



Detection of *V. melitensis* and *B. abortus* Culture through Bacteriological Investigations Common in Kostanay Region

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ABSTRACT

This study investigated the prevalence of *Brucella abortus* and *Vibrio melitensis* pathogens among farm animals in the Kostanay region of Kazakhstan in 2024. A total of 200 animals (120 cattle and 80 sheep/goats) were sampled from various farms. Blood and tissue samples were analyzed using bacterial culture, serological agglutination tests, and PCR for the detection of pathogens. Results showed that 45% of cattle, 40% of sheep, and 40% of goats were infected, with the highest infection rates observed in animals aged two to five years. Younger animals had a significantly lower infection rate. Serological tests indicated 46% seropositivity for *Brucella abortus* in cattle and 38% for *Vibrio melitensis* in sheep/goats. PCR confirmed pathogen presence in 43.3% of cattle and 38.75% of small ruminants. These findings underscore the importance of regular monitoring, vaccination, and enhanced animal welfare to reduce infection rates and mitigate economic losses in the region.

Key words: Zoonotic infections, Serological tests, Molecular genetic analysis, Pathogenic microflora, PCR diagnostics.

INTRODUCTION

Controlling and diagnosing zoonotic infections like brucellosis and vibriosis remains a major challenge in Kazakhstan's livestock industry, particularly in the Kostanay region. Brucellosis caused by *Brucella abortus* and *vibriosis* is caused by *Vibrio melitensis* those result in significant economic losses and pose health risks to humans. The lack of modern diagnostic and prevention methods, combined with low farmer awareness and limited access to affordable monitoring programs, worsens the situation.

One of the key issues is the low efficiency of traditional diagnostic methods in the early stages of brucellosis (Yespebetov et al. 2019). Shevtsov et al. (2023) reported up to 40% seropositivity for *Brucella abortus* in cattle in Kazakhstan, using agglutination tests that fail to detect infection in asymptomatic animals, allowing the disease to spread. This problem is exacerbated by irregular animal monitoring. Jakipov et al. (2021) confirmed the general effectiveness of serological tests but noted their limitations in early detection and the practical challenges of regular testing in remote areas. Low farmer awareness of preventive measures and diagnostics also remains a concern (Abutalip et al. 2017). Although several

studies call for greater awareness and improved practices, they often overlook the role of modern molecular diagnostics. Adambayeva et al. (2022) demonstrated the value of PCR for early detection of *Brucella abortus*, although their study's small sample size limited its broader applicability. Wider-scale research is needed for regional implementation.

Vibriosis in small ruminants is another major concern (Kirimbayeva et al. 2023). Berdimuratova et al. (2022) reported infection rates of up to 50% in some regions, particularly where monitoring and vaccination efforts were lacking. However, they did not examine age-related susceptibility, which could improve targeted prevention strategies. The cost of modern diagnostics also poses a barrier. Although combining PCR and serological testing has been shown to reduce infection rates, it remains unaffordable for many small farms (Abutalip et al. 2024). Kurmanov et al. (2022) emphasized the value of integrating serological and molecular methods for better accuracy, although their study did not assess the economic feasibility of such an approach for broad adoption.

The study aimed to investigate the prevalence of *Brucella abortus* and *Vibrio melitensis* pathogens among cattle and small ruminants in the Kostanay region. The objectives of the study included analysis of the prevalence

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of these infections among farm animals, study of the level of infection among different age groups of animals and development of recommendations to improve methods of monitoring and prevention of infections to reduce morbidity in the region

