

Epizootic situation of brucellosis in cattle and small ruminants in Kazakhstan (2020-2023) and assessment of serological diagnostic performance and post-vaccination antibody responses

Yerden Shakibayev ^{1*}, Bibizada Orynbaeva ², Maral Kadyrkulova ³, Gulzira Kydyrova ¹, Galina Boiko ⁴

¹ Brucellosis Laboratory, Kazakh Scientific Research Veterinary Institute, Almaty, Republic of Kazakhstan

² Department of Veterinary Medicine, M. Auezov South Kazakhstan Research University, Shymkent, Republic of Kazakhstan

³ West-Kazakhstan Innovative-Technological University, Uralsk, Republic of Kazakhstan

⁴ National Reference Center for Veterinary Medicine of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan, Almaty, Republic of Kazakhstan

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* Corresponding author:

Yerden Shakibayev

Email: yshakibayev8@outlook.com

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Abstract

The aim of the study was to investigate the epizootic situation of brucellosis in Kazakhstan and to assess selected serological diagnostic methods and post-vaccination antibody responses used for brucellosis control in farm animals. Monitoring data and laboratory serological techniques were used to evaluate the epidemiological situation and post-vaccination antibody responses in experimental animals. Monitoring data for 2020-2023 showed that most cases of animal brucellosis were recorded in the northern regions (Kostanay and North Kazakhstan) and southern regions (Zhambyl and Almaty). Regardless of geographical location, small ruminants accounted for the highest proportion of infected animals, reaching 4.2% of all examined animals, whereas morbidity among cattle did not exceed 0.8-1.2%. In the analysed samples, the enzyme-linked immunosorbent assay and complement fixation test demonstrated sensitivity values of 95-100% and specificity of 95%. However, because of the limited sample size, these estimates should be interpreted cautiously. Vaccine preparations based on *Brucella abortus* and *Brucella melitensis* strains induced detectable humoral antibody responses that persisted for up to 12 months; however, protective efficacy was not assessed. These findings provide valuable information on the current epizootic situation and selected serological diagnostic methods and post-vaccination antibody responses in brucellosis control in Kazakhstan.

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1. Introduction

Brucellosis is a zoonanthroponotic infection, which has been endemic in Kazakhstan since 1930. At the same time, more than 1,300 cases of human brucellosis are registered annually in the country (Charypkhan and Rüegg 2022), which makes this disease a significant public health concern. The World Health Organization estimated that approximately 500,000 confirmed cases of human brucellosis are reported annually worldwide (WHO 2020). Therefore, monitoring the incidence of brucellosis in animals and humans and research on the prevention of this disease is an urgent and priority areas of research programmes in Kazakhstan. Forbes Kazakhstan (2023) reported that in 2023, only 17.5 thousand infected animals were detected, indicating a reduction in brucellosis incidence among farm animals compared to 2022, where an opposite trend was observed in the general population. Two species, *Brucella abortus* and *B. melitensis*, are responsible for most human cases. Human infection occurs mainly through the alimentary route, particularly through the consumption of contaminated milk and dairy products that have not undergone heat treatment (Shevtsov et al. 2023; Raushan et al. 2023). Although improved veterinary and sanitary control of cattle products has reduced the risk of transmission of *B. abortus*, the persistence of *B. melitensis* in sheep and goats remains the major public health concern in Kazakhstan because of their continued circulation in endemic regions (Taipova et al. 2023). Though the

distribution of the disease across the country is heterogeneous, in brucellosis-unfavourable regions, the actual incidence may be 10 to 25 times higher than officially reported, which contributes to the further spread of infection among the population of Kazakhstan (Taipova et al. 2023).

