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Trypanosoma evansi Infection in Cattle from the Northern Mountainous Regions of Kyrgyzstan

Infección por *Trypanosoma evansi* en ganado de las regiones montañosas del norte de Kirguistán

Veli Yilgor CIRAK[1,6,*], İhsan KISADERE[2,6], Hüseyin CİHAN[3,6], Mambetaly TURSUMBETOV[4], Christian BAUER[5]

[1]Bursa Uludag University, Faculty of Veterinary Medicine, Department of Parasitology, Bursa, Türkiye
[2]Balikesir University, Faculty of Veterinary Medicine, Department of Physiology, Balikesir, Türkiye
[3]Bursa Uludag University, Faculty of Veterinary Medicine, Department of Internal Medicine, Bursa, Türkiye
[4]Arstanbek Duysheev Kyrgyz Veterinary Research Institute, Bishkek, Kyrgyzstan
[5]Justus Liebig University, Institute of Parasitology, Giessen, Germany
[6]Kyrgyzstan-Turkey Manas University, Faculty of Veterinary Medicine, Bishkek, Kyrgyzstan
[*]Corresponding authors: vcirak@uludag.edu.tr

ABSTRACT

Trypanosoma evansi (surra) is widespread in several continents and affects many wild and domestic animal species. Although it has long been known from camels, horses and cattle in southern Central Asian regions of the former Soviet Union, there are no reports from Kyrgyzstan. The aim of this study was to screen for the presence of *T. evansi* infection in cattle in Kyrgyzstan. Plasma samples were collected from 559 clinically healthy cattle of both sexes and different ages in ten villages in the northern mountainous regions of the country. The samples were tested for trypanosome antibodies using a direct card agglutination test *T. evansi* according to the manufacturer's instructions. A total of 23 samples were found to be positive, giving an apparent seroprevalence of 4.1 % (95 % confidence interval: 2.6 – 6.1 %). Low altitude (< 1000 m) and the Mediterranean climate zone were identified as risk factors for seropositivity. The results of this study suggest for the first time the presumptive presence of *T. evansi* infection in cattle in the sampled regions of Kyrgyzstan.

Key words: Cattle; Kyrgyzstan; seroprevalence; *Trypanosoma evansi.*

RESUMEN

El *Trypanosoma evansi* (surra) está ampliamente distribuido en varios continentes y afecta a numerosas especies de animales, tanto salvajes como domésticos. Si bien se conoce desde hace tiempo en camellos, caballos y ganado vacuno de las regiones meridionales de Asia Central de la antigua Unión Soviética, no existen informes de casos en Kirguistán. El objetivo de este estudio fue detectar la presencia de infección por *T. evansi* en ganado vacuno de Kirguistán. Se recogieron muestras de plasma de 559 bovinos clínicamente sanos de ambos sexos y diferentes edades en diez aldeas de las regiones montañosas del norte del país. Se analizaron las muestras para detectar anticuerpos antitripanosoma mediante una prueba de aglutinación directa en tarjeta para T. evansi), siguiendo las instrucciones del fabricante. Un total de 23 muestras resultaron positivas, lo que arroja una seroprevalencia aparente del 4.1 % (intervalo de confianza del 95 %: 2.6 – 6.1 %). La baja altitud (< 1000 m) y la zona climática mediterránea se identificaron como factores de riesgo de seropositividad. Los resultados de este estudio sugieren por primera vez la presunta presencia de infección por *T. evansi* en bovinos de las regiones muestreadas de Kirguistán.

Palabras clave: Bovinos; Kirguistán; seroprevalencia; *Trypanosoma evansi.*

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INTRODUCTION

Trypanosoma evansi is a blood parasite of a wide range of hosts including camelids, equids and bovids in Africa, South America and Asia [1, 2]. Haematophagous flies, mainly of the family Tabanidae but also of the family *Muscidae*, are the vectors of this pathogen, mechanically transmitting it from host to host during the blood-feeding process [3, 4].

