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Prevalence and Vectors of *Theileria Annulata* in Cattle across Various Ecological Zones of West Kazakhstan Region

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ABSTRACT

A study was conducted on the infection of cattle with piroplasmids and Ixodid ticks in the steppe, semi-desert, and desert zones of Western Kazakhstan. The research utilized microscopy, polymerase chain reaction (PCR), and analysis of epizootiological indicators. Three ecological zones, steppe, semi-desert, and desert, were surveyed, with attention to age-related and seasonal dynamics of infestation. Data were analyzed using descriptive statistics and the chi-square test to assess associations between variables. The seasonal prevalence index varied widely, from a minimum of 10.0% in winter to a maximum of 67.5% in spring and summer. The highest infection rate was observed in the steppe zone and in animals under one year of age. Microscopic examination of erythrocytes revealed *Theileria* spp., while PCR analysis demonstrated higher sensitivity and confirmed the circulation of *Theileria annulata*. Out of 681 tick samples, the infection rate with *Theileria annulata* was 66.1% in *Hyalomma marginatum* and 47.4% in *Dermacentor marginatus*. A total of 2028 head of cattle were examined. Thus, pronounced seasonal and age-related patterns of *Theileria annulata* prevalence have been established, highlighting the epizootiological significance of the parasite and the necessity for timely preventive measures, considering the region's natural and climatic conditions.

Keywords: Cattle, Ixodid ticks, *Theileria annulata,* PCR diagnostics, Seasonal and age dynamics, West Kazakhstan region; Epizootology.

INTRODUCTION

Piroplasmosis is a group of vector-borne diseases caused by non-pigmented intra-erythrocytic protozoan parasites of the genera *Babesia* and *Theileria* (Schnittger et al. 2022). These infections are widely distributed among domestic and wild animals, with cattle and small ruminants being particularly susceptible (Jacob et al. 2020). Clinically, piroplasmosis is characterized by fever, anemia, and jaundice of the mucous membranes, cardiovascular and gastrointestinal disturbances, hemoglobinuria, and a sharp decline in productivity (Bock et al. 2004; Rimal et al. 2025). A hallmark of these diseases is their pronounced seasonality, which is closely associated with the life cycle of Ixodid ticks — the primary vectors of transmission (Monoldorova et al. 2024).

In cattle, the most common causative agents are *Babesia bigemina, Babesia bovis*, and *Theileria annulata* (Bock et al. 2004; Liu et al. 2022). In recent years, Kazakhstan has faced an increasingly unfavorable epizootiological situation regarding piroplasmosis, particularly in the southern and western regions. Climatic conditions in these areas facilitate the proliferation of their tick vectors. The circulation of all known peroplasm species has been documented on livestock farms throughout the country, underscoring the need for enhanced surveillance and control strategies (Perfilyeva et al. 2020; Sultankulova et al. 2022; Kuibagarov et al. 2023).

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The problem is especially acute in regions with high temperatures and humidity, which provide optimal conditions for tick development and activity (Sánchez Pérez et al. 2023; Ebert and Becker 2025). Seasonal outbreaks, primarily occurring in summer and autumn, are associated with high morbidity and mortality rates, decreased milk and meat productivity, reproductive disorders, and significant economic losses (Mahmoud et al. 2015; Bursakov and Kovalchuk 2019). In recent years, a growing number of reported mixed infections involving various combinations of *Babesia* and *Theileria* have been reported, which complicate clinical management and reduce the effectiveness of treatment. Without prompt therapeutic intervention, mortality rates in such cases may reach 40–50% (Asif et al. 2022; Selim et al. 2022).

Annual economic losses caused by bovine babesiosis and anaplasmosis are substantial worldwide and vary across regions. Reported estimates indicate losses of approximately USD 16.9 million per year in Australia, USD 21.6 million in South Africa, and USD 57.2 million in China (Bock et al. 2004). In addition, the economic impact of tropical theileriosis caused by *Theileria annulata* has been estimated at around USD 600,000 annually in Turkey (Inci et al. 2007). In Kazakhstan, particularly in the western regions, piroplasmosis remains endemic and results in significant economic losses in the livestock sector due to reduced productivity, increased mortality, and costs associated with treatment and tick control (Mussayeva et al. 2024; Berdikulov et al. 2024). According to data from the Turkistan region over the past five years, 10,133 cases of babesiosis/piroplasmosis and 14,497 cases of theileriosis were recorded in cattle. The mortality from these diseases amounted to 1,419 heads for babesiosis/piroplasmosis and 1,899 heads for theileriosis (Tursunbay et al. 2024).