Infection prevalence of epidemiological units with brucellosis in regions such as Aktobe, Kostanay, Mangistau, Atyrau, Akmola, and West Kazakhstan exceeded 23.4% (Abutalip et al. 2024). The reason for such a higher prevalence of the disease among the population and animals in these regions could be the relative change in the ratio of the number of farm animals in large, collective and small, and individual farms, where the level of veterinary care differed significantly (Aikimbayev et al. 2021). According to Urazayeva et al. (2018), a similar situation is observed in the neighbouring countries of Kazakhstan – Iraq, Tajikistan, Iran and Kyrgyzstan, where private farms with a low level of veterinary care prevail. These factors contribute to the fact that brucellosis continues to pose a threat to public health in Kazakhstan. Moreover, the presence of epizootic foci of brucellosis on the territory of the Republic of Kazakhstan and other regions of the Central Asian region according to the studies of Mamutova et al. (2020) with a low level of preventive measures creates favourable conditions for infection among the working age population, as well as children and adolescents who are not professionally associated with animal husbandry. The decrease in anti-epidemic measures for brucellosis among the

population also contributed to this. In 2023, cases of the disease were registered in North-Kazakhstan, East-Kazakhstan, West-Kazakhstan, Kostanay, Zhambyl, Pavlodar, Kyzylorda regions and Zhetysay region (Dairy News Today 2023). While in Almaty, Turkestan and Karaganda regions, where most livestock are located in large farms under strict veterinary supervision, the incidence rates did not exceed 3.1%. These observations suggest that systematic control of infection and regular preventive measures may contribute to reducing the incidence of brucellosis in animals and humans.

Accurate diagnosis remains one of the key components of brucellosis control. Previous studies have reported variable diagnostic performance of serological methods, including the Rose Bengal test, complement fixation test (CFT), lateral flow test, and enzyme-linked immuno sorbent assay (ELISA). Differences in sensitivity and specificity have been reported for different *Brucella* species, indicating the need for further assessment of diagnostic approaches (Guzmán-Bracho et al. 2020; Bulashev et al. 2023). Vaccination is an important component of brucellosis control programmes. In many endemic countries, live attenuated vaccines based on *B. abortus* strain S19 and *B. melitensis* strain Rev.1 are used for cattle, sheep, and goats (Heidary et al. 2022). Previous studies have reported favourable immunogenic characteristics of these vaccines, although cases of infection with vaccine strains and other limitations have also been described (Liu et al. 2022; Hassan et al. 2020). In addition to its impact on animal and human health, brucellosis causes considerable economic losses in livestock production. Previous studies indicate that effective control requires integrated approaches combining surveillance, diagnostics, vaccination, culling of seropositive animals, environmental hygiene, and personal protective measures (Gong et al. 2023; Nyerere et al. 2020; Bahmani and Bahmani 2022; Ma and Sun 2024). Nevertheless, the disease remains endemic in many regions of Asia and Africa, and inadequate veterinary surveillance, uncontrolled animal movements, consumption of unpasteurised dairy products, and insufficient coordination between veterinary and public health services contribute to its persistence (Abu Sulayman et al. 2020; Bagheri Nejad et al. 2020).

Recent international initiatives also emphasise the importance of coordinated approaches to brucellosis control in Kazakhstan. In 2025, a technical mission led by the Food and Agriculture Organization (FAO 2025) under the One Health framework involved consultations with national stakeholders and identified key gaps and opportunities for improving surveillance, diagnostics, and intersectoral cooperation. These activities highlight Kazakhstan's participation in regional and global efforts to strengthen brucellosis control and reduce zoonotic risks. Due to the continuing burden of brucellosis in Kazakhstan, the aim of the present study was to investigate the epizootic situation of brucellosis in cattle and small ruminants and to evaluate selected diagnostic and vaccination approaches used in disease control. For this purpose, the distribution and intensity of the epizootic process in brucellosis-affected settlements were analysed, and post-vaccination antibody responses in experimental animals were assessed.

2. Materials and Methods

This study employed a mixed design combining descriptive epidemiological surveillance, comparative assessment of selected serological diagnostic methods, and an experimental evaluation of post-vaccination antibody responses in animals. The epizootic situation of brucellosis in the Republic of Kazakhstan was investigated using

materials from the Bureau of National Statistics (2023), the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan (2023), the Committee of Sanitary and Epidemiological Control of the Ministry of Healthcare of the Republic of Kazakhstan (2024), as well as monitoring data collected by the staff of the Kazakh Veterinary Research Institute during routine and emergency visits to affected localities in different regions of Kazakhstan.