Trypanosoma evansi causes surra disease and is highly pathogenic in camels (*Camelus*) and horses (*Equus caballus*). Cattle (*Bos taurus*) often have asymptomatic infection or mild disease but act as reservoir hosts. Nevertheless, *T. evansi* can also cause economically important chronic wasting disease in cattle (*Bos taurus*) [4]. To detect *T. evansi* infections, various diagnostic tests can be employed, including serological methods such as the card agglutination test for trypanosomiasis (CATT)/T. evansi test, immune trypanolysis, Indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assay (ELISA) using either the purified variant surface glycoprotein RoTat 1.2 or whole cell lysate. Additionally, molecular diagnostic tests targeting sequences within the ribosomal gene locus, such as internal transcribed spacer 1- touchdown - polymerase chain reaction (ITS1 TD PCR) and quantitative polymerase chain reaction targeting the 18S rRNA gene (18S qPCR), can also be utilized [5].

For the accurate diagnosis of an infection, it is advisable to combine various serological and molecular diagnostic tests [6].

In the past, the presence of *T. evansi* infection in cattle was documented in Uzbekistan and Kazakhstan 65 – 110 years ago [3]. In contrast, according to a meta - analysis of available data, there is "no published evidence" of this infection from the Central Asian successor states of the former Soviet Union [3].

However, one review declared *T. evansi* to be present in Uzbekistan and Kazakhstan, and 'inferred' in neighbouring Kyrgyzstan [7]; another one listed all Central Asian countries as its distribution area [5].

There appears to be some uncertainty as to whether this pathogen is currently present in Kyrgyzstan. This mountainous, predominantly agricultural country has 1.783,000 cattle and 554.000 horses (*Equus caballus*), according to the official census of 2022 [8]. The aim of the present study was to investigate the presence of *T. evansi* in cattle in Kyrgyzstan using a serological test.

MATERIALS AND METHODS

Study area

The cross - sectional study was conducted on cattle in ten rural communities in five provinces (‘oblasts’) of Kyrgyzstan. The sampled sites were in the northern mountainous regions of the country, shown in FIG. 1.

FIGURE 1. Map of Kyrgyzstan showing the locations of the sampled rural communities for *Trypanosoma evansi* (red dots)

Depending on the location, there is a humid continental climate with warm summers and long, cold winters ('Dfb' climate zone according to the Köppen - Geiger classification [9], a Mediterranean - like continental climate with dry summers and cold winters ('Dsa' climate zone), or a cold semi - arid (steppe) climate ('Bsk' climate zone). The number of cattle in the five provinces was about 648.000, representing about 48 % of the total cattle population in Kyrgyzstan, according to the official Veterinary census [8].

Most cattle are kept by private households, mainly for subsistence purposes (48 %) and on small farms (51 %), with only 0.1 % and 0.5 % respectively on state or collective farms [8]. Cattle in a village are usually herded together during the day and left to graze, in most cases all year round, weather permitting. There is no regular Veterinary care for the animals. Antiparasitic drugs are usually not used on private farms and village herds or are used sporadically on individual animals.

Sampling and laboratory methods

Cattle belonging to owners who agreed to participate in the study were sampled on each sampling day (d); they came from village herds, small private farms and one state farm. A total of 559 cattle of both sexes and different ages (6 months to 12 years) were sampled. The animals were mostly local breeds, including Alatau, crossbreeds and a few Angus and Holstein breeds.

They appeared healthy on the d of sampling, but no specific clinical examination was performed. Blood samples were drawn from the jugular vein into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and transported at 4 °C (Coleman Xtreme 5 Cooler, Coleman Company Inc., Chicago, IL, USA) to the laboratory for plasma preparation. Plasma samples were stored at – 20 °C (Bosch GSV29VWE0N, Bosch, Germany) until use.

Plasma samples were examined for trypanosome IgM antibodies using a CATT / *T. evansi*, Institute of Tropical Medicine, Antwerp, Belgium [10]. According to a report from the

World Organisation for Animal Health, the CATT/*T. evansi* test is the only diagnostic kit currently available for surra [6]. It can potentially be used across all mammal species and exhibits a high positive predictive value, making it a recommended option for the serodiagnosis of surra. Moreover, CATT/*T. evansi* is rather inexpensive, fast and simple.