The West Kazakhstan region is considered an endemic area for piroplasmosis. Its natural and climatic features, combined with extensive pasture-based livestock farming and the presence of natural tick habitats, create favorable conditions for pathogen circulation (Karmaliyev et al. 2024; Tanitovsky and Maikanov 2024). Ixodid ticks of the genera *Boophilus, Rhipicephalus, Haemaphysalis*, *Dermacentor,* and *Hyalomma* are of particular concern. These vectors not only transmit piroplasm infections but also cause mechanical skin damage, inflammation, and stress-related responses, further weakening animals and predisposing them to secondary infections (Perveen et al. 2021).

Effective control of piroplasmosis in such endemic areas requires a comprehensive and systematic approach, including year-round epizootiological monitoring, identification of dominant tick species, and the timely application of preventive and therapeutic measures (Asif et al. 2022; Sajid et al. 2023). In terms of diagnosis, while traditional microscopy using Romanowsky-Giemsa-stained blood smears remains in use, its sensitivity is limited in cases of chronic or mass infections (Wittekind 2020). Therefore, modern molecular and serological methods are increasingly utilized to improve diagnostic accuracy (Kundave et al. 2014; Ullah et al. 2022).

Given the significance of piroplasmosis for livestock health and productivity, especially in endemic areas such as West Kazakhstan, detailed regional studies are essential to inform effective disease control strategies. Despite the wide distribution of both diseases, theileriosis holds key epizootiological significance in the conditions of Western Kazakhstan. Therefore, this study aims to comprehensively investigate the prevalence of *T. annulata*, the role of vector ticks in its circulation, as well as the age-related and seasonal patterns of infection in cattle. The objective of this research is to analyze the epidemiological patterns of theileriosis and identify key tick vectors involved in its spread among cattle across different natural and climatic zones of West Kazakhstan.

MATERIALS AND METHODS

Ethics statement

The present study was conducted in the Testing Center Laboratory of Zhangir Khan West Kazakhstan Agrarian and Technical University. All animal experiments were approved by the Local Biological Ethics Committee of the West Kazakhstan Research Veterinary Station (a branch of Kazakh Research Veterinary Institute LLP) on November 17, 2022 (Protocol No. 1).

All procedures involving animals were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, the Recommendation 2007/526/EC of June 18, 2007, on the Care and Housing of Animals Used for Scientific Research, and the WHO Guidelines for Ethics Committees Reviewing Biomedical Research (World Health Organization 2000).

Study areas

The study of the epizootiology of piroplasmosis was conducted from January to December in 2024 in the West Kazakhstan Region (WKR) of the Republic of Kazakhstan. Research was carried out on livestock farms located in different natural and climatic zones: steppe, semi-desert, and desert (Veselova et al. 2010). Specifically, investigations were conducted in the steppe zone (Taskalinsky District), the semi-desert zone (Akzhaiksky District), and the desert zone (Zhangalinsky District). In the steppe zone, the study was conducted at the peasant farm "Oyan," located at 51°06′28.5″ N, 50°17′31.2″ E, involving 680 animals. In the semi-desert zone, the re-search took place at the peasant farm "Sazhida," located at 50°11′15″ N, 51°10′02″ E, with 676 animals. In the desert zone, the study was carried out at the peasant farm "Nur," located at 49°12′54″ N, 50°18′10″ E, involving 672 animals (Fig. 1).

Sample collection

The examined cattle belonged to the Kazakh White-headed breed (males and females) and were kept under pasture-based grazing conditions. Clinical assessments were carried out, taking into account the animals' age, the season of sampling, and the ecological zone.