MATERIALS AND METHODS

The study took place in Kostanay region, Kazakhstan, from January to December 2024. A total of 200 animals, 120 cattle and 80 sheep/goats, were randomly selected from farms in ecologically significant zones and grouped by age (1-5 years). This ensured representative sampling for evaluating the prevalence of *Vibrio melitensis* and *Brucella abortus*. Blood was collected via jugular venipuncture using sterile needles and 10 mL syringes. Lymph nodes from deceased or culled animals were sampled with sterile instruments. All samples were stored in transport containers at 4°C. In the Veterinary Institute laboratory, specimens were cultured on 5% blood and serum agar (Merck KGaA) at 37°C for 48-72 hours. Colonies were then identified through morphological and biochemical tests. *V. melitensis* identification involved glucose, lactose, and mannitol fermentation, as well as catalase and oxidase tests (BioMerieux). *B. abortus* identification used urease, nitrate reductase tests, and growth on thioglycolate agar. Microscopic analysis was performed using a Nikon Eclipse E100. Serological confirmation employed Wright's agglutination and complementary tests (Sigma-Aldrich). PCR analysis (Qiagen reagents; Applied Biosystems thermocyclers) was used to confirm bacteriological results, ensuring accurate detection of *V. melitensis* and *B. abortus*. Statistical analysis was done with SPSS v27, including means, standard deviations, and Student's t-tests ($P < 0.05$). Results were visualised using GraphPad Prism.

All procedures followed the European Convention for the Protection of Domestic Animals and the Universal Declaration on the Humane Treatment of Animals (Lounes et al. 2021; Chang et al. 2023).

RESULTS

Bacteriological studies in the Kostanay region revealed a significant presence of *Brucella abortus* and *Vibrio melitensis* among farm animals. A total of 200

animals were tested, including 120 cattle and 80 sheep and goats. *Brucella abortus* was detected in 45% of cattle, with a higher prevalence in animals aged two to three years (46.7%). Infected cattle showed symptoms such as loss of appetite, reduced productivity, and general health decline. These findings underscore the significant epidemiological threat posed by these pathogens, accompanied by substantial economic risks for farms in the region.

Infection of sheep and goats with the pathogen *Vibrio melitensis* was also significant, covering 40% of the sample, representing 32 out of 80 animals. In this group, the highest percentage of infections was recorded among animals in the two to four years age group, representing 42.5% of the total number of animals examined in this category. This suggests that adult animals are at a greater risk of infection, likely due to prolonged contact with sources of infection on farms. Animals in the younger age group (under one year of age), both among cattle and small ruminants, deserve special attention. Infection rates among young animals were significantly lower compared to older animals. The rate was 33.3% in cattle and 25% in sheep and goats. These data suggest that young animals either have limited contact with pathogens at an early age or their immune system is more successful in resisting infection at this stage of development. This observation underscores the significance of the age factor in evaluating the epidemiological situation and the necessity of developing targeted preventive measures based on the age of the animals.

The distribution of infections by animal age groups demonstrated that adult animals, especially those of productive age (two to five years), are at the highest risk of infection with both *Brucella abortus* and *Vibrio melitensis* (Table 1). This is possibly related to the fact that such animals, being involved in active farming activities, are exposed to other infected individuals, as well as to contaminated feed and water. In addition, the physiological characteristics of adult animals may make them more susceptible to infections.

In addition, serological studies have shown that some animals may have developed antibodies after an infection, even if an active infection is no longer present (Table 2). This is especially relevant for animals that have been prophylactically vaccinated. In such cases, seropositivity could indicate not an active infection, but an immune defence developed after previous exposure to the pathogen.

Table 1: Distribution of infection among animals by species and age group

Animal species	Age group	Percentage of <i>B. abortus</i> infections (cattle)	Percentage of <i>V. melitensis</i> infections (sheep and goats)
Cattle	1 year and under	33.3	-
Cattle	2-3 years old	46.7	-
Cattle	4-5	41.7	-
Sheep and goats	1 year and under	-	25
Sheep and goats	2-3 years old	-	42.5
Sheep and goats	4-5	-	40

Table 2: Distribution of seropositivity among animals by species and age groups

Animal species	Age group	Seropositivity (%) to <i>B. abortus</i> (cattle)	Seropositivity (%) to <i>V. melitensis</i> (sheep and goats)
Cattle	1 year and under	33	-
Cattle	2-3 years old	48	-
Cattle	4-5	44	-
Sheep and goats	1 year and under	-	25
Sheep and goats	2-3 years old	-	40
Sheep and goats	4-5	-	38

