ORIGINAL RESEARCH ARTICLE

Parasites and rickettsiae in Creole horses from the flooded savanna of the Arauca department, Colombia: risk factors

Parásitos y rickettsias en caballos Criollos de la sabana inundable del departamento de Arauca, Colombia: factores de riesgo

Parasitas e rickettsias em cavalos Crioulos da savana inundada do departamento de Arauca, Colômbia: fatores de risco

Arlex Rodríguez-Durán[1,2]*; Daniel E, Blanco Sarmiento[2]; Omar R, Blanco Álvarez[2]; Delicht Y, Paredes Garrido[2]; Leidy T, Qüenza Vageon[2]; Jhon C, Tineo Barrios[2]; Gary S, Barriga Sanabria[2]; Jannet Bentez-Molano[3]; Jenny J, Chaparro-Gutiérrez[4]; Jesús A, Cortés-Vecino[1]

[1]*Grupo de Investigación Parasitología Veterinaria, Laboratorio de Parasitología Veterinaria, Universidad Nacional de Colombia (UNAL), Bogotá D.C., Colombia.*

[2]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Cooperativa de Colombia (UCC), Arauca, Colombia.*

[3]*Grupo de Investigación Los Araucos, Facultad de Medicina Veterinaria y Zootecnia, Universidad Cooperativa de Colombia (UCC), Arauca, Colombia.*

[4]*Grupo de Investigación CIBAV, Facultad de Ciencias Agrarias, Universidad de Antioquia (UdeA), Medellín, Colombia.*

Received: November 20, 2024. Accepted: March 17, 2025

***Corresponding author:** Laboratorio de Parasitología Veterinaria, Universidad Nacional de Colombia (UNAL), Carrera 45 No. 26-85, Bogotá D.C., Colombia. Email: arrodriguezdu@unal.edu.co

eISSN: 2256-2958

https://doi.org/10.17533/udea.rccp.e358966

To cite this article:

Rodríguez-Durán A, Blanco-Sarmiento DE, Blanco-Álvarez OR, Paredes-Garrido DY, Qüenza-Vageon LT, Tineo-Barrios JC, Barriga-Sanabria GS, Bentez-Molano J, Chaparro-Gutiérrez JJ, Cortés-Vecino JA. Parasites and rickettsiae in Creole horses from the flooded savanna of the Arauca department, Colombia: risk factors. Rev Colomb Cienc Pecu. Year, Vol, number, and pages pending. DOI : https://doi.org/10.17533/udea.rccp.e358966

Abstract

Background: In the flooded savannah of Colombia's Orinoquia region, Creole horse breeds are used for transportation, livestock handling or sporting events, which makes them valuable in animal production systems and part of the Llanero culture. However, health care is scarce, being susceptible to diseases caused by different pathogens or parasites. **Objective:** To identify the different groups of parasites, rickettsias and risk factors that affect Creole breed horses in the flooded savannah subregion of the department of Arauca, Colombia. **Methods:** Coprological (i), hematological (ii) and ectoparasite (iii) samples were taken from 94 horses from 24 farms in three municipalities of this region. Questionnaire was applied to relate risk factors (sex, season control and location) to the presence or absence of parasites or rickettsiae. To determine the statistical differences between the parasite species, the Student t test and ANOVA were used ($p \leq 0.05$). **Results:** 91.5% of horses were infected or infested with one or more parasites, while 87.5% of farms were positive. A total of 24 parasite species and one rickettsia species were identified. *Strongylus* spp., *Oxyuris equi*, *Neobalantidium coli* and *Blepharocorys valvata* were associated with the sex of the horses. While *Strongylus edentatus*, *Strongylus equinus*, *Dictyocaulus arnfieldi*, *Triodontophorus* spp., *Cycloposthium compressum*, *Cycloposthium elongatum*, *Blepharocorys* spp., *Amblyomma mixtum* and *Dermacentor nitens* were associated with previously dewormed horses. *Anaplasma phagocytophilum* was associated with all risk factors evaluated in this study. **Conclusions:** This study identified a variety of parasite species that could represent a risk to the health of this breed of horse, given that species that are not common in these domestic animals were recorded. On the other hand, highlighting the presence of *A. phagocytophilum*, *A. mixtum*, *Entamoeba* spp. and *Strongylus* spp., species registered as zoonotic, so additional studies would be necessary to classify these species.

Keywords: *Arauca; Amblyomma mixtum; Anaplasma* spp.*; Horsefly; Orinoquia; Stomoxys calcitrans; Strongylus equinus; Ticks; Trypanosoma evansi.*

Resumen

Antecedentes: En la sabana inundable de la Orinoquia colombiana, los caballos de raza Criolla son utilizados para transporte, manejo de ganado o eventos deportivos, lo que los hace valiosos en los sistemas de producción animal y parte de la cultura Llanera. Sin embargo, la atención sanitaria es escasa, siendo susceptibles a enfermedades causadas por diferentes patógenos o parásitos. **Objetivo:** Identificar los diferentes grupos de parásitos, rickettsias y los factores de riesgo que afectan a los caballos de raza criolla en la subregión sabana inundable del departamento de Arauca, Colombia. **Métodos:** Se tomaron muestras coprológicas (i), hematológicas (ii) y de ectoparásitos (iii) de 94 caballos de 24 fincas de tres municipios de esta región. Se aplicó un cuestionario para relacionar los factores de riesgo (sexo, época climática, control y localización) con la presencia o ausencia de parásitos o rickettsias. Para determinar las diferencias estadísticas entre las especies de parásitos se utilizó la prueba t de Student y ANOVA ($p \leq 0,05$). **Resultados:** El 91,5% de los caballos estuvo infectado o infestado con uno o más parásitos, mientras que el 87,5% de las granjas fueron positivas. Se identificaron 24 especies de parásitos y una especie de rickettsia. *Strongylus* spp., *Oxyuris equi*, *Neobalantidium coli* y *Blepharocorys valvata* se asociaron con el sexo de los caballos. Mientras que *Strongylus edentatus*, *Strongylus equinus*, *Dictyocaulus arnfieldi*, *Triodontophorus* spp., *Cycloposthium compressum*, *Cycloposthium elongatum*, *Blepharocorys* spp., *Amblyomma mixtum* y *Dermacentor nitens* se asociaron con caballos previamente desparasitados. *Anaplasma phagocytophilum* se asoció con todos los factores de riesgo evaluados en este estudio. **Conclusiones:** En este estudio se identificaron una variedad de especies de parásitos que podrían representar un riesgo para la salud de esta raza de caballos, dado que se registraron especies que no son comunes en estos animales domésticos. Por otro lado, destaca la presencia de *A. phagocytophilum*, *A. mixtum*, *Entamoeba* spp. y *Strongylus* spp., especies registradas como zoonóticas, por lo que serían necesarios estudios adicionales para clasificar estas especies.

