevalence of CCHFV antibodies in cattle across the Turkestan region of Kazakhstan, considering demographic and seasonal factors. These findings aim to refine the understanding of virus circulation and to support targeted surveillance and prevention strategies.

## Materials and Methods

### Research region

This cross-sectional study was conducted from May to October 2024 in the Turkestan region of Kazakhstan, where the cattle population was estimated at approximately 1232500 head across various administrative districts. The study encompassed 42 rural settlements distributed across 14 districts of the Turkestan region (Figure 1). To ensure accurate spatial referencing and reproducibility of sampling locations, the geographic coordinates (latitude and longitude) of each sampling site were recorded in situ using a Garmin GPS navigator (GPSmap 65, Garmin Ltd., USA).

### Collection of samples

Blood serum samples were collected from cattle during two distinct periods: 420 samples in the spring and 420 in the autumn. All samples were obtained from cattle older than 3 months that grazed regularly on communal pastures. Cattle data, including species, age, sex, and geographic location, were recorded using a pre-printed questionnaire. Blood samples (~5–7 mL) were collected from the jugular or tail vein using standard veterinary procedures. Serum was separated and stored at -20 °C until analysis. All laboratory studies were conducted at the Laboratory of Especially Dangerous Infectious Diseases (biosafety level BSL-3+) at the Research Institute for Biological Safety Problems (RIBSP), located in Gvardeyskiy, Kordai District, Zhambyl region.

### Enzyme-linked immunosorbent assay (ELISA) configuration

Collected sera were analyzed for antibodies against CCHFV using a commercial ELISA kit (ID Screen® CCHF Double Antigen for Multi-Species, I.D. Vet, Grabels, France) for the detection of CCHF-specific antibodies in cattle. In each microplate well, 50 µL of a buffer solution coated with the recombinant CCHF nucleoprotein was dispensed. Positive and negative control samples were then added to designated wells, and 30 µL of test sera were added to the remaining wells. The plate

was incubated for 45 minutes at 21 °C, followed by 5 washing cycles with 300 µL of buffer per wash. Subsequently, 50 µL of a 10X conjugate (diluted 1:10) was added, and the plate was incubated for 30 minutes, after which another washing step was performed. Next, 100 µL of substrate was added to each well and incubated for 15 minutes at 21 °C in the dark, followed by the addition of 100 µL of stop solution. The optical density (OD) was measured at 450 nm. Finally, the threshold for seropositivity or seronegativity was determined according to the kit's instruction.

### Statistical analysis

ArcGIS software, version 10.8 (ESRI, Redlands, CA, USA) was used to create thematic maps illustrating spatial variation in seroprevalence and to geolocate sampling sites accurately. Statistical analyses were conducted using R software, version 4.1.0 and GraphPad Prism software, version 8.0. Descriptive statistics were used to summarize the overall and district-level seroprevalence rates. Associations between CCHFV seropositivity and potential risk factors (age, sex, season, and district) were evaluated using the chi-square ( $\chi^2$ ) test and multivariable logistic regression. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength of the associations. A  $P=0.05$  was considered statistically significant.

Seasonal differences in seroprevalence were analyzed by comparing the spring and autumn datasets using the chi-square test for proportions. Additionally, a two-proportion z-test was used to confirm overall seasonal differences in seropositivity between the two sampling periods. District-level comparisons of seasonal seroprevalence were performed using either the chi-square or Fisher exact test, depending on sample size and distribution.

The logistic regression model also included "season" as a categorical independent variable to estimate the adjusted effect of seasonality on seropositivity. Potential interaction effects (e.g. age  $\times$  sex, season  $\times$  district) were tested but found to be statistically non-significant. All tests were two-tailed.

## Results

The results were presented as a bar chart (Figure 2) to make them easier to understand. The graphs clearly show that the Kazygurt District has the highest number of positive samples, while also identifying districts with a minimal number of positive samples.

Seroprevalence percentages obtained by ELISA are shown separately for samples collected during spring (red bars,  $n=420$ ) and autumn (blue bars,  $n=420$ ). Each bar represents the proportion (%) of positive cattle samples from each district.