During the period from 2020 to 2023, 46 outbreaks of brucellosis in humans and animals were observed in 11 regions of the Republic of Kazakhstan. Outbreaks were selected based on official reports of confirmed brucellosis cases provided by veterinary and sanitary authorities. A convenience sampling strategy was used in confirmed outbreak areas; therefore, the findings represent affected localities rather than the national livestock population. When a diagnosis of brucellosis was confirmed, all animals were sampled to determine the intensity of infection and its spread in the controlled area. All susceptible farm animals located within the outbreak focus were examined. No formal sample size calculation was performed because the study analysed all available confirmed outbreaks during the study period. A total of 3,893 animals were examined during the study period, including 2,040 cattle, 1,820 small ruminants, and 33 other types of agricultural and domestic animals.

One of the principal methods used for the identification of infected animals was ELISA testing for the presence of specific antibodies in blood serum. For this purpose, a commercial test kit Ingezim *Brucella* Compac 2.0 from Immunologia y Genetica Aplicada S.A. (Spain) was used. Blood samples were collected in sterile vacuum tubes, and serum was obtained after sedimentation. A commercial multispecies test system allowed the determination of antibody levels in the blood of cattle, sheep, goats, pigs, and other farm animals. The ELISA was performed using a Wellwash Versa microplate washer and a Multiskan FC microplate photometer (Thermo Fisher Scientific, Finland). Complement Fixation Test (CFT) was performed according to a standard procedure recommended by the World Organisation for Animal Health (2021). For comparative purposes, ELISA and CFT results were evaluated using descriptive measures of sensitivity and specificity.

To determine post-vaccination antibody titres, five calves and five lambs aged 6-7 months were selected using the matched-pair principle based on age and body weight. After a one-month acclimatization, they were vaccinated with the appropriate vaccine according to the manufacturers' instructions and recommended dosage schedules. The antibody titre was determined using a commercial *Brucella* Antibody Test Kit, *Brucella* OPS iELISA (USA) (Ellie LLC 2020) and a set of equipment for immunological and serological studies from Thermo Fisher Scientific (Finland).

All procedures involving animals were performed in accordance with national regulations on animal welfare and the manufacturers' recommendations for vaccine administration. The study protocol was approved by the Ethics Commission of the Kazakh Scientific Research Veterinary Institute (Approval No. 02933939). Descriptive statistical analysis was performed using Microsoft Excel 365 and Tibco Statistica 14.0.1 software. Results are presented as numbers and percentages.

3. Results

Since Kazakhstan is a country with an endemic occurrence of

brucellosis, outbreaks of this disease among both animals and humans are frequently detected in different regions of the country. Between 7-8 and 15-20 outbreaks of this disease are registered annually in the country. These findings indicate a significant persistence of the pathogen in the population of farm and possibly wild animals. Insufficient diagnostics associated with the use of less informative serological approaches, particularly agglutination-based tests, may contribute to delayed detection of infected animals. Therefore, accurate and timely laboratory diagnosis remains an important component of brucellosis control. Thus, monitoring all cases of disease in susceptible livestock and humans with obligatory documentation of all veterinary measures taken by sanitary-epidemiological authorities to prevent and eliminate brucellosis forms an important component of brucellosis control. Analysis of confirmed cases of disease in animals and humans in the period from 2020 to 2023, 46 outbreak foci in 11 regions of Kazakhstan were studied. The distribution of brucellosis infection foci is shown schematically in Fig. 1.



Fig. 1. Region-wise distribution of human and animal brucellosis outbreaks in the Republic of Kazakhstan during 2020-2023 (confirmed cases)

The presented map-scheme of the distribution of animal and human cases of brucellosis infection indicates that brucellosis is widespread throughout most of the Republic of Kazakhstan. In recent years, foci of brucellosis infection have been registered in almost all regions except for the western region – Atyrau, Mangistau and Aktope regions, although in previous years outbreaks of the disease were registered here as well. Also, during the observation period, there is a tendency that the bulk of outbreaks of the disease are registered in the northern and southern regions, where the majority of the animal population is concentrated. It should be noted that in these regions there is also a significant concentration of population in Kazakhstan, which also contributes to the spread of brucellosis. When studying the species composition of animal morbidity for brucellosis, the results showed that the disease was mainly registered in small ruminants (Table 1).

