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PREVALENCE OF THEILERIA ANNULATA AMONG CATTLE IN THE TURKESTAN REGION

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Abstract

Theileriosis is a severe blood-parasitic disease, an important problem in veterinary protozoology, since the damage it causes remains significant. The recently widespread practice of importing breeding stock to improve local breeds leads to the fact that in the first summer season, upon contact with infested ticks, the imported animals become very seriously ill, with a mortality rate of up to 90-100%. In this work, 738 samples of cattle were examined using the polymerase chain reaction method, which showed the presence of infection in 598 DNA samples isolated from cattle from 10 districts, 19 settlements of the Turkestan region. Whole blood samples were taken from cattle aged 3 years and older. Upon examination, the animals seemed healthy. The overall positivity rate for the entire region was 81%, highlighting the prevalence of infection among livestock. There are different levels of infection in the regions; in 6 settlements the infection rate of cattle was 100%. In addition, 7 villages showed high PCR positivity rates, ranging from 70% to 97%. While the lowest prevalence of infection showed from 18% to 69% in 6 villages, respectively.

Key words: cattle; polymerase chain reaction; theileriosis; *Theileria annulata*.

Introduction

Theileriosis is an acute or subacute vector-borne disease of domestic and some wild animals caused by pigmented protozoa of the genus *Theileria*. In sick animals, an increase in lymph nodes, high fever, parasitemia of lymph node cells and parenchymal organs, and then red blood cells, impaired function of the cardiovascular and digestive systems are noted [1].

Theileria spp. pathogens are specific to different species and affect both wild and domestic animals. Several species of *Theileria spp.* have been identified that are parasitic in cattle: *Theileria parva*, *T. annulata*, *T. mutans*, *T. velifera*, *T. sergenti*, *T. taurotragi* and *T. orientalis*. The most pathogenic are *T. parva* and *T. annulata*, which are highly lethal and induce the transformation of infected lymphocyte cells or macrophages. Other types of theileria do not cause uncontrolled proliferation of infected white blood cells, but instead multiply predominantly in infected red blood cells. *T. parva* is found in southern

Africa, while *T. annulata* has been recorded in southern Europe, North Africa and Asia [2]. Infection of *Theileria orientalis* (*T. orientalis*) occurs in the Far East, Central Asia and in cattle usually occurs subclinically [3].

Bovine theileriosis caused by *Theileria annulata* (*T. annulata*) is an economically important infection causing serious damage to livestock. Theileriosis causes pathological changes in the organs and systems of the animal body, which lead to the fact that they do not recover to the physiological norm for a long time. Milk yields in sick cows do not return to normal during this lactation. Among the livestock of the meat sector, this disease leads to a sharp emaciation of animals, loss of up to 30% of body weight and deterioration in the quality of meat products from slaughtered animals [4,5]. In Kazakhstan, theileriosis caused by *T. annulata* is common in Turkestan, Kyzylorda, Almaty, Zhambyl regions [6]. Every year in the South Kazakhstan region, there is a case among cattle from theileriosis. Over-sick cattle restore productivity for a long time (1-2 months) only up to 80%. A high percentage of infection leads to the fact that imported highly productive cattle are very difficult to tolerate the disease with a high level of lethality. This, of course, hinders the intensification of animal husbandry [7]. Since ticks of the genus *Hyalomma*, which are carriers of *T. annulata*, are registered in almost all regions of Kazakhstan, there is a danger of spreading the invasion to new regions [8]. Most of the works describing the spread of theileriosis in Kazakhstan are based on microscopic research methods and affect different ages of animals [9]. The purpose of our work was a monitoring study the carriage of *T. annulata* in cattle older than three years in the Turkestan region, which will allow to assess the infection rate of animals that have had contact with ticks for at least two pasture seasons.

Materials and methods

Ethical approval

This study was approved by the local ethics committee in the Kazakh National Center for Biotechnology (Protocol #1 dated April, 2022). The respective cattle owners gave their approval for sampling. No animal was harmed during the sampling.