Blood sampling

The study randomly selected 2028 cattle, all of which had known identification numbers. Each animal was individually identified using numbered ear tags to ensure traceability and reliability of sample collection from the selected population. The blood samples were collected via the tail (coccygeal) vein. Animals were restrained in a

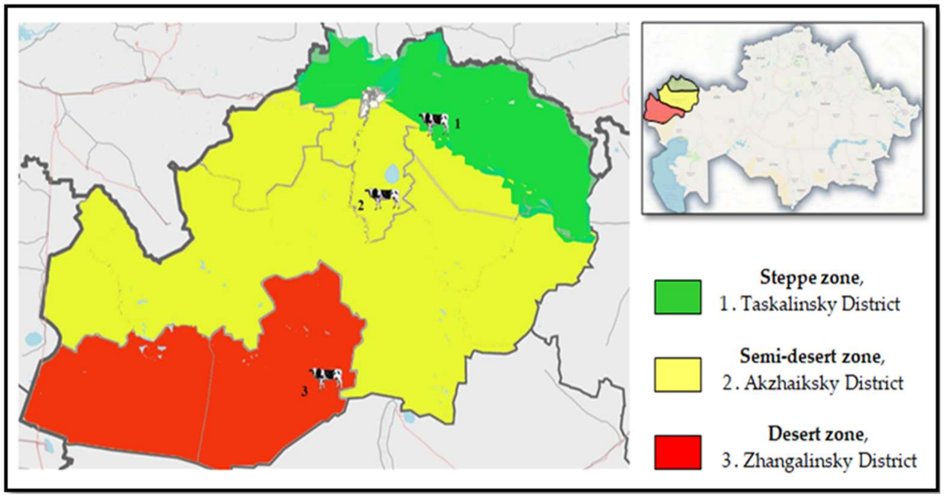

Fig. 1: Location of research objects in various natural zones of the West Kazakhstan region.

chute, and the tail was elevated at its midsection to facilitate optimal access. The puncture site, located between the second and fifth coccygeal vertebrae, was disinfected using 70% ethanol. Blood was drawn into sterile vacuum tubes (Vacutainer®) containing the anticoagulant K_2-EDTA. The tubes were gently inverted 5–8 times to ensure proper mixing and to prevent coagulation. Samples were stored at +4 to +8°C in a TM-9 thermal transport container (the manufacturer is Termo-Kont MK LLC, Moscow, Russia) and delivered to the laboratory within 24 hours.

Collection of Ixodid ticks from cattle

Ixodid ticks were collected quarterly directly from cattle, with attention focused on body regions most susceptible to tick attachment, including the inguinal area, udder (in cows), and scrotum (in bulls). Ticks were removed using forceps by grasping them at the base and gently twisting to avoid damaging the specimen. Collected ticks were placed into 100mm³ containers lined with moistened cotton wool and filter paper to maintain appropriate humidity. Each container was labeled with the date and time of collection, animal identification number, and anatomical site of tick attachment. Storage and transportation conditions were identical to those used for blood samples.

Collection of ticks from vegetation

Ticks were collected from vegetation using the standard flagging method (Dantas-Torres et al. 2013; Bespyatova and Bugmyrin 2021). A flag made of light-colored, textured fabric was dragged over grassland vegetation to stimulate tick attachment. The flag was inspected for attached ticks every 10–20 meters. Collected ticks were transferred into containers lined with moistened cotton wool and filter paper to maintain a suitable humidity level. At the end of each sampling transect, a label was prepared indicating the habitat type, route, date, and time of collection. Storage and transportation conditions matched those used for other sample types. The relative abundance of ticks was calculated as the number of ticks per flag per 1 flag-kilometer. Tick species were identified based on morphological characteristics using The Atlas of Ixodid Ticks and The Guide to Blood-Sucking Insects and Ticks (Vasilevich et al. 2023).

Semi-quantitative assessment of tick infestation intensity by anatomical localization, seasonal, and age dynamics

The anatomical distribution of Ixodid ticks on cattle was assessed with consideration of the parasite's developmental stages: larva (L), nymph (N), and adult (A). During the study, animals were systematically examined, and ticks were counted across specific body regions. The intensity of infestation was categorized semi quantitatively based on the number of ticks observed per anatomical area: low ("+") for 2–4 individuals, moderate ("++") for 5–10 individuals, and high ("+++") for 10–20 individuals per animal. This approach is commonly used when counts are performed by anatomical body zones (head, neck, withers, groin, etc.) in order to compare localization and the degree of infestation.

Microscopic detection of piroplasmida in blood smears

Detection of Piroplasmida in cattle was performed via direct microscopic examination of blood smears stained using the Romanowsky-Giemsa method (Wittekind 2020). Microscopic analysis was carried out at 1000× magnification using immersion oil. The primary diagnostic criterion was the presence of intracellular forms of Piroplasmida, including ring-shaped, pear-shaped, or amoeboid forms, within erythrocytes. Evaluation was based on the identification of characteristic morphological features of the parasite and their prevalence within the field of view. All findings were documented as digital photomicrographs.