On the one hand, the high incidence in small ruminants was due to the fact that sheep and goats are predominantly kept on small private farms where they do not receive adequate veterinary care. Therefore,

brucellosis is more common in this species than in other species. The fact that the total number of sheep and goats is more than 3 times higher than the number of cattle in the country is also an important epizootic factor in the spread of brucellosis among the population of this species, which also increases the likelihood of the disease. These predisposing factors have led to significant infection of small ruminants.

Meanwhile, in the northern regions of Kazakhstan, livestock farming is represented by large collective enterprises that breed dairy cattle and are characterized by strict veterinary supervision. Veterinary regulations provide for regular examinations of both the productive animals and the thermal treatment of all dairy products obtained from cows at milk processing plants. Such approaches are expected to contribute to a lower incidence of disease among livestock. However, a significant number of outbreaks among cows may have been caused by the lack of vaccination of susceptible animals. This is due to the fact that under the terms of the Customs Union, trade in animals that have been vaccinated against brucellosis is prohibited, so veterinary specialists limit preventive measures in breeding farms. Of the other animal species that were also examined for brucellosis infection in the outbreak foci included small numbers of dogs, camels, and pigs. The prevalence of brucellosis among these species was low, with only one animal (dog) out of 33 animals examined.

When analysing the species-specific prevalence of farm animals by geographical distribution, as presented in Fig. 2, it can be observed that both in the northern and southern regions of Kazakhstan, the higher prevalence is observed in small ruminants.

Thus, in the northern regions of the country, the incidence rate in cattle was 0.7% of all animals of this species examined, while in small ruminants it was 2.3%. A similar pattern was observed in the southern regions. The prevalence among cattle was 1.4%, and among sheep and goats it was 4.2%. Thus, brucellosis infection of animals was almost twice as high in the southern regions. These differences may be

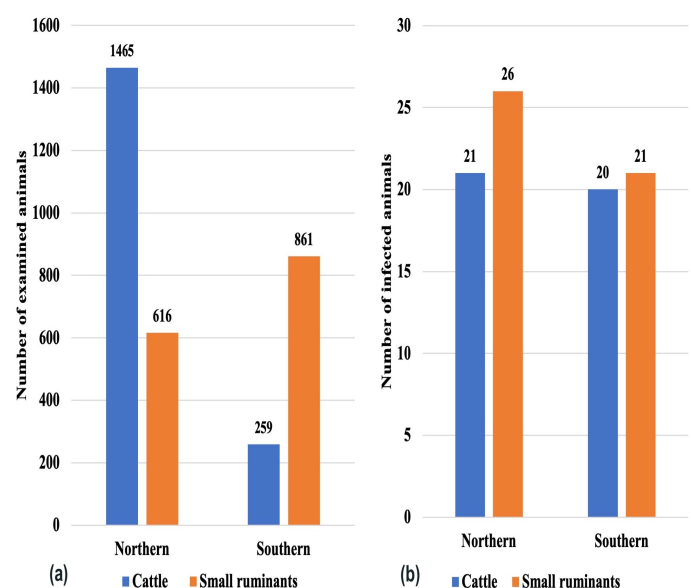


Fig. 2. Distribution of examined and infected cattle and small ruminants in the northern and southern regions of Kazakhstan: (a) examined animals and (b) infected animals

Table 1. Species composition of animals infected with brucellosis in outbreak foci in different regions of Kazakhstan

Area	Animals examined (n)	Infected animals (n)	Prevalence (%)	Cattle (total/infected)	Small ruminants (total/infected)	Other species (total/infected)
Akmola	64	3	4.7%	37/1	24/2	3/0
Almaty	361	7	1.9%	89/1	263/6	9/0
West Kazakhstan	118	4	3.4%	63/2	55/2	0/0
Zhambyl	541	4	4.4%	91/0	443/4	7/0
Karaganda	341	18	5.3%	179/6	162/12	0/0
Kostanay	1,303	26	1.9%	839/9	456/16	8/1
Kyzylorda	149	8	5.4%	53/1	95/7	1/0
Pavlodar	96	5	5.2%	36/2	60/3	0/0
North Kazakhstan	629	14	2.2%	553/9	76/5	0/0
South Kazakhstan	89	3	3.4%	26/0	60/3	3/0
East Kazakhstan	202	9	4.5%	74/5	126/4	2/0
Total	3,893	101	3.8%	2,040/36	1,820/64	33/1

associated with several factors, including variations in livestock density, farm management practices, veterinary surveillance, and the distribution of susceptible animal populations. Further studies are required to clarify the factors contributing to regional differences in disease occurrence.

