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CASE REPORT
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Trypanosoma evansi in Horses from Colombia - Associated Infection Factors

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ABSTRACT

***Background*:** *Trypanosoma evansi* is the most common protozoan in tropical and subtropical regions of the world, due to its ability to maintain and be transmitted by vectors such as *Stomoxys* spp. and *Tabanus* spp. This protozoan causes high morbidity and mortality rates in horses in African, American and Asian countries. In the years 2021 and 2022, a high mortality rate was reported among horses with symptoms associated with *Trypanosoma* spp. in the municipality of Arauca, department of Arauca, Colombia. The investigation described here was therefore carried out, seeking to identify the pathogens and risk factors that led to the death of the horses in this region of Colombia.

***Cases*:** Blood samples were collected from Colombian criollo horses and dogs, as were samples of ticks, flies and horse-flies that infested the horses. A variety of tissue samples were removed from the horses a few min after their death for histopathological analysis. Two questionnaires were applied to obtain information about the horses and the environment in which they live. The results of the clinical examination revealed pale mucous membranes, jaundice, high fever, dehydration and lethargy. The horses were also infested with *Amblyomma mixtum* (17.6%) and *Dermacentor nitens* (82.4%) ticks, and with *Tabanus pungens* (74%), *Tabanus* spp. (26%), and *Stomoxys calcitrans* flies (100%), while the dogs were infested with *Rhipicephalus sanguineus* s.l. (77.7%) and *Amblyomma mixtum* (22.2%) ticks. The blood smear test results revealed the presence of *Trypanosoma* spp. in 66.6% (n = 4) of the horse blood samples, and in 50% (n = 1) of the dog blood samples. PCR performed to identify the *Trypanosoma* species confirmed the presence of *T. evansi*. Histological examination of the spleen revealed the involvement and dissemination of *T. evansi* in the tissues. The horses also showed the presence of Equine Infectious Anemia Virus (EIAV).

***Discussion*:** This is the first updated specific report of *T. evansi* in criollo horses in the savannah flood zone of the municipality of Arauca, Colombia. The main risk associated with *T. evansi* infection in horses was found to be infestation with the natural vector *T. pungens* and the mechanical vector *S. calcitrans*, which are efficient ectoparasites for the transmission of this parasite. The presence of *T. evansi* in dogs represents a constant risk to horses, because dogs may serve as a reservoir for the maintenance of the hemoparasite in the population under study. Another risk factor for horses could be the presence of vampire bats (*Desmodus rotundus*), a species of bat that has been described as a vector and reservoir of *T. evansi* in Colombia. The presence of EIAV antibodies in the horses under study can be attributed to the exposure of sick horses to vectors of this virus, such as *Tabanus* spp., *S. calcitrans* and inanimate needle-shaped fomites. This is the first study that identifies the coinfection of *T. evansi* and EIAV in horses in the floodplain region of Colombia. In view of the importance of these 2 pathologies to the health of horses, a greater number of tests and a larger animal population will be required to determine if this coinfection is the cause of the death of criollo horses in this region of Colombia. Lastly, the owners reported that pharmacological control with trypanocides has not been successful in most of the outbreaks that occurred during the years 2021 and 2022. This may suggest that *Trypanosoma evansi* is developing resistance to these drugs; therefore, specific studies will be required in the future to test this hypothesis.

Keywords: Arauca, Equine Infectious Anemia Virus, floodplain savannah, mortality, surra.

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INTRODUCTION

Trypanosoma evansi is a hematoprotozoan flagellate that causes trypanosomosis, a disease also known as "surra" in horses [9]. *T. evansi* is the most prevalent protozoan in tropical and subtropical regions of the world, due to its ability to be transmitted mechanically by the biting flies *Stomoxys* spp., *Haematopota* spp., *Chrysops* spp. and *Tabanus* spp. [5, 11]. This parasitic protozoan engenders significant economic losses in African, American and Asian livestock production systems, given the high morbidity and mortality rates it causes among horses used for working with livestock [2,19]. Added to this is the high cost of pharmacological treatment for the recovery of the animals health [10,20]. In the years 2021 and 2022, in the municipality of Arauca, department of Arauca, Colombia, horses exhibiting symptoms associated with *Trypanosoma* spp. suffered high mortality rates. The investigation reported here was therefore conducted in order to identify the pathogens and risk factors that led to the death of the horses in this region of Colombia.