Palabras clave: *Arauca; Amblyomma mixtum; Anaplasma* spp.*; garrapatas; Orinoquia; Stomoxys calcitrans; Strongylus equinus; tábano; Trypanosoma evansi.*

Resumo

Antecedentes: Na savana inundada da região de Orinoquia, na Colômbia, a raça de cavalos Crioulo são usadas para transporte, manejo de gado ou eventos esportivos, o que os torna valiosos em sistemas de produção animal e parte da cultura Llanero. No entanto, os cuidados de saúde são escassos, sendo suscetíveis a doenças causadas por diferentes patógenos ou parasitas. **Objetivo:** Identificar os diferentes grupos de parasitas, rickettsias e fatores de risco que afetam cavalos de raça Crioula na sub-região de savana inundada do departamento de Arauca, Colômbia. **Métodos:** Amostras coprológicas (i), hematológicas (ii) e ectoparasitárias (iii) foram coletadas de 94 cavalos de 24 fazendas em três municípios desta região. Foi aplicado um questionário para relacionar os fatores de risco (sexo, localização, clima e controle) à presença ou ausência de parasitas ou rickettsias. Para determinar as diferenças estatísticas entre as espécies de parasitas, foram utilizados o teste t de Student e a ANOVA ($p \leq 0{,}05$). **Resultados:** 91,5% dos cavalos estavam infectados ou infestados com um ou mais parasitas, enquanto 87,5% das fazendas foram positivas. Um total de 24 espécies de parasitas e uma espécie de rickettsia foram identificadas. *Strongylus* spp., *Oxyuris equi*, *Neobalantidium coli* e *Blepharocorys valvata* foram associados ao sexo dos cavalos. Enquanto *Strongylus edentatus*, *Strongylus equinus*, *Dictyocaulus arnfieldi*, *Triodontophorus* spp., *Cycloposthium compressum*, *Cycloposthium elongatum*, *Blepharocorys* spp., *Amblyomma mixtum* e *Dermacentor nitens* foram associados a cavalos previamente vermifugados. *Anaplasma phagocytophilum* foi associado a todos os fatores de risco avaliados neste estudo. **Conclusões:** Este estudo identificou uma variedade de espécies de parasitas que podem representar um risco à saúde desta raça de cavalo, uma vez que foram registradas espécies que não são comuns nestes animais domésticos. Por outro lado, destacando a presença de *A. phagocytophilum*, *A. mixtum*, *Entamoeba* spp. e *Strongylus* spp., espécies registradas como zoonóticas, portanto estudos adicionais seriam necessários para classificar essas espécies.

Palavras-chave: *Arauca; Amblyomma mixtum; Anaplasma* spp.*; carrapatos; Orinoquia; mutuca; Stomoxys calcitrans; Strongylus equinus; Trypanosoma evansi*.

Introduction

Creole horse breed are of vital importance for the inhabitants of Orinoquia, the flooded savanna region of the department of Arauca, Colombia, since they are used as working horses in livestock handling, in equine sports, or in transporting humans (Rodríguez-Qüenza *et al*., 2019). This region is home to a total of 23,694 horses, representing 1.6% of the country's equine population (ICA, 2024).

Colombia's flooded savannah region of Orinoquia has ideal environmental conditions and host diversity for the establishment and development of pathogens that can affect the health of horses (Acevedo-Gutiérrez et al., 2018; Uribe *et al*., 2021; Rodríguez-Durán et al., 2023). One of the problems that silently deteriorate equine health are parasites (Castillo *et al*., 2015; Chaparro-Gutiérrez *et al*., 2018). Not only do they comprise a variety of species that cause nutritional losses in animals, but they are also vectors of numerous pathogens that give rise to different equine diseases (Bowman, 2004; Jongejan and Uilenberg, 2004; Guglielmone *et al*., 2023).

Most farms in this region use only scant health practices to ensure the welfare of Creole horses, which are dewormed and given vitamins on average twice a year (Rodríguez-Durán *et al*., 2023). In fact, some horse owners give only preventive vaccines against Venezuelan Equine Encephalitis (VEE), Equine Influenza (IE) or Equine Infectious Anemia (EIA), since this vaccination is required by the country's public health authoritys for horses to be taken from one farm to another or when there is a change in ownership (ICA, 2020).

The current list of species of parasites or rickettsiae that can affect Creole horse breed in this region of Colombia is scanty and outdated. Therefore, in this study, we focused on identifying the different groups of parasites, rickettsiae and risk factors that affect this horse breed, considering the health, cultural and economic importance that the Creole horse represents for the people of the flooded savannah subregion of the Colombian Orinoquia.

Materials and Methods

Ethical considerations

All the experiments carried out on the horses were approved by the Ethics Committee for Animal Experimentation of the Universidad de Antioquia (UdeA), registered under permit CEEA No. 24-2-957.

Study area and horses

In the flooded savanna region of the Colombian Orinoquia, fecal, blood and ectoparasite samples were collected from 94 horses at 24 farms distributed in the municipalities of Arauca (6°55'43.97'' N; 70°27'34.00'' W), Cravo Norte (6°18'15.09'' N; 70°12'14.12'' W) and Puerto Rondón (6°16'48.00'' N; 71°06'01.10'' W) (Figure 1). This region is situated at about 120 m above sea level, with an average annual rainfall of 1477 mm, 90% relative humidity and an average temperature of 30.9°C (Rangel-Ch *et al*., 2017).