Preparation of Ixodid tick homogenate

For molecular analysis, Ixodid ticks collected from cattle underwent preliminary processing. Each specimen was rinsed twice with sterile phosphate-buffered saline (PBS, pH 7.4) to remove surface contaminants and potential microorganisms. Ticks were then transferred into individual sterile 1.5mL Eppendorf tubes. Homogenization was performed mechanically using sterile disposable plastic pestles in 200–300µL of chilled PBS. All procedures were conducted on ice to prevent degradation of proteins and nucleic acids. Following homogenization, the samples were centrifuged at 8000rpm for 10 minutes at +4°C. The resulting supernatant was used for subsequent PCR analysis. For long-term storage, homogenates were frozen at −20°C. All manipulations were carried out under sterile conditions in a laminar flow cabinet (Pichon et al. 2003).

DNA extraction and PCR analysis

DNA was extracted from cattle blood samples and Ixodid ticks using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's instructions.

To amplify a fragment of the 18S rRNA gene, specific to protozoan parasites, the forward primer BaF (5′-AATACCCAATCCTGACACAGGG-3′) and reverse primer BaR (5′-TTAAATACGAATGCCCCCAAC-3′) were used (Armstrong et al. 1998). The expected PCR product size was 400 base pairs. The PCR reaction mixture contained 5µL of 5X ScreenMix, 1µL of each primer (10 µM), 5µL of genomic DNA (5–50ng/µL), and nuclease-free water to a final volume of 25µL.

For the specific detection of *T. annulata*, the forward primer TanF3 (5′-AAGTTTCTACTGTCCCGTT-3′) and reverse primer TanR3 (5′-GATGACTTGCGCATACTAGG-3′) were used, producing an expected amplicon of 250 base pairs (Ertleuova et al. 2025). For detection of *T. sergenti*, the primers used were: forward primer Tser-U (5′-CACGCTATGTTGTCCAAGAG-3′) and reverse primer Tser-R (5′-TGTGAGACTCAATGCGCCTA-3′) (Zakimi et al. 2006). To identify *T. orientalis*, the primers MPSP-F (5′-CTTTGCCTAGGATACTTCCT-3′) and MPSP-R (5′-ACGGCAAGTGGTGAGAACT-3′) were used, generating an amplicon of 776 base pairs (Ota et al. 2009). For detection of *B. bovis*, the forward primer (5′-TGAACAAAGCAGGTATCATAGG-3′) and reverse primer (5′-CCAAGGAGATTGTGATAATTCA-3′) were used (Wu et al. 2025). The PCR conditions and reaction protocol were identical to those used in the authors' previous studies (Zakimi et al. 2006; Ota et al. 2009; Ertleuova et al. 2025; Wu et al. 2025).

The tick species were confirmed using conventional PCR targeting mitochondrial cytochrome C oxidase subunit 1 (COX1) or 16S rRNA as DNA barcoding genes for selected members of tick species. The fragment of the COX1 gene was amplified using primer sets Cox1-F- (5'-GGAACAATATATTTAATTTTTGG-3') and Cox1-R- (5'-ATCTATCCCTACTGTAAATATATG −3'), amplifying approximately 820bp (Chitimia et al. 2010). The 16S rRNA gene was amplified using primer sets T16SF- (5'-TTAAATTGCTGTRGTATT-3') and T16SR- (5'-CCGGTCTGAACTCASAWC-3'), amplifying approximately 455 bp (Mwiine et al. 2020). Amplified products were separated by electrophoresis on a 1.5% agarose gel prepared with 1×TBE buffer, stained with ethidium bromide (8ng/µL) and visualized under UV light.

Sequencing of amplified DNA fragments

All PCR-positive products were purified using the Quick PCR Purification Kit (QIAGEN, Germantown, MD, USA) according to the manufacturer's instructions, then subjected to sequencing and genotyping. Sequencing was performed on a 3730xl DNA Analyzer 96-Capillary Array (Thermo Fisher Scientific, Applied Biosystems, and Foster City, CA, USA). The genetic sequences obtained in this study have been deposited in GenBank under the following accession numbers: *T. annulata* from cattle (PX246732, PX246733, PX246734, PX246735, PX246736, and PX246737), *H. marginatum* (PX242965, PX242966, PX245473, and PX242973), *D. marginatus* (PX242967, PX242968, PX243341, and PX243342), *T. annulata* from ticks (PX246738, PX246739, and PX246740). The resulting nucleotide sequences were manually edited and compared with reference sequences from the GenBank database using the BLAST algorithm (https://www.ncbi.nlm.nih.gov/).