CASES

Blood samples were collected from 6 pastured Colombian Criollo horses and 2 dogs from 3 farms where horse deaths had previously been recorded. The horses were selected according to their owners convenience and availability, while the dogs were selected as a secondary reservoir of *Trypanosoma* spp. [21]. Samples of ticks, house flies and horse-flies infesting the horses were also collected.

Each horse underwent a physical examination and approximately 5 mL of blood was drawn by jugular venipuncture with and without EDTA anticoagulants. In addition, 3 mL of blood was collected from the dogs by cephalic venipuncture, also into EDTA tubes. All the samples were kept in a cooler containing ice packs and taken to the laboratory before noon.

Different tissue samples were taken from 1 of the horses a few min after its death for histopathological analysis. All the samples were processed in the Laboratory of Animal Pathology of the University of Antioquia (UdeA), the Laboratory of Veterinary Parasitology of the University of Antioquia (UdeA), and the Laboratory of Veterinary Parasitology of the National University of Colombia (UN).

Horse blood smears positive for *Trypanosoma* spp. were subjected to a molecular analysis using genetic material extracted with the IndiSpin Pathogen Kit[1] [INDICAL®], as recommended by the manufacturer. The quality and quantity of DNA was verified using Take[3] plates on the EPOCH kit[2] and Gen52 software [Biotek®]. The DNA was stored at -20°C until PCR analysis.

The primers TBR F (5′-GAA TAT TAA ACA ATG CGC AG-3′) and TBR R (5′-CCA TTT ATT AGC TTT GTT GC-3′) were used to detect *Trypanozoon* [13], and TP1 GAATCAGTGTCTTTTGAGGG and TP2 AACCGTGTGTGTATTACA to detect *T. evansi* [4]. The initial denaturation temperature was 95°C for 5 min followed by 30 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The final extension was 10 min at 72°C. The PCR product was examined in 1% agarose gel stained with GelRed[3] [BioSalab®].

Two questionnaires were subsequently created to obtain information about the horses and the environment in which they live. The first questionnaire served to garner information about individual horses, such as their age, sex, breed, zootechnical purposes, herd structure, treatment, environmental description and tick/insects control program. The purpose of the second questionnaire was to obtain information at the stable level, and included questions about the location of the stable, the horses exercise schedules, stabling conditions, provenance, their owners actions for the care of sick horses, and type of neighboring farms.

The results of the survey showed that horses are used for work tasks such as herding cattle and transporting people from one farm to another. They are not managed, as such, but are simply allowed to roam freely in 3 pastures of approximately 50 hectares each to feed on natural grasses. These pastures are also shared with other animals, mainly cattle and pigs, as well as wild animals. Actions aimed at preserving the health of these horses are almost nonexistent, since they were only dewormed against gastrointestinal parasites and vitaminized a year ago. In terms of nutrition, they are not provided any type of supplementary food such as silage or feed. The farms involved in this study do not control the entry or exit from the premises of their own horses those coming from other farms. Moreover, they have no quarantine or treatment facilities for sick animals.

The clinical examination of the horses revealed poor body condition, pale mucous membranes, jaundice, high fever, dehydration, and lethargy, all symptoms