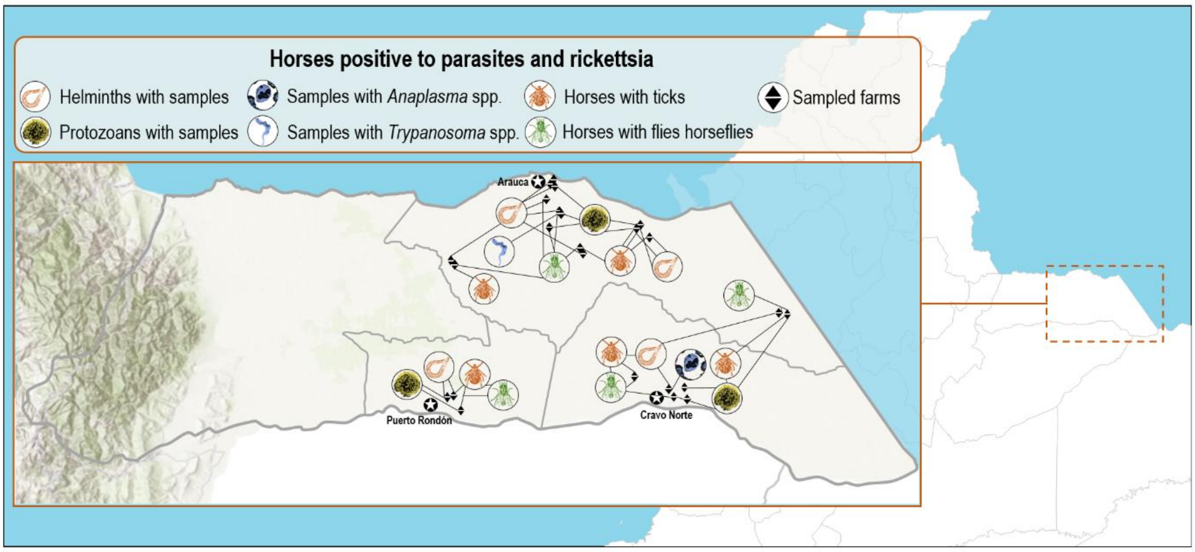

Figure 1. Location of the farms studied in the municipalities of Arauca, Cravo Norte and Puerto Rondón in the Orinoquia floodplain region of Colombia. Farms sampled by municipality: Arauca: n = 16/24, Cravo Norte: n = 5/24, and Puerto Rondón: n = 3/24 farms studied.

Creole breed horses found in the flooded savannah region of the Arauca department, Colombia, are small, non-elliptical animals, with variability in the color of their coat and with a unique conformation of their head characteristics, which distinguishes them from other horse breeds in Colombia (Jiménez et al., 2012; Salamanca et al., 2017; Salamanca et al., 2024). For the development of this study, only farms were included in which no type of genetic intervention was carried out on the horses. Then, an evaluation of the phenotypic characteristics compatible with the Creole breed was carried out, such as: triangular head, medium-sized ears pointed forward, comma-shaped nostrils, wide and flat forehead (Canelón, 2005; Salamanca et al., 2015). While the height and weight were determined by means of an inextensible tape and equinox rule. The determination of age was based on the methodology proposed by Cardona and Álvarez (2010).

Creole breed horses were sampled, males and females and aged between 1 to 10 years. The body condition score of the animals was between 3-3.5 (scale of 1 to 5). Likewise, animals that appeared to be healthy at the time of physical inspection were selected. Horses that showed symptoms compatible with parasites, rickettsiosis or other diseases were not selected. This was done to avoid the validity and reliability of the results, as well as to increase the suffering of the horses. The 24 farms engaged in similar horse management practices, i.e., grazing on natural open pastureland (Figure 2A), application of some officially controlled vaccines, minerals or vitamins and sharing space with other domestic and wild animals (Figure 2B).

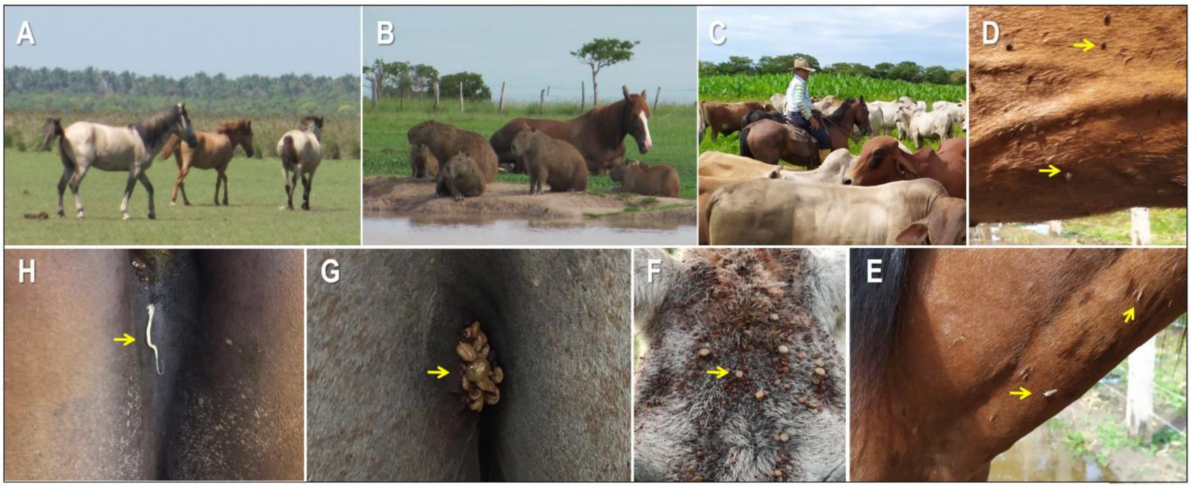

Figure 2. Habitat, use and horses infected or infested with parasites. (A) Creole breed horses in a natural state in the flooded savannah region of the Orinoquia of Colombia. (B) Horse sharing habitat with wild animals: capybaras (*Hydrochoerus hydrochaeris*). (C) Use of horses in cattle management. (D-G) Horses infested by ectoparasites: (D) flies, (E) horseflies, and (F and G) ticks; and (H) Horse with the presence of a species of gastrointestinal parasites.

Sample collection

Each horse was subjected to a physical examination, and various systems were used for a detailed semiological analysis (Bedoya *et al*., 2011; Chaparro-Gutiérrez *et al*., 2018). Three kinds of samples were collected during the dry and rainy climatic seasons. (i) Coprological: approximately 5 grams of fecal matter were collected directly from all the horses (n = 94/94). (ii) Hematological: approximately 5 ml of blood were collected from 18 horses and stored without and with EDTA anticoagulants, via jugular venipuncture (n = 18/94). (iii) Ectoparasites: samples were collected from all the horses (n = 94/94) at the time of the semiological evaluation. These samples consisted of ticks, flies and/or horseflies (no less than 10 specimens per ectoparasite group) found at the time of inspection.