Study area and objects

The territory of the Turkestan region is in the south of Kazakhstan, within the eastern part of the Turan lowland and the western spurs of the Tien Shan and has a warm and mild climate, which is favorable for the development and reproduction of many pathogens and vectors of invasive diseases of farm animals, which cause great economic losses to livestock. The number of cattle in Turkestan at the end of 2023 was 1 082 702, respectively, which is 12.6% of the total number of cattle in Kazakhstan according to the Bureau of National Statistics of the Republic of Kazakhstan [10].

Sampling

The sampling period is from mid-June to the end of July 2023. Whole blood samples were taken from cattle aged 3 years and older. Upon examination, the animals seemed healthy, special clinical examination or investigation for current tick infestation was not carried out. The blood was collected in vacuum tubes with ethylenediaminetetraacetic acid (EDTA) and delivered at a temperature of 4 °C to the laboratory within 48 hours. 738 DNA samples isolated from cattle from 10 districts and 19 settlements of the Turkestan region were examined.

DNA isolation

DNA was isolated from 300 µl of whole blood using a modified method of Boom using silica powder with preliminary lysis of erythrocytes [11]. Erythrocytes were lysed RBL (Red Blood Cell Lysis Buffer, NH₄Cl (150 mM), NaHCO₃ (10 mM), EDTA (1 mM, pH 8.0), H₂O) in a ratio of 1:3. Lysis of leukocyte mass was carried out in a lysing buffer (30mM Tris-HCl, 30mM EDTA, 5% Tween20, 0,5% Triton X-100, 3,2 M GuaSCN), followed by sorption of DNA onto silica. The silica with bound DNA was washed twice with a washing buffer (75 % Ethanol, 10mM Tris), dried and eluted in 100 µl of 1 x TE (10 mM Tris-HCl, 1 mM EDTA (pH 8.0)) buffer.

Polymerase chain reaction

For the screening of samples, a standard polymerase chain reaction was used using primers that were previously described in the article by Kuibagarov M. et al. 2023 [9]. Matched to the enolase gene: Eno_T.anul_F 5'-ttgcgagatggagacaaaagc-3' and Eno_T.anul_R 5'-tcagggtgtgataaacttctgcc-3'. Distilled sterile water was used as a negative control. DNA samples in which the corresponding pathogen was confirmed by PCR and direct Sanger sequencing were used as a positive control. The PCR reaction

was performed in a total volume of a reaction mixture of 25 μ l: 10 pmol of each primer, 5 μ l of matrix DNA, 12.5 μ l of UDG HS-qPCR BioMaster (2x) and water up to 25 μ l. The PCR amplification program included: anticontamination treatment of the PCR mixture for 2 minutes at 50 °C, prolonged denaturation of 95 °C for 5 minutes, 40 cycles of 95 °C – 30 seconds, 60 °C – 40 seconds, 72 °C – 50 seconds, final elongation of 5 minutes at 72 °C, the PCR program was performed using an amplifier Mastercycler ProS (Eppendorf, Germany).

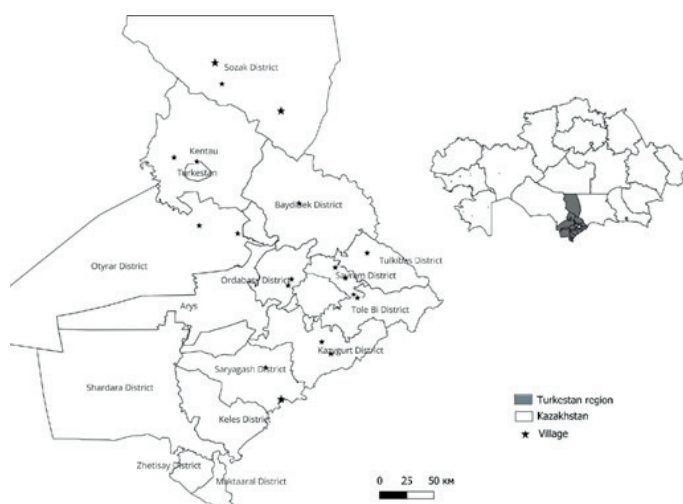
The analysis of DNA fragments amplified by PCR was performed by electrophoresis in 1.5% agarose gel containing ethidium bromide. Electrophoresis was performed in a horizontal Powerpack electrophoresis chamber using a current source for Bio Rad electrophoretic bath. A 1X TAE buffer was used as a buffer for electrophoresis. The results were documented using the Gel Doc (Bio-Rad) gel documentation system and Quantity One (Bio-Rad) software. The sizes (bp) of PCR amplifications were determined by comparing their electrophoretic mobility in a gel with the mobility of a marker ladder (Biolabmix, a marker with a molecular weight of 100 – 3000 bp, in increments of 100 bp).