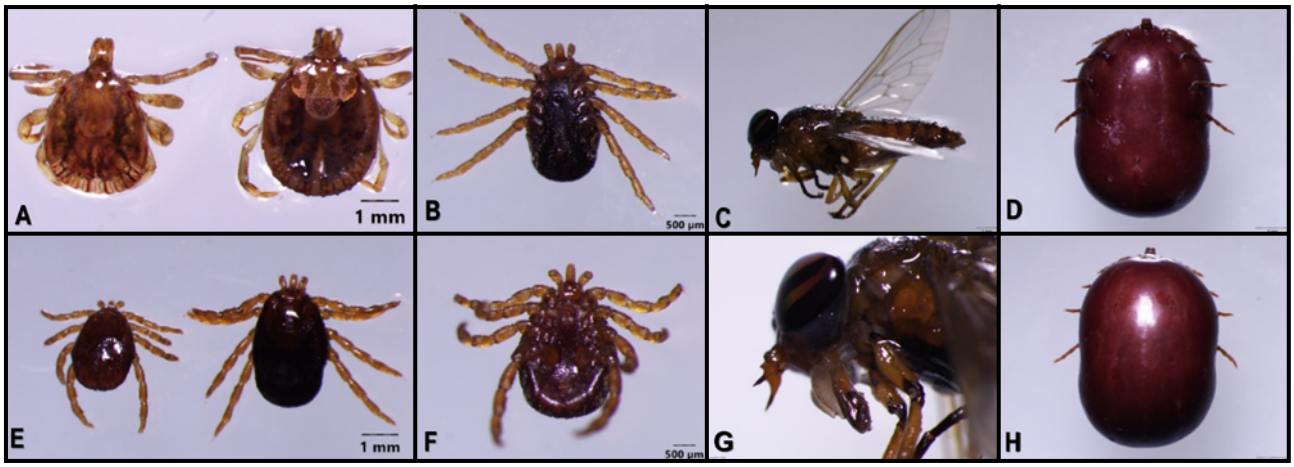

Figure 1. Details of the arthropods: A- *Amblyomma mixtum* female and male dorsal view. B- *Dermacentor nitens* female ventral view. C- *Tabanus* spp. D- *D. nitens* dorsal view, fully fed. E- *D. nitens* female and male dorsal view. F- *D. nitens* male. G- *Tabanus* spp. Head, and H- *D. nitens* dorsal view, fully fed.

consistent with trypanosomiasis. In addition to these symptoms, 1 of the horses exhibited hindlimb incoordination caused by hip problems, known in Spanish as "mal de caderas", which is a symptom caused by *Trypanosoma* spp. [12].

The horses also presented infestations with *Amblyomma mixtum* (17.6%) [Figure 1A] and *Dermacentor nitens* (82.4%) [Figure 1B] ticks. *Tabanus pungens* (74%) [Figure 1G] and *Tabanus* spp. (26%) and *Stomoxys calcitrans* flies (100%), while dogs were infested with *Rhipicephalus sanguineus* s.l. (77.7%) and *Amblyomma mixtum* (22.2%). Of the horse blood smears, 66.6% (n = 4) were positive for *Trypanosoma* spp. (Figure 2A), while 50% (n = 1) of the dog blood smears tested positive for this protozoan. PCR was performed to identify the species of *Trypanosoma* spp., using the primers TBR in the 164 bp band for *Trypanozoon* [13] and TP1 in the 500 bp band for *T. evansi* [4], which revealed the presence of *T. evansi* (Figure 2B).

A histological examination of the spleen revealed the involvement and spread of *T. evansi* in the tissues (Figure 3C). An infiltrate of mononuclear leukocytes with a predominance of histiocytes and plasma cells was observed in the red pulp (Figure 3A), which was found to be affected by moderate lymphoid depletion (Figure 3B). On the other hand, the blood serum samples were positive for antibodies against Equine Infectious Anemia Virus (EIAV).

DISCUSSION

In a previous study carried out in the department of Arauca, infection by *T. evansi* was reported in horses [14]. However, this is the first updated specific report of *T. evansi* in Criollo horses in the floodplain region of the municipality of Arauca.