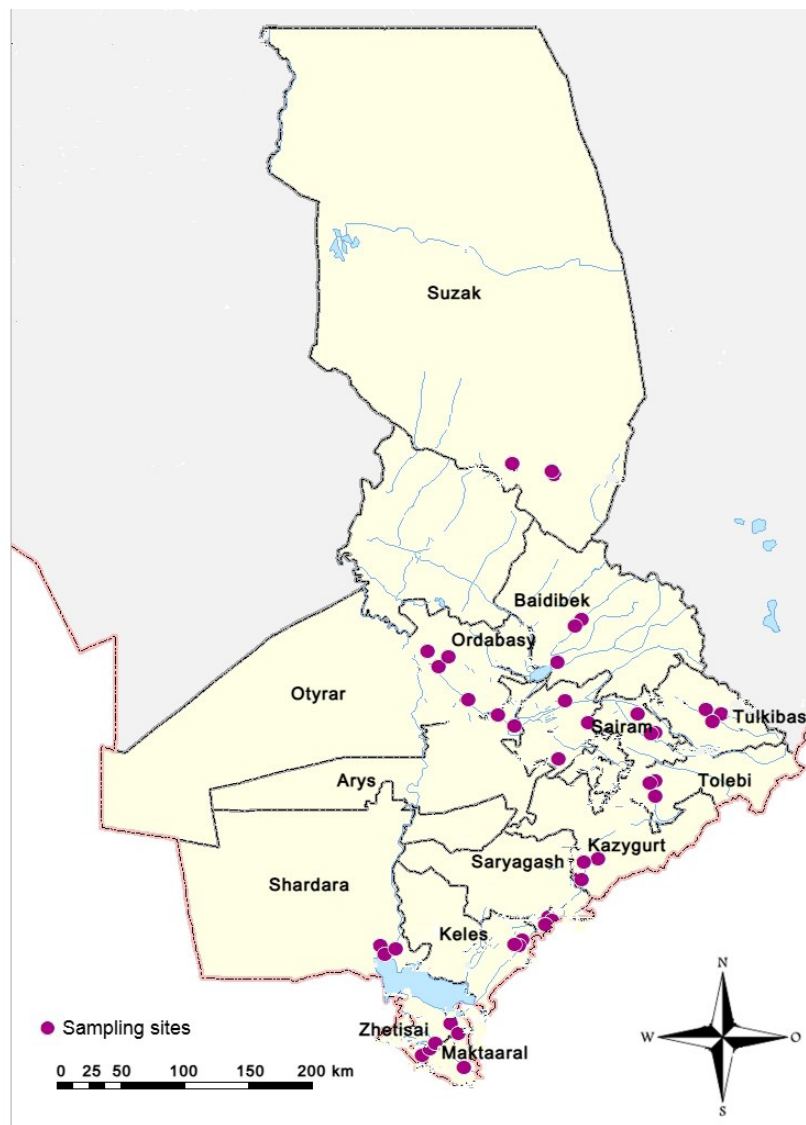
A significant seasonal variation in CCHFV seroprevalence was observed across the study sites. In the spring season, 181 out of 420 cattle serum samples (43.09%) tested seropositive, while in the autumn, only 123 out of 420 samples (29.28%) were positive. This seasonal difference was statistically significant ( $\chi^2=16.9$ ,  $df=1$ ,  $P=0.001$ ), indicating a strong association between sampling season and the likelihood of detecting CCHFV antibodies. Geographically, antibodies were detected in nearly all districts of the Turkestan region except for Sairam. The highest seroprevalence rates, exceeding 50%, were recorded in Kazygurt (76.7%), Ordabasy (73.33%), Baidibek (70%), Arys (63.33%), Otyrar (60%), Suzak (60%), and Saryagash (50%). Moderate seroprevalence (20–40%) was observed in Keles, Zhetisay, Shardara, and Tolebi, while Maktaaral and Tulkibas had the lowest rates (below 20%). These seasonal and regional patterns are clearly illustrated in Figure 2.

The study comprised 661 adult cattle (554 females and 107 males) and 179 juvenile cattle (122 females and 57 males). Table 1 presents descriptive statistics about the correlation between antibody presence and variables such as gender and age.