Fecal and blood samples were placed in a cooler box containing ice packs and taken to the laboratory before noon on the day of collection. The ectoparasites were preserved in 70% alcohol and stored at room temperature until morphological identification. The samples were processed in the Veterinary Parasitology Laboratory of the Universidad Cooperativa de Colombia (UCC), Arauca, Colombia, the Veterinary Parasitology Laboratory of the Universidad de Antioquia (UdeA), Medellin, Colombia, and the Veterinary Parasitology

Laboratory of the Universidad Nacional de Colombia (UNAL), Bogotá D.C., Colombia.

Diagnostic tests

The following diagnostic techniques were employed, according to the type of sample. (i) Coprological: to identify the largest number of eggs, larvae or cysts of helminths and protozoans, flotation techniques were applied using Sheather's sugar solution (specific gravity = 1.27) (Mariño-González *et al*., 2017) and Telemann Modified MTM (Feldman and Guardis, 1999) on 1 g of fecal matter. The samples were examined under an optical microscope (Olympus SZ61, USA), starting under 10x magnification and ending with 100x magnification. The phenotypic keys described by Levine *et al*. (1980), Thienpont *et al*. (1986), Ito and Imai (2000) and Vélez (2006) were used.

(ii) Hematological: blood smears stained with Giemsa and Hemacolor® were analyzed (Luckins, 1988). Horse blood smears positive for *Trypanosoma* spp. were subjected to molecular analysis using genetic material extracted using an IndiSpin Pathogen Kit (INDICAL®, LE, Germany), as recommended by the manufacturer. The primers TBRF (5′-GAA TAT TAA ACA ATG CGC AG-3′) and TBRR (5′-CCA TTT ATT AGC TTT GTT GC-3′) were used to detect *Trypanozoon* (Masiga *et al*., 1992), and TP1F (5′-GAA TCA GTG TCT TTT GAG GG-3′), and TP2R (5′-AAC CGT GTG TGT ATT ACA-3′) to detect *Trypanosoma evansi* (Diall *et al*., 1992). 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.

(iii) Ectoparasites: these were identified taxonomically under a stereoscopic microscope (Olympus SZ61, USA) at 100x magnification, considering the phenotypic characteristics described by Strickland *et al*. (1976), Barros-Battesti *et al*. (2006, 2024), Kleinjan and Lane (2008), Estrada-Peña (2015), and Nava *et al*. (2014).

Risk factors for the presence or absence of parasites or rickettsiae

A questionnaire was applied to obtain as much information as possible about the horses, such as age, sex, breed, zootechnical uses, herd structure, preventive healthcare, environmental details, parasite control program, stable conditions (if applicable) and origin of horses. Grouping the results of this information into four risk factors associated with (i) sex, (ii) season (dry or rainy), (iii) control (administration or not of a type of anti-protozoal, anti-helminthic, chemical acaricide, anti-*Rickettsial* or anti-*Trypanosoma* control), and (iv) location (Arauca, Cravo Norte or Puerto Rondón), the above would allow us to establish some statistical relationship with respect to the presence or absence of species of parasite or rickettsia species identified in the three types of samples collected from the horses.

Statistical analysis

Student's *t* test and ANOVA were used to analyze the differences between the risk factors and the presence of each species of parasite or rickettsia recorded in the horses, using IBM SPSS Statistics version 19.0 (Statistical Package for the Social Sciences) software (IBM, NY, USA). Sex and control were established as dependent variables and the season and location of the horses as independent variables. Confidence intervals (C.I.) were calculated for 95% prevalence. The observed differences were considered statistically significant with a threshold value of 0.05 ($p < 0.05$). While to determine the prevalence of the frequency of observation of parasites and rickettsiae, according to Kier (2011), multiplying the number of positive animals in relation to the total number of samples by 100 (%). In addition, descriptive statistics were used to identify differences between species, genera and percentage frequency of parasites identified in the samples studied.

Results

Gastrointestinal parasites

Among the 24 farms that participated in this study, 87.5% (*n* = 21/24) had horses positive for one or more species of gastrointestinal parasites (GIP). Among the horses, 91.5% (*n* = 86/94) were infected with one or more species of GIP. A total of 17 GIP species were identified, distributed among 11 different families (Figure 3 and supplementary Table 1). Of these species, 52.9% (*n* = 9/17) were classified as helminths, and 47.1% (*n* = 8/17) as protozoans. The most frequent parasite observed was *Strongylus* spp., which infected 42.6% (*n* = 40/94) of the horses.

The species *Strongylus equinus* and *Cycloposthium* spp. came in second place, representing 24.5% of the observations (*n* = 23/94) for each, respectively. These were followed by *Cycloposthium compressum*, with 20.2% (*n* = 19/94), *Triodontophorus* spp., with 14.9% (*n* = 19/94), *Strongylus edentatus* and *Eimeria leuckarti*, with 12.8% (*n* = 12/94) and *Anoplocephala* spp. with 10.6% (*n* = 10/94). Less frequently identified were *Oxyuris equi* and *Trichostrongylus* spp., each with 9.6% (*n* = 9/94), followed by *Entamoeba* spp. with 8.5% (*n* = 8/94), *Parascaris* spp., *Dictyocaulus arnfieldi*, *Cycloposthium elongatum* and *Blepharocorys* spp., each with 6.4% (*n* = 6/94), *Blepharocorys valvata* with 3.2% (*n* = 3/94) and *Neobalantidium coli* with 2.1% (*n* = 2/94).