An important approach to the prevention and elimination of brucellosis in susceptible animals is timely and accurate diagnosis. In the Republic of Kazakhstan, several methods based on antigen-antibody reactions are mainly used to detect antibodies to the causative agent of the disease: CFT, agglutination reaction (AR), Rose Bengal Test (RBT) and ELISA using commercial kits. Owing to their reported diagnostic performance, CFT and ELISA are more widely used in routine practice. In comparative studies (Table 2) of the diagnostic performance, no substantial differences between them were observed.

These studies showed sensitivity values ranging from 95% to 100% and specificity values of 95%, with a low false-positive rate. In both cases of testing with different serological methods, the same sample from a healthy animal (previously tested by polymerase chain reaction as the most accurate diagnostic method and given brucellosis-free status) showed a doubtful result. Such a result may indicate the presence of sensitization of the antigen-antibody system by the presence of non-pathogenic forms of microorganisms belonging to the Brucellaceae, namely the genus *Ochrobactrum* and having similar immunogenic characteristics. Therefore, studies of this phenomenon, which may significantly influence the quality of diagnostic results, may be a future direction of research in the diagnosis and prevention of brucellosis in Kazakhstan.

Thus, both methods demonstrated satisfactory diagnostic performance under the conditions of the present study and can be used to identify infected animals in the outbreak foci. However, considering the fact that the technique of ELISA includes significant automation of the diagnostic process, namely the availability of devices such as washer, thermo-shaker and photometer from different manufacturers, as well as robotic systems for enzyme immunoassay, this method is more attractive in comparison with the CFT. In addition, the results of an ELISA are automatically recorded by determining the optical density difference between the negative and positive control samples included in the ELISA reagent kit and then comparing them with the result obtained from the test animal. Meanwhile, most manufacturers of laboratory equipment and commercial tests offer software to simplify the evaluation of the test results and their statistical processing. This makes the immunoassay method more objective compared to the subjective evaluation of the CFT. Therefore, ELISA may offer practical advantages for rapid diagnosis because of the greater potential for automation and standardisation of result interpretation.

Vaccination remains an important component of brucellosis control in Kazakhstan, although its implementation may be restricted in some breeding farms because of Customs Union regulations affecting trade in vaccinated animals. Taking into account the fact that in Kazakhstan there are cases of both animal and human diseases caused by two types of brucellosis – *B. abortus* and *B. melitensis*, different biopreparations are used for vaccine prophylaxis of cattle and small ruminants. The immunological properties of the following biological preparations for active immunization of animals were analysed: vector vaccine against brucellosis of cattle (Kazakhstan), vaccine Brucon (SPE “Antigen” LLP,

Table 2. Comparative results of sensitivity and specificity of CFT and ELISA methods in the diagnosis of brucellosis

Blood serum samples	Compliment Fixation Test (CFT)		Enzyme-Linked Immunosorbant Assay (ELISA)	
	Positive tests (%)	Negative/doubtful tests (%)	Positive tests (%)	Negative/doubtful tests (%)
From animals with confirmed diagnosis of brucellosis, n=20	95% (19 / 20)	5% (1 / 20)	100% (20/20)	0
From healthy animals, n=20	5% (1 / 20)	95% (19 / 20)	5% (1 / 20)	95% (19 / 20)

Note: Owing to the limited number of samples (20 positive and 20 negative sera), only descriptive estimates of sensitivity and specificity are presented. Confidence intervals and agreement statistics were not calculated

Kazakhstan) and two variants of vaccine Bruvac for conjunctival and subcutaneous administration. The levels of post-vaccinal immunity to the administration of these vaccines are shown in Table 3.