The test is based on the direct agglutination of antibodies present in the serum or plasma of infected animals against fixed and stained bloodstream-form trypanosomes expressing the RoTat 1.2 variable antigen type. Briefly, the test was performed on a plastified card. Twenty-five microliters of diluted plasma were mixed with one drop of reconstituted antigen. When antibodies were present in the test sample, trypanosomes agglutinated within 5 minutes of rotation at 70 rpm. All samples were tested at a screening dilution of 1 : 4, which is considered positive [10]. Depending on the species of tested animals and the diagnostic techniques compared, the test sensitivity and specificity range from 12 % to 95.2 % and from 77.8 % to 100 %, respectively, [5 ,11].

Statistical analysis

Explorative data analysis was performed using the commercially available statistical software BIAS for Windows [12]. Apparent seroprevalence and its 95 % confidence interval (CI) were calculated. Associations of the percentage of seropositive cattle with sex, age group and breed, and with altitude and climate zone of rural communities were tested for significance using the Chi - square, Haldane - Dawson or Craddock - Flood tests. The odds ratio (OR) was calculated for altitude and climate zone of rural communities to assess their effect on the percentage of seropositive cattle. Differences with P values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In total, 23 of the 559 cattle tested by card agglutination test were positive at the 1:4 screening dilution, giving an apparent seropositivity of 4.1 % (95 % CI: 2.6 – 6.1 %), shown in TABLE I.

The percentage of seropositive cattle did not differ significantly between sexes, age groups or breeds. However, the percentage tended to be associated, although not significantly, with the altitude of the rural community and was significantly associated with climate zone, shown in TABLE II.

TABLE II

Effect of sex, age class and breed of cattle, and altitude and climate zone of the rural community on the percentage of cattle positive for Trypanosoma evansi

Factor	N positive/ N tested	% positive	P value (test used)
Sex			0.245 (CST)
Male	7/106	6.6	
Female	16/453	3.5	
Age (years)			0.168 (CST)
< 1	3/26	11.5	
1–2	5/198	2.5	
3–5	9/208	4.3	
> 5	6/127	4.5	
Breed			0.579 (HDT)
Local	16/369	4.3	
Alatau	3/77	3.9	
Angus	0/19	0	
Holstein	1/12	8.3	
Local x Alatau	3/33	9.1	
Local x Holstein	0/44	0	
Local x Simmental	0/5	0	
Altitude (m)			0.095 (CST)
< 1000	13/199	6.5	
1000–2000	4/122	3.3	
> 2000	6/238	2.5	
Climate zone			0.005 (CFT)
Dsa	11/117	9.4	
Dfb	3/102	2.9	
Bsk	9/340	2.6	

CST: Chi-square test; HDT: Haldane-Dawson test; CFT: Craddock-Flood test.

In direct comparison, both low altitude and Mediterranean climate zone were risk factors for seropositivity: the probability of detecting seropositive animals was almost three times higher (OR 2.7) in rural communities with an altitude < 1000 m than in those with an altitude > 2000 m and almost four times higher (OR 3.8) in climate zone 'Dsa' than in climate zone 'Bsk', shown in TABLE III.

TABLE I

Rural communities sampled in Kyrgyzstan, noting altitude, climate zone, cattle origin, sampling date, and seropositivity of Trypanosoma evansi

Province	Rural community	Altitude (m)	Climate zone	Date of sampling	Origin of cattle	N positive/ N tested	% positive
Talas	Köpürö-Bazar	2130	Bsk	Mar 2013	Village herd	3/103	2.9
Chui	Jaiyl	740	Dsa	Dec 2012	Village herd	3/32	9.4
	Kayyngdy	780	Dfb	Mar 2013	Private farm	2/45	4.4
	Sokuluk	700	Dfb	Dec 2012	State farm	1/57	1.8
	Tokmok	1200	Dsa	Mar 2013	Private farm	1/20	5.0
Bishkek	Bishkek	650	Dsa	Jun 2013	Private farm, village herd	7/65	10.8
Naryn	Kochkor	2100	Bsk	Mar 2013	Village herd	2/81	2.5
Yssyk-Kul	Kara-Shaar	1650	Bsk	Feb 2013	Village herd	2/48	4.2
	Tamga	1730	Bsk	May 2013	Village herd	1/54	1.9
	Tong	2820	Bsk	Jul 2012	Private farm, village herd	1/54	1.9
Total						23/559	4.1