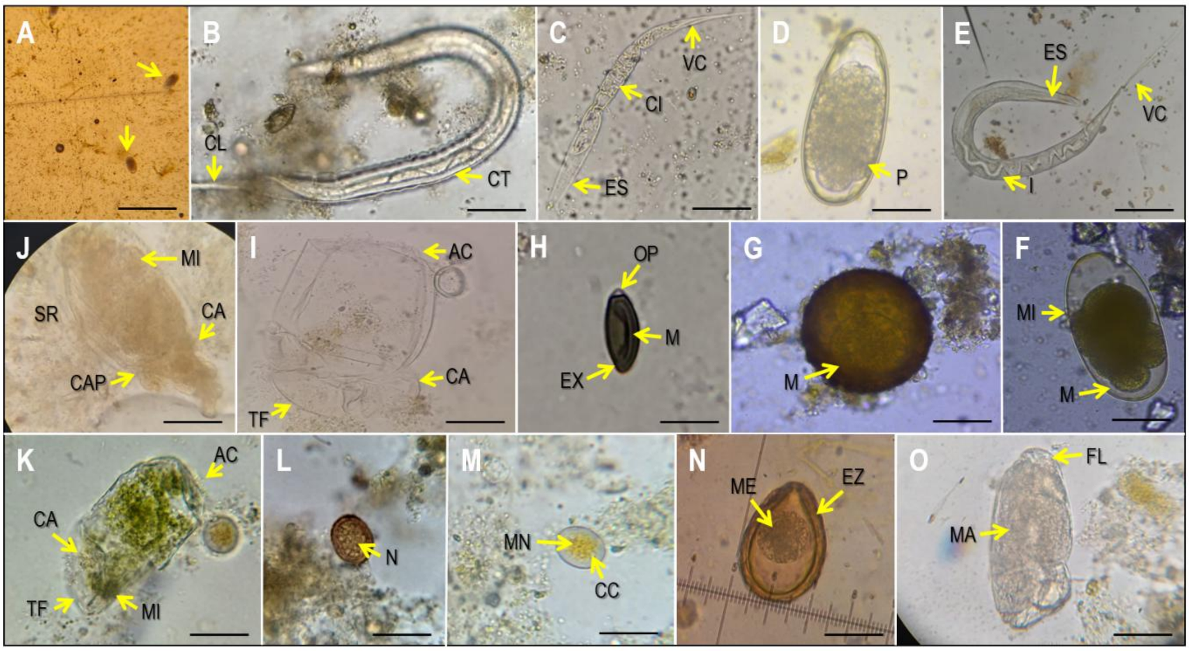

Figure 3. Gastrointestinal parasites identified in horses in Colombia's flooded savanna region of Orinoquia. (A) Eggs of *Strongylus* spp. under 10x magnification. (B) *Strongylus equinus*, L2 stage, under 40x magnification. CT: intestinal cells; CL: long whip-shaped tail. (C) *Strongylus edentatus* (*Alfortia edentata*), stage L2, under 40x magnification. IC: intestinal cells; VC: tail sheath; EN: esophagus. (D) Eggs of *Trichostrongylus* spp. under 40x magnification. Ellipsoid egg. P: morulae. (E) *Cyathostoma* spp., larval stage, under 40x magnification. I: intestinal cells; VC: tail sheath; EN: esophagus. (F) Eggs of *Triodontophorus* spp. under 40x magnification. MI: thin capsule with smooth surface, M: morula. (G) Eggs of *Parascaris* spp. under 40x magnification. M: morula. (H) Eggs of *Oxyuris equi*, under 100x magnification. OP: transparent polar operculum at a single point; M: morula; EX: thick capsule with smooth surface. (I) *Cycloposthium* spp., adult stage, under 100x magnification. AC: adoral cilia; AC: caudalium; TF: tail fin. (J) *Cycloposthium elongatum*, adult stage, under 100x magnification. AC: caudalium; CAP: caudal polybrachygyny; MI: micronucleus; SR: skeletal rod. (K) *Cycloposthium compressum*, adult stage, under 100x magnification. AC: adoral cilia; MI: micronucleus; AC: caudalium; TF: tail fin. (L) *Entamoeba* spp., immature cyst, under 100x magnification. N: typical nuclear structure and irregular chromatoid body. (M) *Neobalantidium coli*, cyst, under 100x magnification. MN: large macronucleus; CC: cytoplasmic contractile vacuoles. (N) *Eimeria leuckarti*, small and rounded non-sporulated oocyst, under 100x magnification. EZ: sporont; ME: outer membrane. Lastly, (O) Eggs of *Blepharocorys valvata*, under 100x magnification. FL: frontal lobe; MA: macronucleus.

Hemoparasites and rickettsiae

Among the 24 farms, 20.8% (*n* = 5/24) had horses positive for one or more species of hemoparasites, while 12.5% (*n* = 3/24) had horses positive for rickettsiae. One species of hemoparasite and two rickettsiae distributed in two different families were identified (Figure 4 and supplementary Table 1). Among the horses, 8.5% were infected with *Anaplasma phagocytophilum* (*n* = 8/94), 5.3% were infected with *Anaplasma* spp. (*n* = 5/94) (Figure 4A), 2.1% were infected with *Trypanosoma* spp. (*n* = 2/94) (Figure 4B), and 2.1% were infected with *Trypanosoma evansi* (*n* = 2/94) (Figure 4C).