The conducted study indicates that all the biological preparations used for vaccination of animals have a significant immunological effect and induced detectable antibody responses in both cattle and small ruminants. Vaccines for immunization of cattle are made from attenuated strains of *B. abortus* S19. Both vaccines already on day 21 induced detectable antibody titres that increased up to 6 months after vaccination. The live dry vaccine against bovine brucellosis from *B. abortus* strain S19 was associated with higher mean antibody titres throughout the observation period. Twelve months after vaccination, the mean antibody titre remained higher than that observed in animals receiving the vector vaccine. In addition, the use of this vaccine is less stressful for the animals and detectable antibody titres persisted for up

to 12 months.

For the vaccination of sheep and goats, two variants of the vaccine Bruvac from the Kazakh manufacturer Scientific-Production Enterprise “Antigen” developed from the attenuated *B. melitensis* Rev.1 strain. Higher antibody titres after vaccination were observed after subcutaneous administration of this vaccine. Detectable antibody titres after subcutaneous administration persisted throughout the 12-month observation period. When the conjunctival route of vaccine administration was used, antibody responses were detected early after vaccination, but lower mean antibody titres were observed after conjunctival administration. Moreover, lower antibody titres were observed after 6 months compared with earlier time points. Earlier decline in antibody titres may facilitate earlier serological monitoring after vaccination. Thus, the results indicated that diagnostic methods currently used in Kazakhstan enable the identification of infected

Table 3. Post-vaccination antibody titres at different time periods in animals following administration of selected brucellosis vaccines

Vaccine	Animal	21 days	3 months	6 months	12 months
Vaccines for immunization of cattle based on <i>B. abortus</i> strains					
Vector vaccine against brucellosis of cattle (Kazakhstan), based on <i>B. abortus</i> strain S19	1	19.3	24.2	31.6	6.9
	2	21.5	23.9	33.7	8.4
	3	22.7	27.8	29.4	9.3
	4	16.4	17.3	26.7	8.9
	5	23.1	27.3	31.8	8.6
	Average	20.6±1.24	24.1±1.87	30.6±1.19	8.4±0.41
Brucon live dry vaccine against bovine brucellosis based on <i>B. abortus</i> strain S19 for conjunctival administration	1	21.5	34.5	38.7	11.6
	2	25.6	38.4	39.5	13.7
	3	19.7	24.1	33.6	16.5
	4	26.3	37.5	37.9	11.8
	5	22.4	29.7	31.4	17.4
	Average	23.1±1.25	32.8±2.66	36.2±1.58	14.2±1.19
Vaccines for immunization of small ruminants based on <i>B. melitensis</i> strains					
Bruvac live dry vaccine for conjunctival administration based on <i>B. melitensis</i> strain Rev.1	1	16.3	27.4	15.3	3.6
	2	14.9	22.1	13.1	3.8
	3	13.8	19.4	15.3	2.6
	4	11.4	14.1	12.4	4.1
	5	17.9	27.4	14.3	3.5
	Average	14.7±1.10	22.1±2.52	14.1±0.58	3.5±0.25
Bruvac live dry vaccine for subcutaneous administration from <i>B. melitensis</i> Rev.1 strain	1	21.7	32.3	34.5	18.5
	2	21.5	27.5	31.4	21.6
	3	22.1	29.4	30.7	19.6
	4	23.4	31	33.8	18.7
	5	19.5	26.8	27.5	16.8
	Average	21.6±0.63	29.4±1.03	31.6±1.24	19.0±0.78
Note: Values represent relative antibody titre units obtained using the Brucella OPS iELISA kit. No validated protective antibody titre threshold was defined, and antibody titres were used only as indicators of humoral immune response. Therefore, these values should not be interpreted as direct evidence of protective immunity. No challenge infection or protective efficacy assessment was performed					

animals, whereas the evaluated vaccines were associated with detectable post-vaccination antibody responses in cattle and small ruminants. These findings provide additional information on selected diagnostic and vaccination approaches used in brucellosis control in Kazakhstan.