Statistics

The exact Clopper-Pearson method, which is quite conventional and tends to produce wider intervals than necessary, was used based on the beta distribution to calculate the 95% confidence interval (CI).

Results

A total of 738 DNA samples isolated from cattle from 10 districts and 19 settlements of the Turkestan region were examined (Figure 1).



1 – Map of Kazakhstan (as of 2024), presenting the location of selected villages in the provinces of Turkestan

A PCR fragment characteristic of the *T. annulata* genome was detected in 598 DNA samples by PCR (Figure 2).

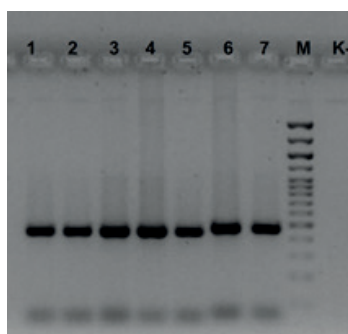


Figure 2 – Electrophoregram of PCR products of 7 positive samples; (M) molecular weight marker (Biolabmix) (100 – 3000 bp, in increments of 100 bp), (K-) negative control sample

Figure 1 shows the result of PCR with species-specific primers of 7 samples, in all samples a specific fragment of 450 bp was amplified. No PCR products were observed in the negative control sample, which indicates the absence of contamination in the reaction.

Thus, in our study, the infection rate was 81%. At the same time, there is a difference in infection rates in different localities (Table 1).

Table 1– Data on the spread of theileriosis in the Turkestan region

District	Village	N cattle examined	N cattle positive by PCR	% cattle positive (CI) 95% confidence interval
Sayram	Karabulak	41	36	87.8 (73.8-95.9)
	Karamurt	20	7	35.0 (15.4-59.2)
	Mankent	20	12	60.0 (36.0-80.9)
Ordabasy	Badam	39	38	97.4 (86.5-100)
	Burdjar	40	40	100 (91.1-100)
Otyrar	Aktobe	30	25	83.3 (65.3-94.4)
	Talapy	39	30	77 (60.7-88.9)
Tulkibas	Abai	88	83	94.3 (84.2-98.1)
Kazygurt	Karzhhan	50	43	86.0 (73.3-94.2)
	Zhanabazar	70	35	50.0 (37.8-62.2)
Tole Bi	Kogaly	23	8	34.8 (16.4-57.3)
Sauran	Karashik	17	17	100 (80.5-100)
	Shornak	49	49	100 (92.7-100)
Saryagash	Saryagash	45	38	84.4 (70.5-93.5)
	Zhilga	49	49	100 (92.7-100)
Baydibek	Shayan	49	33	67.5 (52.5-80.0)
Sozak	Syzgan	27	27	100 (87.2-100)
	Sozak	25	25	100 (86.3-100)
	Sholakkorgan	17	3	17.7 (0.38-43.4)
Total		738	598	81.0 (78.0-83.9)

In 6 settlements (Kaushik, Shornak, Syzgan, Sozak, Zhilga and Burjar), the infection rate of cattle over 3 years of age was 100%. While the lowest prevalence of infection (18%) was observed in animals from Sholakkorgan in the Sozak region. In addition, in 7 villages (Karabulak, Aktobe, Talenty, Abay, Karazhan, Badam) high PCR positivity rates were detected, ranging from 70% to 97%. The percentage of positive PCR results varied from 30% to 69% in 5 villages, respectively (Karamurt, Mankent, Zhanabazar, Shayan, Kogaly).