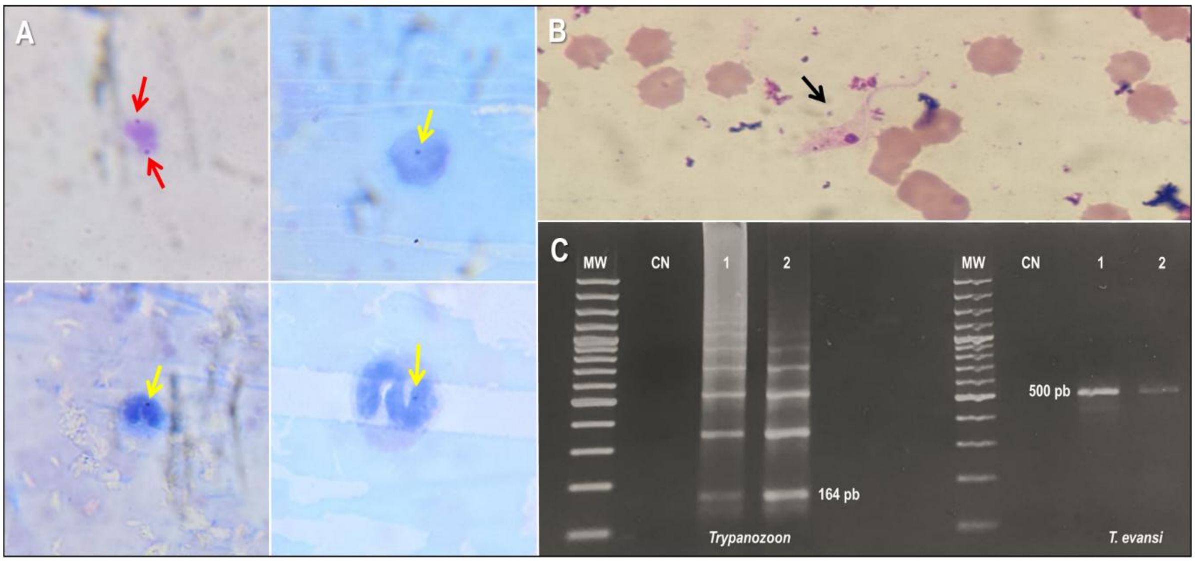

Figure 4. Identification of hemoparasites and rickettsiae in horses in Colombia's flooded savanna region of Orinoquia. (A) Detection of *Anaplasma* spp. and *A. phagocytophilum* in blood smear. Granulocyte surrounded by erythrocytes. In the cytoplasm of the granulocyte, note the presence of a morula with grouped *A. phagocytophilum* (yellow arrows), as well as free *A. phagocytophilum* (red arrows). (B) Blood smears positive for *Trypanosoma evansi* (black arrows), and (C) 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* (Masiga *et al*., 1992). Right side: TP primers expected band 500 bp corresponding to *T. evansi* (Diall *et al*., 1992).

Ectoparasites

Among the 24 farms, 79.2% (n = 19/24) had horses positive for one or more species of ectoparasites. Five species were identified, distributed in three different families (Figure 5 and supplementary Table 1). A total of 2598 ectoparasites were identified, classified into the two large groups of arthropods, insects and arachnids. The presence of the tick species *Amblyomma mixtum* (n = 98/2598) (Figure 5A-B) and *Dermacentor nitens* (n = 1241/2598) (Figure 5C-D) was recorded, as was that of the dipterans *Stomoxys calcitrans* (n = 783/2598) (Figure 5E), *Haematobia irritans* (n = 317/2598) (Figure 5F) and *Tabanus* spp. (n = 159/2598) (Figure 5G-H).

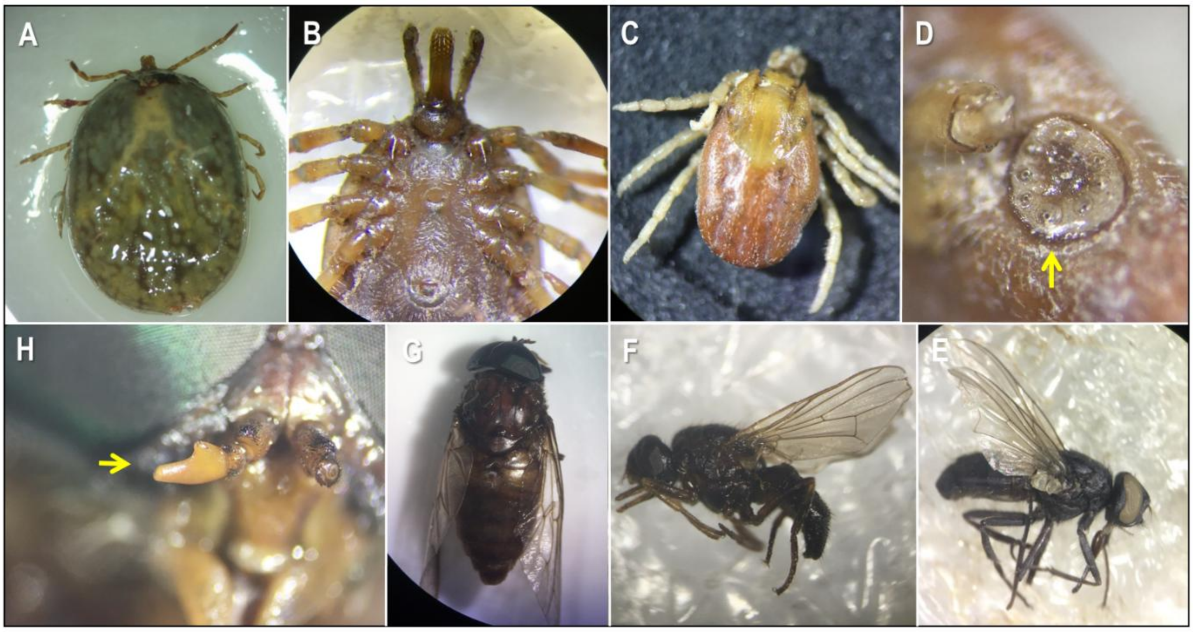

Figure 5. Ectoparasites identified infesting Creole horses in Colombia's flooded savannah region of Orinoquia. Ticks: (A) *Amblyomma mixtum*. Engorged adult female, dorsal view. (B) *Amblyomma mixtum*. Unengorged adult female, ventral view. (C) *Dermacentor nitens*. Adult female, partially engorged, dorsal view. (D) Peritreme (arrow) of *Dermacentor nitens*. Flies: (E) *Stomoxys calcitrans*. (F) *Haematobia irritans*, Horseflies: (G) *Tabanus* spp. and (H) Antennae (arrow) of *Tabanus* spp.

Analysis of risk factors

The presence or absence of each species of parasite in the horses was variable, with statistical differences according to each risk factor, (i) sex, (ii) season (dry or rainy), (iii) control (control or absence of control), and (iv) location (Arauca, Cravo Norte or Puerto Rondón)

(Figure 6 and supplementary Table 1). As for GIP, the only differences were in the presence of *Strongylus* spp., *O. equi*, *N. coli* and *B. valvata* (*n* = 4/17), depending on the sex of the horses, while the presence of *Strongylus* spp., *Parascaris* spp., *E. leuckarti*, *Cycloposthium* spp., *Blepharocorys* spp. and *B. valvata* (*n* = 6/17) was dependent on the season.