Table 3: PCR diagnostic results among animals of different age groups

Animal species	Age group	Positive (%) PCR results for <i>B. abortus</i> (cattle)	Positive (%) PCR results for <i>V. melitensis</i> (sheep and goats)
Cattle	1 year and under	30	-
Cattle	2-3 years old	45	-
Cattle	4-5	42	-
Sheep and goats	1 year and under	-	25
Sheep and goats	2-3 years old	-	39
Sheep and goats	4-5	-	36

Table 4: Epidemiological picture among animals of different age groups

Animal species	Age group	Percentage of <i>B. abortus</i> infections (cattle)	Percentage of <i>V. melitensis</i> infections (sheep and goats)
Cattle	1 year and under	33.3	-
Cattle	2-3 years old	46.7	-
Cattle	4-5	41.7	-
Sheep and goats	1 year and under	-	25
Sheep and goats	2-3 years old	-	42.5
Sheep and goats	4-5	-	40

Another important feature of molecular studies was the detection of pathogen genes in animals that did not show positive results in bacteriological studies (Table 3). This is associated with the fact that molecular methods can detect pathogen DNA even when the active phase of the disease has passed or the concentration of bacteria in the organism is too low to isolate them by conventional methods. This makes PCR a valuable method for early detection of infections and accurate diagnosis in complex cases.

The importance of the age factor in the spread of infections requires the development of differentiated approaches to prophylaxis depending on the age of the animals (Table 4). Young animals, although less susceptible to infection, also require regular testing to prevent potential infection.

These results highlight the necessity for continuous monitoring and targeted diagnostics, especially for productive-age animals. Implementing preventive programs aimed at these age groups can help reduce infection rates and mitigate economic losses. Serological testing confirmed a high level of exposure to both pathogens. Among cattle, 46% (55 out of 120) were seropositive for *Brucella abortus*, aligning with bacteriological findings and reinforcing the importance of serological testing as a confirmatory method. In sheep and goats, 38% (30 out of 80) were seropositive for *Vibrio melitensis*, indicating a widespread presence of the pathogen despite the absence of clinical signs in some cases. Seropositivity was age-dependent. In cattle, the highest rate (48%) was found in animals aged two to three years, followed by 44% in the four to five-year group and 33% in animals under one year. Among sheep and goats, seropositivity was 40% in two to three-year-olds, 38% in the four to five-year group, and 25% in younger animals. These patterns support previous findings that adult animals are more frequently exposed to infection, possibly through longer contact with contaminated environments or infected individuals. It is also noteworthy that some animals may have recovered from infection, retaining antibodies without having active disease. The high seropositivity among adult animals reinforces the need for sustained diagnostic efforts and long-term monitoring strategies.

PCR testing confirmed previous diagnostic results, detecting *Brucella abortus* DNA in 43.3% of cattle (52/120) and *Vibrio melitensis* genes in 38.75% of sheep and goats (31/80). PCR proved more sensitive than

bacteriological methods, identifying infections even in asymptomatic or test-negative animals. In cattle, *B. abortus* was most common in the 2 to 3 year group (45%), followed by 4 to 5 years (42%) and under one year (30%). Among small ruminants, *V. melitensis* was detected in 39, 36, and 25% of these respective age groups, showing higher infection in adults, likely due to prolonged exposure. The consistency of PCR results with other methods underscores its value for early detection. Overall, 45% of cattle and 40% of sheep and goats were infected, with the highest rates in productive-age animals, 46.7% in 2 to 3-year-old cattle and 42.5% in 2 to 4-year-old sheep and goats, posing a threat to farm productivity. These findings highlight the need for regular PCR-based screening, age-specific prevention strategies, vaccination, and improved sanitary conditions to reduce infection and maintain livestock health.

The introduction of mandatory vaccination and regular testing of animals for *Brucella abortus* and *Vibrio melitensis* will significantly reduce infection rates in cattle and small ruminant populations. In addition, the use of molecular diagnostic techniques, such as PCR allowed early detection of infections, which is particularly important to prevent large-scale outbreaks in animals. These studies also support the need for increased epizootological control and improved animal housing conditions. It is necessary to ensure strict sanitary measures on farms, including improved water quality, feed quality and housing conditions, to minimise the risk of spreading infections among farm animals. These measures will not only reduce infection rates but also improve the overall health of animals, which will have a positive impact on farm productivity. Thus, the epidemiological situation in the Kostanay region requires intervention and development of comprehensive measures to control the spread of *Brucella abortus* and *Vibrio melitensis* among farm animals. Regular testing, vaccination and improvement of housing conditions will be key elements in the strategy to control these infections, which will minimise economic losses and improve animal health in the region.