Statistical analysis

Descriptive statistics were used to calculate prevalence. Associations between hemoparasitic infection and categorical variables (age group, agro-ecological zone, and tick species) were assessed using the Chi-square (χ^2) test. A P-value <0.05 was considered statistically significant.

RESULTS

Microscopic examination of Giemsa-stained blood smears revealed intraerythrocytic stages of *Theileria* spp. in cattle from all studied ecological zones (Fig. 2). PCR screening of all 2,028 blood samples targeting the 18S rRNA gene detected hemoparasitic DNA in 213 animals. Species-specific PCR confirmed *T. annulata* in all positive samples, while *T. sergenti*, *T. orientalis*, and *Babesia bovis* were not detected, indicating that *T. annulata* was the only circulating piroplasmid species (Table 1). Most infected animals were clinically asymptomatic, suggesting a high prevalence of latent or chronic infections.

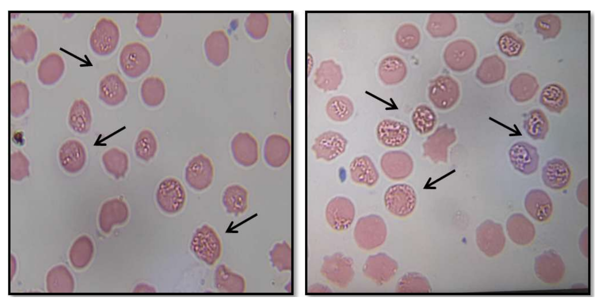

Fig. 2: Detection of *Theileria spp.* in cattle erythrocytes (×1000).

Infection prevalence decreased with age. The highest rates were recorded in cattle younger than one year, with PCR positivity of 58.0–59.6% across steppe, semi-desert, and desert zones. In animals aged 1–3 years, prevalence declined to 29.5–41.9%, while the lowest rates were observed in cattle aged 4–5 years (26.3–33.9% by PCR) (Table 1).

Clear seasonal variation was observed. During winter, infection prevalence remained low, with PCR detection below 13% in all zones. In spring, prevalence increased to 21.4–26.4% and peaked in summer, when PCR positivity reached 55.3–59.4%, coinciding with peak tick activity. In autumn, infection levels declined to 17.8–20.5%, remaining higher than winter values.

Two Ixodid tick species, *H. marginatum* and *D. marginatus*, were identified in all ecological zones of the West Kazakhstan region (Fig. 3) and confirmed by PCR amplification of 16S rDNA and COX1 gene fragments.

Table 1: Findings from the analysis of cattle blood samples for hemoparasitic infections.

Determinant		Zone	Examined	Microscopy (n)	Microscopy (%)	P-value	PCR (n)	PCR (%)	P-value
Age	<1 year	Steppe	208	116	55.7	0.4452	124	59.6	0.7403
		Semi-desert	204	112	54.9	0.5588	120	58.8	0.8667
		Desert	200	104	52	-	116	58	-
	1–3 years	Steppe	248	88	35.3	0.1576	104	41.9	0.1824
		Semi-desert	252	84	33.3	0.3592	100	39.6	0.4066
		Desert	244	72	29.5	-	88	36.1	-
	4–5 years	Steppe	224	56	25	0.1451	76	33.9	0.0783
		Semi-desert	220	48	21.8	0.5094	64	29.1	0.5118
		Desert	228	44	19.3	-	60	26.3	-
Season	Winter	Steppe	680	80	11.8	0.0879	88	12.9	0.1052
		Semi-desert	676	68	10.1	0.4792	76	11.2	0.5045
		Desert	672	60	8.9	-	68	10.1	-
	Spring	Steppe	680	164	24.1	0.0238	180	26.4	0.0301
		Semi-desert	676	140	20.7	0.4446	152	22.5	0.6394
		Desert	672	128	19	-	144	21.4	-
	Summer	Steppe	680	376	55.3	0.1960	404	59.4	0.1318
		Semi-desert	676	368	54.4	0.3293	392	57.9	0.3298
		Desert	672	348	51.8	-	372	55.3	-
	Autumn	Steppe	680	124	18.2	0.2916	140	20.5	0.2030
		Semi-desert	676	116	17.6	0.5915	132	19.5	0.4319
		Desert	672	108	16.1	-	120	17.8	-

Table 2: Distribution of Ixodid ticks in the West Kazakhstan region.