After adjusting for other variables, adult cattle were found to be 4.06 times more likely to be seropositive than juveniles ( $OR=4.06$ ; 95% CI, 2.62%, 6.29%). This effect was highly statistically significant, as demonstrated by the Wald test statistic ( $z=6.86$ ,  $P=0.001$ ).

Regarding gender, 254 out of 676 females (37.57%) and 50 out of 164 males (30.49%) were seropositive. While females showed a higher apparent seroprevalence, the difference was not statistically significant in the logistic regression model ( $OR=1.37$ ; 95% CI, 0.95%, 1.98%;  $z=1.59$ ;  $P=0.11$ ). This finding suggests that sex is not a strong determinant of exposure risk in the current cattle population, possibly due to similar grazing patterns and shared tick environments for both males and females.

To investigate potential effect modification, an interaction term between age and gender was included in the logistic regression model. The result was non-significant ( $P=0.48$ ), indicating that the association between age and seropositivity was independent of gender. In



**Figure 1.** Map of sampling sites in 14 districts of the Turkestan region

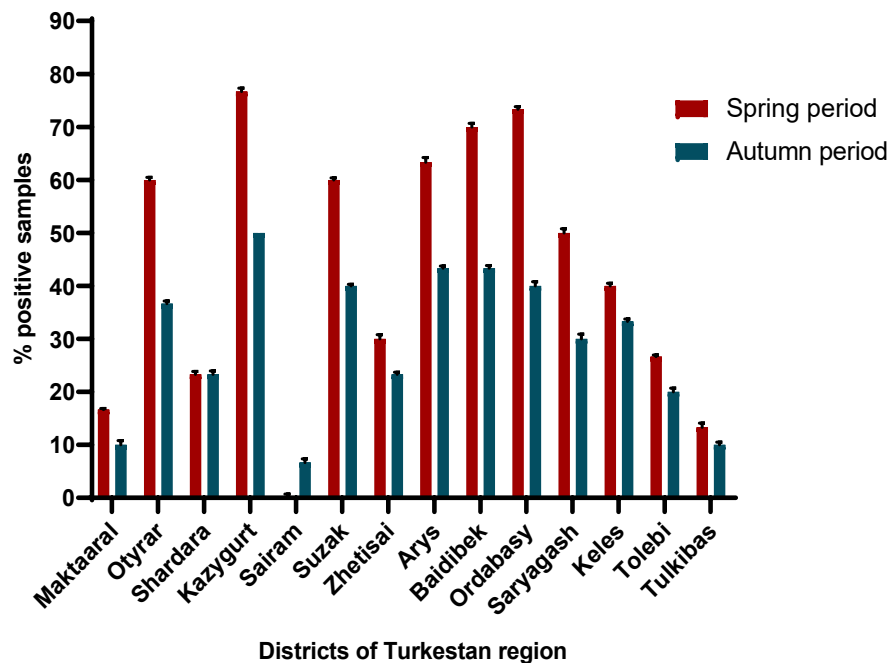
other words, both male and female adult cattle exhibited similar odds of seropositivity compared to juveniles, without synergistic or antagonistic interactions between the two variables.

## Discussion

Our findings confirm the high endemicity of CCHFV in southern Kazakhstan. In [Argimbayeva et al. \(2023\)](#), seropositivity in cattle in the Turkestan region reached 78.6%, the highest nationwide, whereas in the Zhambyl region it was 22.5% ([Bryant-Genovese et al., 2022](#)). Earlier data from [Nurmakhanov et al. \(2017\)](#) on human cases likewise point to the long-standing presence of natural foci in the region. Marked year-to-year fluctuations have been observed: In 2022, cattle seropositivity was 14.5%;

it peaked at 78.6% in 2023, and declined to 36.2% in 2024. These differences are likely driven by *Hyalomma* tick activity and changes in animal husbandry practices.