Discussion

The territory of the Turkestan region is highly endemic for bovine theileriosis. A study of 738 samples of cattle using the polymerase chain reaction (PCR) method showed the presence of infection in 598 DNA samples isolated from cattle from 10 districts and 19 settlements of the Turkestan region. The results are divided into different villages and districts, allowing a more detailed assessment of the spread of infection in different parts of the region. The overall positivity rate for the entire region was 81%, highlighting the significance of the infection in livestock. Our results coincide with previously obtained data from Kuibagarov et al, [9] who determined 84.4% of the prevalence of infections caused by *T. annulata* in the Turkestan region using the molecular genetic method. Also, one of the works on the prevalence of theileriosis in cattle in Kazakhstan is a microscopic study of blood smears of cattle in the Kyzylorda region; the authors described 23.7% of cases of infection of animals [12].

Differences in positivity rates between different villages and districts show the heterogeneity of infection spread across the region. This may indicate different factors influencing the spread of infection related to relief and microclimatic conditions in different areas. For example, in mountainous areas such as Tole bi and some Sairam villages, positive results were lower, while in steppe areas such as Ordabasy, Sauran, Sozak, Saryagash, positive results were higher.

Attention must be paid to marked geographic differences in pathogen distribution. However, it is worth noting that in addition to the general climate, local microclimatic conditions play a significant role in the spread and maintenance of ixodid tick infestations [13].

The significant range of absolute altitudes of the territory and the peculiarities of the water regime determine the presence in the Turkestan region and have a significant impact on the species composition and number of ixodid ticks parasitizing farm animals. Farm animals in Southern Kazakhstan are parasitized by 12 species of ixodid ticks. The richest fauna is of ixodid ticks of the genus *Hyalomma*, species *H. anatolicum*, *H. detritus*, *H. scupense*, *H. aziatic*, *H. plumbeum*, which are potential carriers of the causative agent of theileriosis in cattle grazing in low-mountain steppe and tugai agricultural landscapes - 10 species. The smallest number of tick species is recorded in the desert landscape - 5 species; in the semi-desert landscape, 9 species parasitize [14]. A recent study of 2809 ticks collected from cattle and livestock buildings in the Turkestan region showed a high abundance of *H. anatolicum* (47.3%), *H. scupense* (26.3%) and *H. asiaticum* ticks (7.9%) with infection rates of 0.5%, 0.1% and 0.9%, respectively [15].

In studies by Sang C. et al, it was indicated that *D. marginatus* and *H. asiaticum* are the most common tick species in the five border regions of Kazakhstan. *D. marginatus* and *H. asiaticum* are also dominant in the neighboring Xinjiang Uyghur Autonomous Region, as South-East Kazakhstan and North-West China share a 1,783 km border [16].

Conclusion

In conclusion, our results show that theileriosis in cattle is widespread in the Turkestan region. A study of 738 samples of cattle using the polymerase chain reaction (PCR) method showed the presence of infection in 598 DNA samples isolated from cattle from 10 districts and 19 settlements of the Turkestan region. The overall positivity rate for the entire region was 81%, highlighting the significance of the infection in livestock. The difficult situation regarding blood-parasitic diseases in the Turkestan region must be taken into account when planning to regulate the movement of animals to other regions. Since the species of ticks *H. anatolicum*, *H. asiaticum*, *H. scupense*, *H. aziatic*, *H. plumbeum* are distributed over 90% of the territory of Kazakhstan, there remains a potential threat of shifting the area of theilariasis to the northern regions of Kazakhstan.

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