DISCUSSION

This study found that *Brucella abortus* infected 45% of cattle, indicating a high prevalence. Notably, infection peaked at 46.7% in animals aged two to three years, suggesting increased vulnerability during this stage, an

observation supported by Chang et al. (2023), who reported similar patterns in adult goats. Lounes et al. (2021) also identified *B. abortus* as a dominant cattle pathogen in Algeria, confirming its wide distribution. These findings underscore the need for targeted preventive measures for at-risk age groups.

PCR diagnostics confirmed *B. abortus* in 43.3% of cattle, echoing Ntivuguruzwa et al. (2022), who emphasized the method's sensitivity and specificity. Kumari et al. (2021) also demonstrated PCR's effectiveness in identifying *B. abortus* and *B. melitensis* strains in India. Our findings support PCR's broader adoption in routine veterinary screening, particularly for large farms. Younger animals under one year showed lower infection rates (33.3%), possibly due to immune resistance or reduced exposure.

Serologically, 46% of cattle and 38% of small ruminants tested positive for *B. abortus* and *V. melitensis*, respectively. This aligns with Papapaskevas et al. (2023), who reported 78% seropositivity in Greek small ruminants, and Deniz and Oruç (2023), who emphasized that seropositivity reflects infection duration and the need for ongoing surveillance. Genetic studies, such as those by Umizhanov et al. (2022) and Naseer et al. (2023), revealed regional variability in *Brucella* strains, underscoring the necessity of region-specific vaccines and diagnostics. This is consistent with findings from Rostami et al. (2023), who highlighted the role of genomic diversity in shaping control strategies.

In Kazakhstan, high seropositivity levels mirror findings from Schelling et al. (2020) in Iran, calling for enhanced preventive measures, especially in unvaccinated populations. Notably, vaccination in Kazakhstan reduced infection rates, consistent with Long et al. (2023), whose peptide vaccine showed promise against *B. melitensis*, *B. abortus*, and *B. suis*. The introduction of such vaccines could greatly improve the epidemiological situation in the region. Turmagambetova et al. (2017) emphasized the importance of IgM antibodies in enhancing immune responses, though they noted MHCII expression might hinder long-term vaccine efficacy. Monitoring immune markers could help evaluate vaccine performance more accurately in Kazakhstan. Molecular methods remain key to early detection. Bogoyavlenskiy et al. (2012) and Xue et al. (2023) both demonstrated PCR's effectiveness in identifying *Brucella* infections before symptom onset, critical for large-scale farms. Similarly, Basybekov et al. (2018) highlighted that early diagnostics play a vital role in containing outbreaks.

Conclusion

The study highlighted a significant epidemiological threat from *Brucella abortus* and *Vibrio melitensis* infections among farm animals in the Kostanay region. It revealed infection rates of 45% in cattle for *Brucella abortus* and 40% in sheep and goats for *Vibrio melitensis*, emphasizing the need for ongoing monitoring and preventive measures, particularly for productive-age animals. Infection was most common in animals aged two to five years, with cattle showing a 46.7% infection rate in two-to-three-year-olds and sheep and goats at 42.5%. Younger animals had lower infection rates (33.3% in cattle and 25% in sheep and goats), indicating greater

susceptibility among older individuals. Serological tests revealed high seropositivity, 46% in cattle for *Brucella abortus* and 38% in sheep and goats for *Vibrio melitensis*, confirming the widespread prevalence of these pathogens.

PCR testing identified pathogens in 43.3% of cattle and 38.75% of sheep and goats, demonstrating the method's high accuracy and its importance in early detection. The study recommends a comprehensive approach to disease control, encompassing mandatory vaccination, regular testing and enhanced animal housing conditions. Preventive programs should be implemented to reduce economic losses and enhance regional economic stability. The study's scope was limited to the Kostanay region, so further research is needed to assess the effectiveness of preventive measures in different climates and economies

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