West Kazakhstan region zone	Animais studied/ infected	*Hyalomma marginatum* Range of intensity	Prevalence, CI%	Mean intensity±SD	Mean (SD) abundance	*Dermacentor marginatus* Animals Studied/ Infected	Range of intensity	Prevalence, CI%	Mean intensity±SD	Mean (SD) abundance
Steppe	680/512	2-49	75.3 (71.9-78.5)	25.5±13.6	19.2±13.6	680/456	2-18	67.0 (63.4-70.6)	10.0±4.6	6.7±4.6
Semi-desert	676/460	1-21	68.1 (64.4-71.5)	11.0±5.8	7.5±5.8	676/404	1-11	59.8 (56.0-63.5)	6.0±2.9	3.6±2.9
Desert	672/388	1-44	57.7 (54.0-61.5)	22.5±12.4	13.0±12.4	672/316	1-13	47.0 (43.2-50.9)	7.0±3.5	3.3±3.5
Total	2028/1360		67.0 (65.0-69.1)			2028/1176		58.0 (55.8-60.1)		

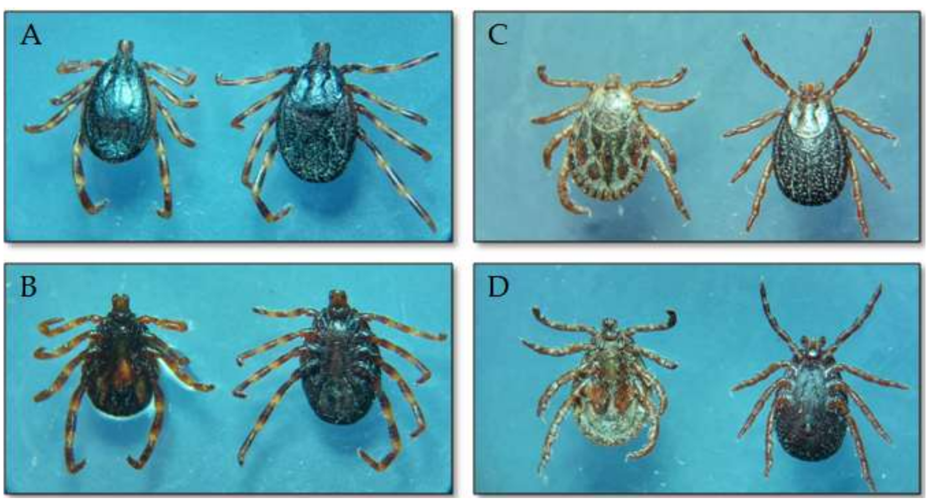

Fig. 3: Morphological views of adult ixodid ticks. *Hyalomma marginatum*: (A) dorsal view, (B) ventral view; *Dermacentor marginatus*: (C) dorsal view, (D) ventral view (×2.5).

Tick infestation was highest in the steppe zone, where *H. marginatum* infested 75.3% of animals (mean abundance 19.2±13.6) and *D. marginatus* 67.0% (6.7±4.6). Lower prevalence was recorded in semi-desert and desert zones (Table 2).

Tick localization depended on developmental stage (Table 3). Larvae and nymphs were mainly found on the head, neck, withers, and shoulders at low to moderate intensities, whereas adult ticks showed the highest infestation levels on the ventral body, udder, inguinal, perineal, and subcaudal regions.

A total of 681 Ixodid ticks were collected, including 369 *H. marginatum* and 312 *D. marginatus* specimens (Table 4). PCR detected *T. annulata* DNA in 66.1% of *H. marginatum* and 47.4% of *D. marginatus*. No tick sample was positive for *T. sergenti*, *T. orientalis*, or *B. bovis*, confirming *H. marginatum* and *D. marginatus* as the principal vectors of *T. annulata* in the region.

DISCUSSION

The results of the microscopic blood smear analysis revealed a widespread occurrence of piroplasmid (*Theileria*) infections among cattle in various ecological zones of the West Kazakhstan region. Stages of *T. annulata* were detected in the erythrocytes of the animals, confirming the presence of the disease in the studied population. The study encompassed three zones – steppe, semi-desert, and desert and included animals of different age groups.