4. Discussion

The endemic nature of brucellosis in Kazakhstan is accompanied by continuing occurrence of the disease among the population of the country, which makes research aimed at improving disease control relevant and a priority. The initial stage of such work is monitoring studies, the purpose of which is not only the registration of disease foci, but also collection of data necessary for understanding the mechanisms of persistence of the pathogen in the population of susceptible animals, mechanisms of its transmission among animals and from animals to humans. Studies conducted as part of this work indicate that the highest disease occurrence in Kazakhstan for the period from 2020 to 2023 was in the northern and southern regions of the country. This is due to the presence of large agricultural enterprises or a significant number of livestock that are compactly located in small areas, which may facilitate the disease transmission. Despite the fact that there are different types of susceptible farm and domestic animals in these areas, the disease predominantly occurred in small ruminants. The predominance of brucellosis among small ruminants observed in the present study suggests that these species may play an important role in maintaining the epizootic process under endemic conditions. Among other animal species, one case of a dog was also detected in the disease focus. Such a high prevalence in sheep, on the one hand, can be explained by the largest population of this species in the country, but the proportion of infected sheep was higher in other regions as well, where other animal species were outnumbered. Regional differences in the distribution of infected animals may reflect variations in livestock structure and farming practices.

High susceptibility of small ruminants to brucellosis is also confirmed in the works of other researchers. In particular, in the studies of Ma et al. (2022), the authors use a sheep model to study the effect of different control measures on brucellosis transmission in China, and in the work of Akinyemi et al. (2022), the authors indicate that this particular animal species is predominant among patients in Nigeria. The relatively low proportion of seropositive animals in outbreak foci may indicate that disease expression is influenced by host immunity and pathogen virulence. Adem and Duguma (2020) suggested that the development of the infectious process is associated with the level of immunity in the affected population and the pathogenicity of the pathogen itself. According to the protocol, the immunological status of animals in the brucellosis outbreak focus of infection was not given due attention in the studies conducted, so this point will be taken into account in future studies to clarify all factors in the development of the epizootic process. In addition, many cases were recorded in border regions. However, the present study did not specifically investigate the role of transboundary transmission. Regional differences may also reflect variations in livestock density, management practices, and veterinary surveillance. Further studies are needed to clarify these factors. This fact is also confirmed in the works of Kydyshov et al. (2022), who indicate that even though the coverage of the country's livestock by diagnostic testing was low, the number of positive animals in Kyrgyzstan was 0.35%. Also in China, in the border areas with Kazakhstan, there is a high incidence of the disease in both animals and

the population. Sun et al. (2023) indicate that a high incidence of brucellosis in the population was observed mainly in northern China, especially in Inner Mongolia, Shanxi and Heilongjiang.

The next stage of the study was to analyse the efficiency of diagnostic approaches in controlling the spread of the disease among animals in the disease focus. For this purpose, serological methods based on antigen-antibody interaction were used as one of the most rapid and inexpensive diagnostic approaches. This was also pointed out by such researchers as Di Bonaventura et al. (2021), whose work supports the information that serological tests remain the main diagnostic tool, especially in endemic areas, because they are inexpensive and have a high predictive value of a negative result. The studies used ELISA and CFT. The studies did not use approaches that had low sensitivity and specificity – AR and RBT, as confirmed in the work of Alamian et al. (2023). The observed diagnostic performance of ELISA and CFT was generally consistent with previous reports, supporting their continued use in endemic settings. Similar results were reported by Yin et al. (2021) using ELISA. However, the ELISA may offer practical advantages due to the possibility of automation and more objective interpretation of results. At the same time, the diagnostic comparison was based on a limited number of samples and relied only on descriptive estimates of sensitivity and specificity. Therefore, the results should not be interpreted as a formal validation of these methods. From a practical perspective, a specificity of 95% implies that false-positive reactions may occur in a proportion of non-infected animals. In large herds and regions with extensive livestock populations, such reactions may result in unnecessary culling and additional economic losses if serological findings are interpreted without confirmatory testing. False-positive serological reactions remain a recognised limitation of brucellosis diagnostics and may arise because of cross-reactivity and the use of common lipopolysaccharide antigens in serological assays (Bányász et al. 2023). Therefore, serological results should be interpreted together with epidemiological information and, when necessary, additional diagnostic procedures.