The ELISA-based serological survey revealed statistically significant seasonal and spatial differences in the distribution of CCHFV antibodies among cattle in the Turkestan region, as supported by the chi-square test ( $\chi^2=101.6$ ,  $df=13$ ,  $P=0.001$  for inter-district variation;  $\chi^2=16.9$ ,  $df=1$ ,  $P=0.001$  for seasonal differences). The observed epizootic heterogeneity likely reflects the influence of regional ecosystems, climatic conditions, and livestock management practices. Seasonally, seroprevalence declined from spring (43.1%) to autumn (29.3%), consistent with *Hyalomma* biological cycle. After overwintering, adult ticks are more active in spring, increas-



**Figure 2.** Seasonal distribution of CCHF virus antibody seroprevalence in cattle across 14 districts of the Turkestan region, Kazakhstan (2024)

ing the probability of virus transmission to animals, whereas by autumn, transmission declines. This seasonal pattern likely reflects changes in tick activity and contact risk. *Hyalomma* ticks, the principal vectors of CCHFV, are most active in spring and early summer, which correlates with higher antibody detection during this period. Conversely, tick populations tend to decrease in late summer and early autumn due to environmental factors such as temperature, humidity, and vegetation cycles. The statistically significant reduction in antibody detection in autumn likely reflects reduced contact intensity after the spring transmission peak.

District-level differences confirm the localized nature of transmission: in Kazygurt District, seropositivity consistently reached the highest levels (76.7% in spring and 50.0% in autumn); in Baidibek and Ordabasy districts, it

also exceeded 60%, whereas in Maktaaral and Tulkubas districts, it was  $\leq 16.7\%$ . These spatial trends demonstrate geographic clustering of risk and underscore the need for surveillance and preventive measures tailored to individual districts.

Logistic regression showed that age is a statistically significant and independent risk factor: Adult animals were 4.06 times more likely to be seropositive than juveniles (OR=4.06; 95% CI, 2.62%, 6.29%;  $P=0.001$ ). Although seropositivity was somewhat higher in females than in males (38.3% vs 30.0%), this difference was not statistically significant (OR=1.37; 95% CI, 0.95%, 1.98%;  $P=0.11$ ). Thus, age reflects cumulative exposure to the virus, whereas sex plays a secondary role and is likely linked to herd demography.

**Table 1.** Seroprevalence and logistic regression study of risk variables for CCHF virus in cattle throughout the Turkestan region

Risk Factors	Total Samples	Total Positive	Apparent Prevalence (%)	True Prevalence (%)	OR (95% CI)
Age	Adult	661	277	41.90	4.06 (2.62, 6.29)
	Juvenile	179	27	15.08	
Gender	Female	676	254	37.57	1.37 (0.95, 1.98)
	Male	164	50	30.49	

CCHF: Crimean-Congo hemorrhagic fever.



Because domestic studies have not examined seroepidemiological features by age, sex, and season, we compared our results with international research that analyzed these risk factors in greater detail.

Our age-related findings agree with results from other endemic regions. In Iran, [Lotfollahzadeh et al. \(2011\)](#) reported seropositivity of 78.8% in cattle older than 3 years versus only 21.2% in animals younger than 2 years, indicating cumulative exposure to tick bites. In Mali, [Diakite et al. \(2024\)](#) found similar patterns: Seropositivity was 49.1% in adult cattle and 34.8% in juveniles (OR=1.73). In Mauritania, [Schulz et al. \(2021\)](#) reported seropositivity exceeding 90% in animals older than 5 years, corroborating the universal importance of age as a risk factor. These observations are consistent with our results, which showed that adult animals were 4 times more likely to be seropositive than juveniles.

Sex was not a statistically significant determinant in our study, although females showed slightly higher values. Similar conclusions were reached in Iran ([Lotfollahzadeh et al., 2011](#)) and Uganda ([Nyakarahuka et al., 2023](#)), where sex was not an independent risk factor. However, in The Gambia, [Matthews et al. \(2023\)](#) reported higher seropositivity in female cattle (64.2%) than in males (46.4%), and in Senegal ([Gahn et al., 2024](#)), a comparable trend was observed in sheep and goats. These discrepancies are likely related to herd demography and management practices—females typically remain in herds for breeding longer, rather than to inherent biological differences.