Table 3: Anatomical localization and developmental stages of ixodid ticks in cattle

Body part	Larva (*L*)	Nymph (*N*)	Imago (*I*)
Head	+	+	
Ears			+
Neck	+		+
Withers	+	+	+
Neck			+
Sides of neck	+		+
Lower neck		+	+
Dewlap	+		++
Chest			++
Shoulders	+	+	+
Back			+
Forequarters	++		++
Hindquarters			++
Lower body			+++
Udder			+++
Milk mirror			+++
Groin			+++
Scrotum			++
Perineum			+++
Base of tail			++
Tail			++
Sub-tail region			+++
Sides			++

Table 4: PCR detection results of hemoparasites in collected Ixodid ticks

Type of parasite	*Hyalomma marginatum*		*Dermacentor marginatus*	
	Researched specimen	Positive PCR (n/%)	Researched specimen	Positive PCR (n/%)
T. annulata	369	244(66.1)	312	148(47.4)
T. sergenti	369	0	312	0
T. orientalis	369	0	312	0
B. bovis	369	0	312	0

A distinct age-related dynamic in *T. annulata* infection was identified: the highest rates were observed in animals under one year of age (54.2% by microscopy; 58.8% by PCR), followed by a decrease in the 1–3 years age group (32.7-39.2%), with the lowest rates found in animals aged 4–5 years (22.0-29.7%) (Table 1).

Similar findings have been reported in Pakistan, a significantly higher prevalence of infection in young animals (≤1.5 years, 40.4%) compared to adult animals (14.5%) (Ullah et al. 2021). Other authors have also noted that young ruminants are infected more frequently, while older animals exhibit lower infection rates, suggesting the development of acquired immunity (Sajid et al. 2023). In the Punjab, Sargodha, and Multan provinces of Pakistan, overall rates were lower than in our study (17.1% by PCR and 9% by microscopy), but the age-related trend was consistent; young animals were infected more often (Ahmad et al. 2023).

Conversely, some regions report an opposite trend. For example, in Egypt, an increase in *T. annulata* infection with age: 3.5% in animals under 2 years, 16.8% in the 2–4 years age group, and 31.8% in animals over 4 years (Selim et al. 2022). Comparable data were obtained in India (Odisha state), where the maximum infection rate (64.1%) was noted in older age groups, including animals >8 years (85.7%), while in animals aged 1–3 years, it was approximately 50% (Selim et al. 2022). These discrepancies may be attributed to agroclimatic variations and cumulative exposure to the pathogen.

Our results also demonstrated that the PCR method possesses higher sensitivity compared to microscopy, enabling the detection of latent infection forms. In Spain, PCR detected *T. annulata* in 75% of samples, whereas microscopy detected it in only 22%, and ELISA in 40% (Ullah et al. 2022). In India, a study of cattle and buffaloes revealed 63.8% positive samples by PCR, compared to merely 12.9% by microscopy (Kundave et al. 2014).

In our study, *T. annulata* was the only species detected. Other hemoparasites (*T. sergenti, T. orientalis, B. bovis*) were not found. This aligns with data from southern regions of Kazakhstan, where *T. annulata* was identified most frequently (83.0%), while *T. orientalis* and *Babesia* spp. were less common (33.3% and 13.5%, respectively), and co-infections were recorded in 49% of animals (Kuibagarov et al. 2023). Furthermore, tick studies have confirmed the presence of several *Theileria* species in Kazakhstan (*T. annulata, T. ovis, T. orientalis)*; however, *T. annulata* was the dominant species in cattle (Zeng et al. 2025).

Thus, the obtained results confirm the leading role of *T. annulata* as the primary causative agent of bovine piroplasmosis in the West Kazakhstan region, attributable to the natural and climatic features of the area's ecological zone. It was also established that PCR is the most informative diagnostic method for this infection. The absence of other hemoparasites in our samples may indicate their limited local distribution or temporary absence in the studied populations.

T. annulata is an obligate intracellular protozoan parasite and the etiological agent of tropical theileriosis – a tick-borne disease affecting cattle in countries of Asia, the Middle East, North Africa, and southern Europe (Soosaraei et al. 2018; Liu et al. 2022). The parasite has a complex life cycle involving development in mononuclear leukocytes and erythrocytes of cattle, as well as transstadial and transovarial transmission by Ixodid ticks, primarily from the species *Hyalomma* and *Dermacentor* (Yin et al. 2018; Ali et al. 2024).

