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Parasitological and molecular diagnosis of *Trypanosoma theileri* in cattle sampled in the extreme south of São Paulo, SP, Brazil

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ABSTRACT

OBJECTIVE

Trypanosoma theileri is the type species of the subgenus *Megatrypanum*, first registered in cattle in South Africa and East Africa. Cattle farms in the extreme south of the municipality of São Paulo and Embu-Guaçu, are characterized as subsistence, on small properties, and 500 head of cattle were registered in 2019. This work aimed to evaluate the occurrence of *T. theileri* in cattle from family farms in the extreme south of the city of São Paulo and in the municipality of Embu-Guaçu, SP, Brazil.

METHODS

Parasitological and molecular diagnoses were carried out on samples taken from 68 cattle in small farms in the extreme south of São Paulo.

RESULTS

The specific molecular diagnosis of *T. theileri* was 17.6% positive (12/68) and the parasitological diagnosis was 5.8% (4/68).

CONCLUSIONS

Although considered non-pathogenic, its presence in the host may favor other parasitic and infectious diseases.

DESCRIPTORS

Trypanosomiasis, Tabanids, Ruminants, PCR.

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INTRODUCTION

Trypanosomes infect several groups of vertebrates; however, the majority do not cause disease in their host and circulate only in the wild. The same animal can be parasitized by several species of trypanosomes and can also present mixed infections. In cattle, infectious species are T*rypanosoma brucei*, *T. vivax*. *T. congolense*, and *T. evansi* found in Africa and *T. vivax* and *T. evansi* in the Americas[1,2,3]. *T. theileri* have a cosmopolitan distribution, however, they have no proven pathogenicity to their host.

The clade *T. theileri* of the subgenre *Megatrypanum* contains *Trypanosoma theileri*, the type species of the subgenus *Megatrypanum*, and was first recorded in cattle in South Africa and East Africa in 1902[3]. The transmission of *T. theileri* occurs during the blood meal of tabanids, through the elimination of feces that release metacyclic trypomastigote forms[1,2].

According to Hoare[3], all trypanosomes of cattle and buffaloes are considered *T. theileri*. However, considering Wells[4], only cattle isolates should be considered synonymous with *T. theileri*, while those isolated from *Megatrypanum* from non-bovine *Artiodactyla* species, including those from other *Bovidae spp.*, except goats (*T. theodori*) and sheep (*T. melophagium*), should be classified as *T. theileri-like*[5].

Studies have demonstrated the separation of *T. theileri* of cattle and *T theileri-like* of buffaloes[5]. Thus, two main phylogenetic strains in the *Megatrypanum* subgenus (TthI and TthII) were proposed based on phylogenetic analyses of *T. theileri* of cattle and *T. theileri-like* of buffaloes[5].

The use of different methodologies for the analysis of the variability of isolates from *T. theileri*, such as RAPD and phylogenetic analyses based on several molecular markers such as SSUrDNA, ITS1 SSUrDNA, Cytochrome B, Mini-exon, and Cathepsin L-*Like*, confirmed the presence of two major groups TthI and TthII, and associated strains according to the geographic origin of the isolates of *T. theileri*[6,7,8]. The occurrence and/or prevalence of *T. theileri* by different methodologies (parasitological and molecular tests) has already been recorded in cattle-producing regions throughout Brazil[5,6,7,8].

Cattle farms in the extreme south of the municipality of São Paulo and Embu-Guaçu, are characterized as subsistence, with small farms, and 500 head of cattle were registered in 2019[9]. The current work aimed to evaluate the occurrence of *T. theileri* in cattle from family farms in the extreme south of the city of São Paulo and in the municipality of Embu-Guaçu, SP, Brazil.

METHODS

Sampling

The samples obtained are from cattle raised in the extreme south of the metropolitan region of São Paulo, on small farms with an extensive cattle breeding system, with dairy and beef production. In the Parelheiros district, 20 animals were sampled (Girolando and Holstein breeds) aged between 2 and 6 years, in Marsilac there were 18 animals (Girolando, Gir, and Holstein) aged 4 to 6 years, in Cipó-Guaçú there were 12 animals (Girolando and Holstein) aged between 2 and 4 years, and in Embu-Guaçú there were 18 animals (Girolando, Holstein, and Nelore) aged between 2 and 4 years.

The region chosen to carry out the current study has forested, humid, flooded areas with a hot climate, being favorable for the development of the vector insect (horseflies).