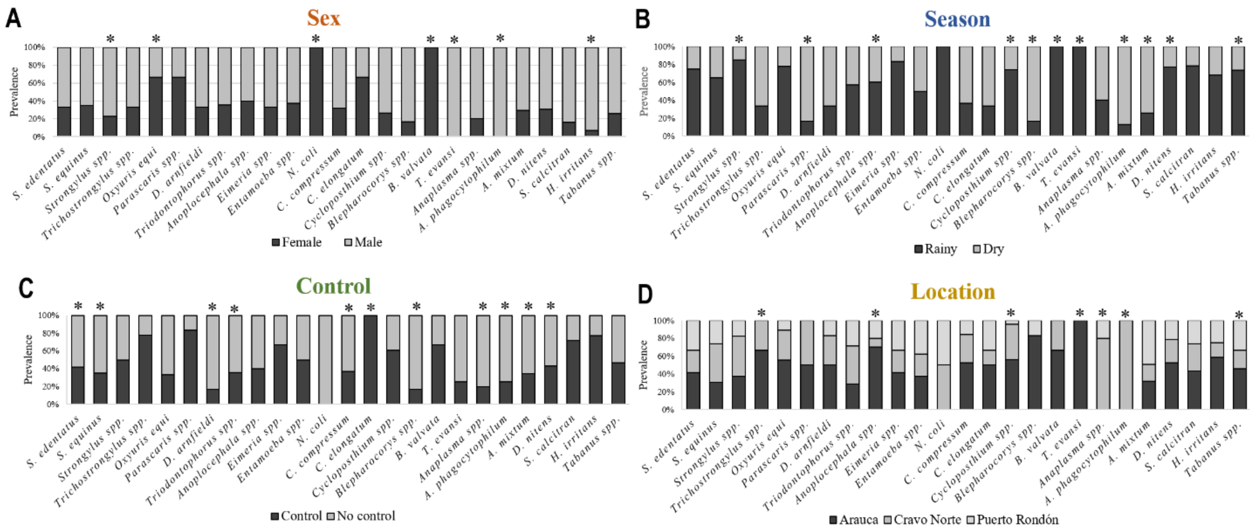

Figure 6. Prevalence rate and risk factors associated with the presence or absence of the different species of parasites and rickettsiae identified in Creole horse breeds in Colombia's flooded savanna region of Orinoquia. Risk factors analyzed: (A) Sex, (B) Season, (C) Control, and (D) Location. Asterisks indicate significant differences between parasite species associated with risk factors (sex, season, control or location), determined by Student's *t* test ($p < 0.05$).

On the other hand, the variable deworming of horses only affected the presence of the species *S. edentatus*, *S. equinus*, *D. arnfieldi*, *Triodontophorus* spp., *C. compressum*, *C. elongatum* and *Blepharocorys* spp. (*n* = 7/17). The analysis of the variable location (Arauca, Cravo Norte and Puerto Rondón) of the horses only affected the presence of the species *Trichostrongylus* spp., *Anoplocephala* spp. and *Cycloposthium* spp. (*n* = 3/17). Meanwhile, the presence of *A. phagocytophilum* showed statistical differences in all the risk factors. The presence of *T. evansi* differed according to the variables time of year and location, while that of *Anaplasma* spp. was identified among the horses according to the variables control and location.

Differences were also detected between the species of ectoparasites that infested horses. For example, the tick species *A. mixtum* and *D. nitens* were only influenced by the factors of climate (season) and type of control, while the presence of *Tabanus* spp. was influenced by the factors of sex, season and location of the horses. The presence of the fly species *H. irritans* was only dependent on the sex of the horses, while that of *S. calcitrans* was not affected by any of the factors evaluated in this study.

Among the 25 parasite species identified in the three parasite groups, the only species that presented no differences in any risk factor analyzed were *Entamoeba* spp. (GIP) and *S. calcitrans* (ectoparasite), while the other species presented differences in at least one risk factor. Surprisingly, only the species *A. phagocytophilum* (rickettsia) revealed a difference in all the risk factors (Figure 6).

Discussion

Infection by the parasite species *E. leuckarti*, *Entamoeba* spp., *Neobalantidium coli*, *C. compressum*, *C. elongatum*, *Cycloposthium* spp., *Blepharocorys valvata*, and *Blepharocorys* spp. were identified for the first time (non-*Strongyloides* parasites, rarely recorded in horses), as well as *Anaplasma* spp., *A. phagocytophilum* and *H. irritans* infestation in Creole horse breeds from this region of Colombia. Unlike our results, previous studies in this region have reported infections by *Habronema* spp., *Paranoplocephala* spp. (Moreno *et al*., 2015), *Trypanosoma equi* (Jaimes-Dueñez *et al*., 2023) and infestations by *A. cajennense* s.l. (Jaimes-Dueñez *et al*., 2023), *Rhipicephalus sanguineus* s.l. (Rivera-Páez *et al*., 2018) and *Tabanus pungens* (Rodríguez-Durán *et al*., 2023).

The variability and frequency of gastrointestinal parasites exceed those of ectoparasites and hemoparasites/rickettsiae. Of these, *Strongyloides* were the most prevalent (42.5%). This finding is in line with reports from other regions of Colombia, where 54.3% prevalence was recorded in horses living in the department of Antioquia (Chaparro-Gutiérrez *et al*., 2018) and 86.4% in horses in Bogotá D.C. (Ramírez-Hernández *et al*., 2019).

The factors driving parasite richness and prevalence are not clear in this study, but perhaps an important factor is the presence of other domestic or wild animals sharing the habitat where the horses live (Figure 2). The use of shared space allows the exchange of parasites through individual or group movements of the definitive hosts or infected intermediates, which can affect the parasite load or their transmission, thus creating an indirect transmission network (Gortazar *et al*., 2015; Sih *et al*., 2018). This fact could be confirmed by our results, since we identified 11 (n = 25) species of unrecorded or rare parasites infecting horses.

Previous reports from this same region have identified the presence of *E. leuckarti*, *C. compressum*, *Strongylus* spp. *Trichostrongylus* spp. and *Neobalantidium coli* infecting wild animals such as capybaras (*Hydrochoerus hydrochaeris*) (Rodríguez-Durán *et al*., 2015; Uribe *et al*., 2021), and *A. mixtum* infesting birds and capybaras (Busi *et al*., 2024; Ossa-López *et al*., 2024). The latter being an amplifying host for different *Rickettsia* spp. species (Luz et al., 2019; de Araújo et al., 2023), as well as a host that maintains different species of ticks in the flooded savannah region of Colombia (Rodríguez-Durán et al., 2025).