TABLE III
Associations of altitude and climate zone of the rural community with the percentage of cattle positive for Trypanosoma evansi

Factor	Odds ratio (95 % CI)	P value
Altitude (m)		
< 1000 vs. 1000 – 2000	2.1 (0.7 – 6.3)	0.207
< 1000 vs. > 2000	2.7 (1.0 – 7.0)	0.041
Climate zone		
Dsa vs. Dfb	3.4 (0.9 – 11.8)	0.052
Dsa vs. Bsk	3.8 (1.6 – 8.9)	0.002

95 % CI: 95 % confidence interval.

Infections with *T. evansi* in camels, horses and cattle have long been known to occur in the southern Central Asian regions of the former Soviet Union [3]. These results indicate the presence of this pathogen in the cattle population of Kyrgyzstan, supporting statements in review papers [5, 7].

The present study was carried out using the card agglutination test. It primarily detects IgM antibodies, which indicate early infection. While IgMs can form complexes with antigens, their phagocytization can cause fluctuations in serum concentration, leading to false negative results [13]. Due to their short half-life, they are reliable indicators of recent trypanosome infections. The test has high genus specificity but limited species specificity, with potential cross-reactions with other Trypanozoon species and *T. vivax* [14].

Additionally, it was found to be highly specific but moderately sensitive in cattle (96 % and 83 % in one study [15] and 100 % and 12 % in another [11]). Based on these figures and the present results, the positive predictive value of the test is 47 % or 100 %. This would mean that at least 10 or even all 23 positive cattle in the current study could have been seropositive for *T. evansi*. CATT/*T. evansi* is known to occasionally produce nonspecific agglutination and it is recommended that this test be combined with an ELISA or other diagnostic methods (e.g. PCR) [6, 16].

However, DNA from this pathogen has recently not been detected by polymerase chain reaction in cattle mainly from the north western regions of Kyrgyzstan [17]. Therefore, more research is required to determine the presence and prevalence of this disease throughout the country, including non - studied regions of Kyrgyzstan.

Assuming that the CATT did indeed identify *T. evansi* positive cattle in the present study, the generally traditional small-scale livestock farming in the study area makes it more likely that these were autochthonous infections rather than introduced by purchased animals from a foreign endemic area. Cattle age and sex were not identified as risk factors, in the current study. This may be explained by the fact that in this region all cattle in a village, regardless of age and sex, are usually allowed to graze together and are equally exposed to biting haematophagous flies.

However, both low altitude (< 1000 m) and Mediterranean climate zone ('Dsa') were identified as risk factors for seropositivity. Unless these are accidental, biased results, they are probably related to the role of haematophagous flies in the transmission of *T. evansi*.

Tabanids are thought to be a major vector [3, 4]. Their breeding habitats require the presence of certain landscape parameters (water, soil moisture, vegetation), and their appearance and flight activity are influenced by, among other things, the location of the pasture and the climatic conditions (humidity, temperature, sunlight, wind) [4, 18, 19].

Certainly, these factors necessary for the occurrence of tabanids vary between latitudes and climatic zones [20, 21]. About 30 species of tabanids are known from Kyrgyzstan, where they are widespread and have been found in low valley semi-deserts and subalpine and alpine meadows [22]. However, data on their breeding habitats and spatial and temporal distributions are still lacking. These would help to explain the risk factors mentioned.

CONCLUSION

In conclusion, this study showed for the first time the presumptive presence of *T. evansi* infection in cattle in the sampled regions of Kyrgyzstan. It should be noted that further tests or complementary tests are necessary to confirm the presence of this pathogen. Additionally, more comprehensive studies should be conducted to assess the impact of this parasitosis on the country's animal population.

Conflicts of interest

The authors of this article declare that there are no potential conflicts of interest.

Author contributions

All authors have contributed equally to the article. They have read and approved the final version of the article.

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