The seasonal and spatial differences identified in our study are also reflected in international publications. We observed a statistically significant decline in seropositivity from spring (43.1%) to autumn (29.3%), which aligns with the *Hyalomma* activity cycle. Similar patterns have been noted in Turkey, where the main period of virus transmission falls in the spring and summer months. In Uganda, [Nyakarahuka et al. \(2023\)](#) demonstrated a direct association between seropositivity and the number of ticks on animals, while African studies more often emphasized geographic mosaics: In The Gambia, [Matthews et al. \(2023\)](#) reported cattle seropositivity ranging from 30% to 84% across villages, and in Mauritania, [Schulz et al. \(2021\)](#) reported 68.7% in cattle and 81% in camels. These data parallel our findings from the Turkestan region, where Kazygurt consistently showed the highest seropositivity, whereas Maktaaral and Tulkubas remained at the lower end.

Taken together, our results are consistent with international evidence, highlighting universal patterns (the influence of age and season, the secondary role of sex) as well as regional specificities (geographic mosaics, very high values in camels in arid Mauritania, and local contrasts among districts within the Turkestan region). For Kazakhstan, these data provide the first comprehensive demonstration of how age, seasonal, and environmental factors interact, underscoring the need to integrate seroepidemiological monitoring with entomological studies and analyses of livestock management practices. Such an approach will enable more effective risk forecasting and the development of biosafety measures within a one health framework.

## Conclusion

This study examined how common the CCHFV is in cattle in the Turkestan region, focusing on differences specific to the area, the time of year, and the types of animals that live there. The findings illustrate the significance of climatic, environmental, and anthropogenic factors in influencing seroprevalence patterns. Efficient surveillance systems, targeted preventive efforts, and cooperation between the veterinary and public health sectors are crucial for controlling the spread of CCHF. To reduce the spread of disease and improve biosecurity in areas where they are common, more research is needed on vector ecology, environmental dynamics, and new management strategies.

Epidemiological surveillance is a crucial tool that must be strengthened not only in Kazakhstan but also in adjacent endemic areas to manage outbreaks effectively. It is essential to educate farmers and private households about the disease, as well as to enhance public awareness of hygiene and tick management. This objective encompasses disseminating information through media, social networks, and advertising channels. Given the potential for CCHF to spread during the pandemic, it is essential to evaluate the risks to populations in endemic areas, standardize diagnostic methods, and enhance cross-border collaboration. The precise identification of the virus, dependable monitoring, and quantitative evaluation of the viral load are crucial for diagnostics. The advancement of multiplex PCR assays and the incorporation of digital PCR as a supplementary method are advised. Molecular identification techniques for the virus and modern bioinformatics strategies will improve outbreak analysis, epidemiological surveillance, and the study of how viruses change over time. They will also lay the groundwork for future research.

## Ethical Considerations

### Compliance with ethical guidelines

Experimental studies involving animals were conducted in strict accordance with national and international standards governing the care and use of laboratory animals. The study protocol was approved by the Animal Experiments Ethics Committee of [Research Institute for Biological Safety Problems \(RIBSP\)](#), Gvardeyskiy, Kazakhstan (Code: 0909/25). No animals were injured during sampling. Data regarding the study materials were obtained with participants' consent. Every cow owner engaged in the study by completing the necessary consent form. The study was presented, and consent was acquired.

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### Authors' contributions

Conceptualization and formal analysis: Lespek Kutumbetov; Data curation: Talshyngul Tlenchiyevaa, Balzhan Myrzakhmetovaa, and Lespek Kutumbetov; Methodology: Lespek Kutumbetov, Balzhan Myrzakhmetovaa, and Kuandyk Zhugunisso; Investigation: Talshyngul Tlenchiyevaa, Aiganym Tussipovaa, Gulzhan Zhaparovaa, Karina Bissenbayevaa, Aslan Kerimbayev, and Sergazy Nurabayev; Project administration: Aslan Kerimbayev and Sergazy Nurabayev; Visualization and writing the original draft: Talshyngul Tlenchiyevaa; Review and editing: Talshyngul Tlenchiyevaa and Aiganym Tussipovaa; Final approval: All authors.

### Conflict of interest

The authors declared no conflict of interest.

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