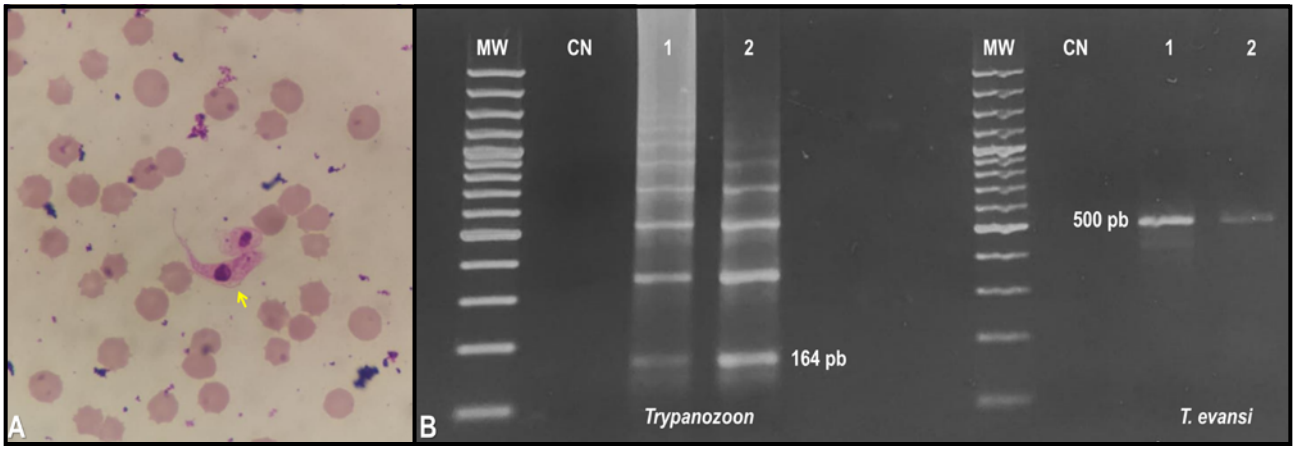

Figure 2. A- Blood smears positive for *Trypanosoma* spp. B- PCR. 1% agarose gel. MW: 100 bp weight marker. CN: negative control. 1: undiluted sample. 2. Sample diluted 1:10. Left side: TBR primers expected band 164 bp (blank: *Trypanozoon* [13]. Right side: TP primers expected band 500 bp (*T. evansi* [4]).