Recent molecular studies have confirmed the wide distribution of *T. annulata* in Kazakhstan, indicating its endemic status. For instance, infection rates reached 83.0% (n = 766) in the Turkistan and Zhambyl regions (Kuibagarov et al. 2023), and as high as 100% in certain villages of the Turkistan region (Tursunbay et al. 2024). Our data supplement these findings, demonstrating characteristic seasonal, zonal, and age-related patterns of pathogen circulation in Western Kazakhstan.

We determined that the highest infection rates occurred during the summer period, coinciding with the peak activity of Ixodid ticks, while prevalence decreased sharply in autumn and winter. Similar seasonal dynamics are characteristic of other endemic regions (Egypt, Pakistan, China, Turkey) (Sutton et al. 2012; Asif et al. 2022; Selim et al. 2022; Chai et al. 2025; Wu et al. 2025), underscoring the pivotal role of climatic factors and vector biology in shaping the epizootic process.

Infection rates in animals varied significantly across natural zones, with higher rates observed in the steppe zone compared to the semi-desert and desert zones. Similar patterns have been previously reported in Central Asia and Africa, where microclimatic conditions and vegetation cover directly influence vector abundance and activity (Khajuria et al. 2015; Maïlaïso et al. 2021; Perveen et al. 2021; Mussayeva et al. 2024).

Our findings indicate that *H. marginatum* and *D. marginatus* are the primary tick species responsible for transmitting *T. annulata* in the West Kazakhstan region. Similar results have been reported in other parts of Kazakhstan and Asia, where these species were identified among the most common Ixodid ticks and confirmed as carriers of *T. annulata* (Hay et al. 2016; Abdiyeva et al. 2020; Karmaliyev et al. 2024). These observations support our conclusion that *H. marginatum* and *D. marginatus* play a key role as the main vectors of bovine theileriosis in the region.

Tick attachment sites on animal bodies also showed a consistent pattern: the largest number of specimens were found in the udder and groin area, followed by the head and neck, while limbs and back were infested significantly less frequently. These patterns are explained by anatomical and physiological factors (thin skin, high vascularization) and animal behavior (limited self-grooming capabilities). Comparable data have been re-ported for Egypt, Turkey, and Pakistan (Khajuria et al. 2015; Maïlaïso et al. 2021).

Molecular analysis demonstrated that *T. annulata* was detected in two tick species; however, the infection levels varied substantially. The highest infection rate was observed in *H. marginatum* (67%), while it was lower in *D. marginatus* (57.9%). This confirms the leading role of these types in the epidemiology of theileriosis in the region.

The totality of our results demonstrates a close relationship between animal age, season, ecological zones, and vector biology in shaping the epizootic situation of *T. annulata* in the West Kazakhstan region. The high susceptibility of young animals indicates the necessity for targeted preventive measures, specifically in this age group, particularly during periods of peak vector activity.

The significance of this study lies in its being the first to comprehensively characterize the epizootic situation of *T. annulata* in Western Kazakhstan, identifying key risk factors (age, season, natural zone, tick species composition) and confirming the leading role of *H. marginatum* and *D. marginatus* as the primary vectors. The obtained results expand existing knowledge about the pathogen's circulation in Central Asia and can be used to optimize veterinary surveillance systems, develop preventive programs, and reduce the economic damage associated with tropical theileriosis.

Conclusion

This study confirmed the active circulation of *Theileria annulata* among cattle and Ixodid ticks in the West Kazakhstan Region. Young animals under one year of age were identified as the most vulnerable group, underscoring the need for targeted veterinary surveillance and protective measures. Seasonal patterns were evident, with infection rates in cattle peaking in summer and tick activity reaching its maximum in spring, indicating a strong link between vector dynamics and disease transmission. Zonal differences were also significant: the highest prevalence occurred in the steppe zone, where environmental conditions favor tick proliferation, while lower rates were observed in semi-desert areas, reflecting harsher ecological constraints. The tick species Hyalomma marginatum and Dermacentor marginatus were the main vectors due to their high infection rates. PCR proved more sensitive than microscopy, especially for latent cases. These findings provide a scientific basis for improving regional theileriosis monitoring, prevention, and control programs.

DECLARATIONS

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Data Availability: Data are available upon request from the corresponding author.

Ethics Statement: The study was approved by the Local Biological Ethics Committee of the West Kazakhstan Research Veterinary Station (Protocol No. 1 dated 17 November 2022).

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