Collection and processing of blood samples

Blood samples were collected by external jugular venipuncture in siliconized vacutainer® tubes containing diethylene tetraacetic acid (EDTA). The blood samples were fractionated in plastic Eppendorf tubes and frozen at -20ºC for further molecular analysis.

Parasitological diagnosis

Blood samples collected by puncture of the jugular or caudal vein were inoculated into tubes with biphasic medium consisting of a solid phase BAB (blood agar base with 10% rabbit blood) and a liquid phase of LIT medium (containing fetal bovine serum and antibiotics)[6]. The samples were examined weekly for a period of 2 months.

Molecular diagnosis

The commercial kit Purelink Genomic DNA (ThermoFisher) was used to extract DNA directly from blood and quantified photometrically using a Nanodrop (ThermoFisher). Oligonucleotides and reaction conditions for molecular diagnosis based on the cathepsin L-*like* gene followed the protocol proposed by Rodrigues and collaborators[10]. As a positive control, a sample of DNA from the isolate of *T. theileri* CBT 111 cryopreserved in the Brazilian Collection of Trypanosomatids from the Department of Preventive Medicine and Animal Health of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo was used.

RESULTS

Samples were collected from 68 cattle on small farms in the extreme south of São Paulo. Of the animals sampled, 5.8% (4/68) were positive for *T. theileri* in blood culture. The blood cultures were positive 8 days after sowing and it was not possible to establish and cryopreserve the cultures. The culture epimastigote forms had a morphology compatible with *T. theileri* (data not shown).

The specific molecular diagnosis for *T. theileri* based on the cathepsin L-*like* gene showed positivity of 17.6% (12/68) in animals from the districts of Parelheiros and Marsilac. All positive samples in blood culture were also positive in molecular diagnosis and came from the same property in Parelheiros.

DISCUSSION

The results obtained demonstrated, both by blood culture and PCR, the presence of *T. theileri* in the extreme south region of the city of São Paulo, with all positive samples coming from the same property. The animals tested are of the Girolando and Holstein breeds, purchased from nearby regions. The property is small, located on the edge of a preserved fragment of Atlantic Forest, in addition to being close to the Serra do Mar State Park. The climate in the region is composed of hot, sunny days, followed by cold nights with drizzle and constant rain (tropical and neotropical), favoring the prevalence of vectors (tabanids and hippoboscidae)[11]. In addition, the herd is reared in an extensive system, stabled only at the time of milking, facilitating contact with ectoparasites.

According to Hoare[3], *T. theileri* is the type species of the subgenus *Megatrypanum*, which includes species that, according to traditional criteria, have in common the presence of large blood trypomastigotes, transmission by contamination by tabanids and/or hippoboscidae vectors (intermediate hosts), restriction to ungulate mammalian hosts, limited pathogenicity, and worldwide distribution, corroborating the results found in this work, in which the positive animals were in good general condition, without showing any pathological alteration. The presence of insects of the Tabanidae family was reported in all the properties where samples for blood culture and PCR were collected.

During the blood meal, the invertebrate host (vector) ingests blood trypomastigote forms of the definitive host, which develop in the final portion of the intestine of these vectors into epimastigotes, and defecates metacyclic trypomastigote forms, which infect the skin or mucosal solutions, infecting a new definitive host. The enormous diversity of hosts reflects its wide geographic distribution, variety of ecosystems, and ecological niches, in addition to the transmission mechanisms described[12].

A previous work reports the isolation of *T. theileri* from cattle and buffaloes (*T. theileri*-Like), in the Vale do Ribeira region, state of São Paulo[4]. However, the present study is the first to demonstrate the presence and occurrence of *T. theileri* in the metropolitan region of São Paulo. The occurrence of *T. theileri* in Brazil is estimated at 90%, being recorded as isolated from T. theileri in the states of São Paulo, Rio de Janeiro, Mato Grosso do Sul, Rondônia, Pará, and Rio Grande do Sul[6,7,8,13].

The fact that *T. theileri* has worldwide distribution and is cyclically transmitted by tabanids, which are geographically widespread and well adapted to the Brazilian climate, along with the large supply of hosts in the country, which are raised in an extensive system, plus the importation of animals from endemic areas, may explain the presence of *T. theileri* in the extreme south region of São Paulo.

The studied region has two large dams, Guarapiranga and Billings, used to supply water to the population of the metropolitan region of São Paulo, with remnants of the Atlantic Forest. The region presents properties with rural characteristics and small properties with family production, where the few animals are the source of milk available. Despite being considered non-pathogenic, the presence of *T. theileri* in the host may favor other parasitic and infectious diseases5. Further studies should be conducted for the isolation and typing of strains that occur in the area.

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