Nevertheless, the application of vaccination in breeding animals may be constrained by trade regulations within the Customs Union, which limits the use of preventive vaccination in some herds. These restrictions create a practical challenge for disease control because breeding farms participating in animal trade may rely primarily on surveillance and culling strategies instead of routine vaccination. As a result, balancing disease prevention with compliance with trade requirements remains an important policy issue in Kazakhstan and may require coordinated approaches combining diagnostics, movement control, and targeted vaccination programmes, which is consistent with current One Health recommendations for brucellosis control (Dadar et al. 2021). For this purpose, vaccines prepared from cultured *B. abortus* strain S19 are used in Kazakhstan for vaccination of cattle, and *B. melitensis* strain Rev.1 for small ruminants. Two vaccines for cattle – vector vaccine against brucellosis of cattle (Kazakhstan) and live dry vaccine from *B. abortus* strain S19 were used in studies on post-vaccination antibody responses. The persistence of post-vaccination antibody responses observed in the present study is consistent with previous reports describing the immunogenicity of S19-based vaccines. The slightly higher mean antibody titre for the live vaccine may have been due to the strong humoral immune response to the live brucellae used to prepare this vaccine. Sustained antibody responses during the observation period suggest that these vaccines are capable of inducing

prolonged humoral responses, although direct protective efficacy was not evaluated. Previous studies have also reported favourable results of vaccine preparations used for brucellosis prophylaxis in cattle. Similar findings were reported by Khurana et al. (2021). Furthermore, in the studies of Heidary et al. (2022), the authors indicate that vaccines from *B. abortus* strain S19, despite the high titre of agglutinins, which interfere with standard serodiagnostic tests, can reduce the level of abortions in cows. In addition, no validated antibody titre threshold corresponding to protective immunity was established. Therefore, antibody titres were used only as indicators of humoral response and should not be considered validated surrogate markers of protection.

The vaccines used for vaccination of small ruminants in the experiments contained the same *Brucella* strain – Rev.1, and differed only in the method of administration – conjunctival and subcutaneous. Both routes of administration were associated with measurable humoral responses, although differences in the duration of antibody persistence were observed. Earlier decline of antibody titres after conjunctival administration may reflect the influence of mucosal immune mechanisms and deserves further investigation. While in the study of Darbandi et al. (2022), a similar method of administration of vaccine preparations was on a par with the intraperitoneal method of administration and had the maximum protective effect. Therefore, the influence of the route of administration on the magnitude and duration of antibody responses requires further investigation. Studies are also planned to investigate vaccine responses under conditions of host immune status with simultaneous infection with *B. abortus* and *B. melitensis*. The interpretation of these observations should also take into account the limited number of animals included in the vaccination experiment, which restricted statistical comparisons and the generalisability of the obtained results. These findings also highlight the importance of a One Health approach, which requires coordinated actions between veterinary and public health services, continuous surveillance, vaccination programmes, and control of zoonotic transmission. Strengthening cooperation between animal and human health sectors remains important for reducing the burden of brucellosis in Kazakhstan. Overall, the present findings support the importance of integrated surveillance, diagnostics, and preventive measures for brucellosis control in Kazakhstan, while also indicating the need for further optimisation of these approaches.

5. Conclusions

Based on the results of the epizootological monitoring of brucellosis in farm animals in Kazakhstan and the assessment of selected diagnostic and preventive approaches, it can be concluded that the main areas of disease occurrence are located in the northern (Kostanay and North-Kazakhstan) and southern (Zhambyl and Almaty) regions, which are associated with high livestock density. At the same time, there is a predominant spread of the disease among small ruminants in both northern and southern regions with a prevalence range of 2.5-4.2% among the examined livestock, while the prevalence in cattle did not exceed 0.8-1.2%. The evaluated ELISA and CFT methods showed high observed sensitivity and specificity values in the analysed samples. The ELISA method had a sensitivity level of 100% and a specificity level of 95%, while the CFT had a sensitivity level of 95%. The vaccine preparations used for active immunisation induced detectable antibody responses that persisted for up to one year in the observed animals. The obtained results characterise selected diagnostic and vaccination approaches assessed in the present study and may contribute to further

improvement of brucellosis control strategies. The vaccination experiment involved a limited number of animals and assessed only post-vaccination antibody titres. Therefore, the findings should not be interpreted as direct evidence of protective efficacy or long-term immunity. Future studies should also assess vaccine responses and disease outcomes under field conditions where exposure to *B. abortus* and *B. melitensis* may occur. Further studies are also required to evaluate additional diagnostic approaches using larger sample sizes.

Declarations

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