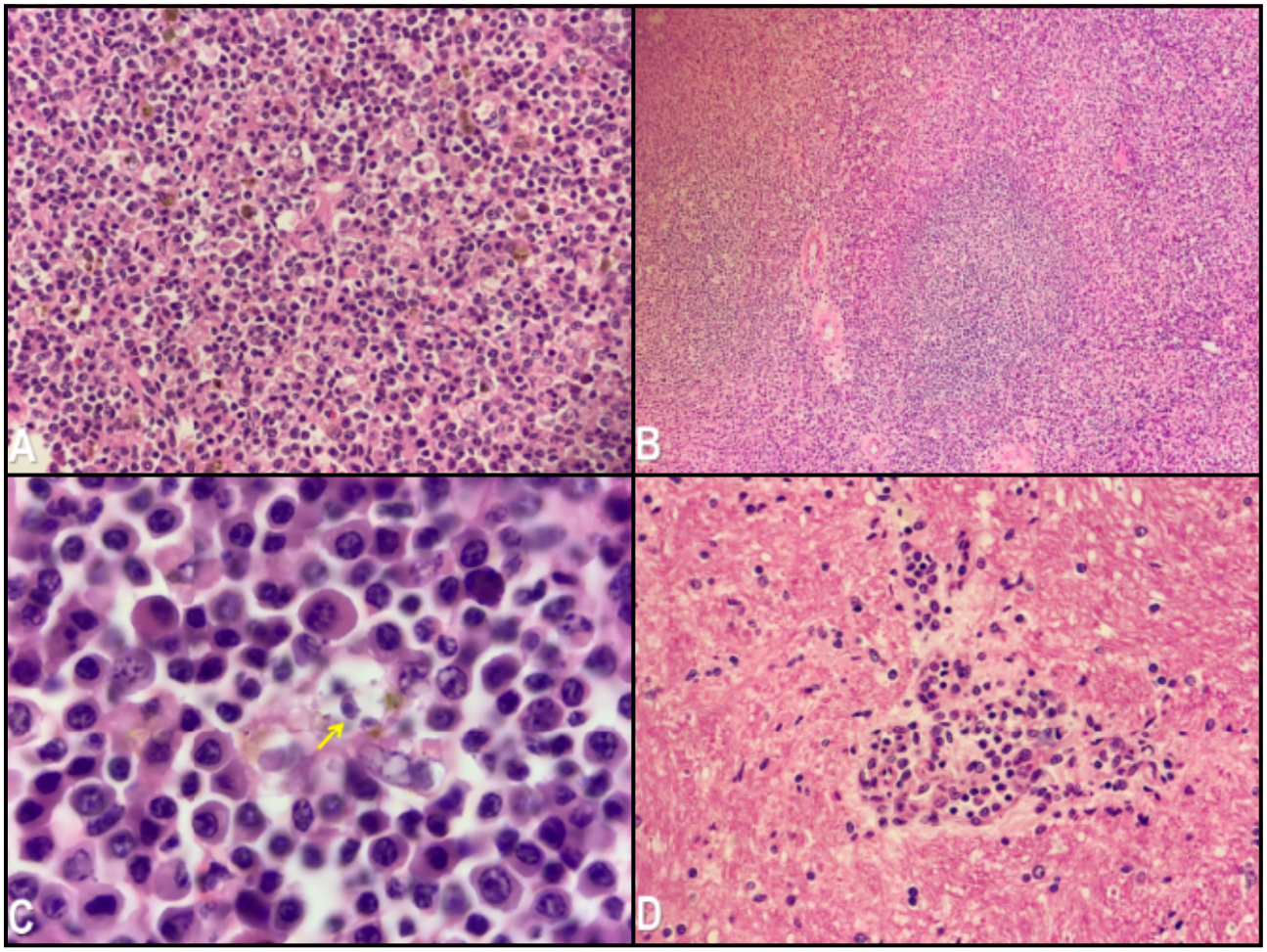

Figure 3. A- Spleen: red pulp. Mononuclear leukocyte infiltration with predominance of histiocytes and plasma cells. B- Spleen: white pulp with mild lymphoid depletion. C- Presence of parasitic structures in the cytoplasm of macrophages. D- Brain: Perivascular mononuclear leukocyte infiltration, consistent with non-suppurative encephalitis.

The main risk associated with *T. evansi* infection in horses was found to be infestation with the natural vector *T. pungens* and the mechanical vector *S. calcitrans*, which are efficient ectoparasites for the transmission of this parasite [15]. Horseflies feed on many vertebrate hosts, including humans [1,9], as well as domestic and wild animals such as capybaras living on the farms under study [16,23].

The identification of *T. evansi* in dogs may reinforce this hypothesis, given the constant risk posed by horseflies, which are the vector and reservoir responsible for infection by *Trypanosoma* spp. in horses, during the seasonal period of greatest proliferation of these biting flies [15].

Another risk factor for horses may be vampire bats (*Desmodus rotundus*), a species of bat that has been described as a vector and reservoir of *T. evansi* in Colombia [3]. This bat may contribute to the rapid dissemination and infection by *T. evansi*, by multiplying in horses not only as blood forms but also in a non-infectious manner, maintaining this hemoparasite without causing clinical signs for long periods of time [8,18].

EIAV in the horses under study may be attributed to exposure of sick horses to vectors of this virus, such as *Tabanus* spp., *S. calcitrans* and inanimate needle-shaped fomites [7,17]. In other regions of Colombia, a prevalence of up to 7.5% of EIAV infection in horses has been recorded [22]; therefore, the aforementioned horses were not free of this pathology.

This is the first study to identify coinfection with *T. evansi* and EIAV in horses in the floodplain region of Colombia. Similar findings were reported in horses in Brazil, registering a seroprevalence of 56% for EIAV and 70% for *T. evansi* [19]. A larger number of tests in horses are required to determine whether this coinfection is the cause of their death in this region of Colombia.

The owners report that pharmacological control with trypanocides has not been successful in most of the outbreaks that occurred during 2021 and 2022 (unpublished data). This suggests that *T. evansi* may be resistant to these drugs. This finding is consistent with the latest FAO report that describes the drug resistance of *Trypanosoma* spp., which poses an increasing problem in different countries around the world [6]. Specific studies will be required in the future to test this hypothesis.

MANUFACTURERS

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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