As for other species of domestic animals, *Strongylus* spp. and *Oxyuris* spp. have been found infecting mules (*Equus asinus* x *Equus caballus*) (Salamanca *et al*., 2017), *Trypanosoma* spp. infecting cattle (Salamanca-Carreño *et al*., 2018) and *A. mixtum* infesting cattle and pigs (Rodríguez-Durán *et al*., 2024; Ossa-López *et al*., 2024).

Treatment with some type of antiparasitic medication was the factor most closely correlated with the absence or presence of parasites in this research. We observed a lower prevalence of infections by the group of gastrointestinal parasites and hemoparasites/rickettsiae (47.6%) among treated horses than among those that received no type of antiparasitic treatment (52.4%). The ectoparasite group showed the opposite finding, i.e., a significant correlation was found among horses that had received some type of antiparasitic control, showing a high prevalence (55.7%), compared to those that had not been subjected to parasite control (44.3%).

Among the horse owners, 87.5% (*n* = 21/24) use some type of chemical acaricide as the only method to control ectoparasites during periods of high infestation levels (mostly in dry). Only 16.6% (*n* = 4/24) of them alternate with another type of non-chemical control. The former practice may have accelerated the multiple resistance of ectoparasites to the different commercial chemical molecules circulating in the region. What leads us to this inference are the findings of resistance studies that have focused on ectoparasites in the region, which have reported that some populations of ticks are already resistant to Amitraz (9.7% efficacy), Deltamethrin (6.7% efficacy) or Trichlorfon (34.9% efficacy) (Villar *et al.*, 2016), as well as genetic resistance to Ivermectin (Villar *et al.*, 2020). However, it is important to clarify that specific resistance studies with populations of ticks, flies or *Tabanus* spp. that specifically infest horses in this region are needed to verify our hypothesis.

The second most prevalent risk factor was the season, with the rainy season (73.3%) being more prevalent than the dry season (26.7%). These findings are consistent with those of other studies in the region, which have also reported a higher frequency of parasitic infections during the rainy season (Rodríguez-Durán *et al*., 2015). The rainy season is crucial for animal and plant development in the flooded savannah region, when conditions of humidity, temperature and precipitation increase (Rangel-Ch *et al*., 2017). However, this season also favors the development of most of the gastrointestinal parasites and ectoparasites identified in our study. Probably, the environmental conditions of this season provide parasites with better opportunities for development, transport, infection and infestation or abundance of hosts in their life cycles (Daugschies, 2000; Henriksen *et al*., 2023).

For example, in horses, ciliated protozoans provide a positive biological interaction, behaving as commensals rather than as parasites. Therefore, horses show a tolerance for these protozoans and not a reaction of resistance and elimination (Gürelli and Göçmen, 2011; Gürelli *et al*., 2019; Cedrola *et al*., 2019). However, this interaction can be reversed in a situation of stress, e.g., during food scarcity (especially during the dry), when the behavior of this group of parasites shifts from mutualism to parasitism (Martínez and Cordero del Campillo, 2008).

On the other hand, the location of the horses only differed when it came to the species *Anoplocephala* spp., *Cycloposthium* spp., *Trichostrongylus* spp. and *Tabanus* spp., while the other gastrointestinal parasite and ectoparasite species were not affected by location. This may be explained by the fact that the three municipalities evaluated have very similar environmental conditions, fauna and flora, animal production, cultural and equine management systems (Rangel-Ch *et al*., 2017; Rodríguez-Qüenza *et al*., 2019), thus possibly explaining the similar prevalence rates among the other parasite species in the three municipalities. However, regarding the hemoparasite/rickettsia group, the findings varied in the municipality of Cravo Norte, showing differences in the three species. As for the horses' sex, males showed a higher parasite prevalence rate (76.2%) than females (23.8%). However, this may be attributed to the fact that, in this study, 24.5% (n = 23/94) of the sampled horses were females, while 75.5% (n = 71/94) were males, thus possibly explaining these results.

The variability of the identified species is of great concern to equine health in this region of Colombia. Although most of the identified parasite species cause an asymptomatic and persistent pathogenesis in their hosts, these parasites may have significant impacts on equine health as their prevalence rates increase (Stringer, 2014; Mehlhorn, 2016). Therefore, our findings serve as basic information to help future prevention programs and research on parasite species of major concern for animal and human health, in view of the importance and close contact with horses that their owners have, thereby potentially becoming reservoirs for zoonotic parasite species.

Our findings also provide valuable information from the perspective of zoonosis, since the species *Strongylus* spp. (Braga *et al*., 2021), *Entamoeba* spp. (Navone *et al*., 2006; Hernanz *et al*., 2023), *A. phagocytophilum* (Dzięgiel *et al*., 2016; Matei *et al*., 2019) and *A. mixtum* are identified (Novakova *et al*., 2015; Castillo-Martínez *et al*., 2020; Chitimia-Dobler *et al*., 2020), the latter identified as a vector of *Rickettsia rickettsii* and *Rickettsia amblyommatis* in this region (Rivera-Páez *et al*., 2018; Chaparro-Gutiérrez *et al*., 2023). The presence of the above species could pose a risk for humans, given that the interaction between hosts, ecosystems and humans is becoming more frequent (Otranto *et al*., 2021; Uribe *et al*., 2021), which makes it is necessary to address this problem with a "One Health" approach in the flooded savanna of Colombia's Orinoquia region. Lastly, additional and specific studies will be required to classify the species found in our study, in particular, which are known to also infect humans.

Conclusions

This research updates and identifies new species of parasites and rickettsiae in Creole horses found in the flooded savannah region of the Arauca department, Colombia. Likewise, we were able to establish some risk factors for the presence or absence of these parasites and pathogens in the horses studied. The previous results can serve as a basis for information to establish different types of anti-parasitic and anti-rickettsiae controls, as well as sanitary measures that could be established in horses of this breed and region, taking into account that some of these species of parasites are reported as zoonotic and the close relationship and interaction that humans and horses present, which could create a risk factor for the presentation of parasitic diseases.

Limitation

This study did not involve the use of additional techniques (for example, molecular or coprological) that would allow us to complement the taxonomic identification of other parasite species or confirm the gastrointestinal parasite species of the genera *Anoplocephala*, *Blepharocorys*, *Cycloposthium*, *Entamoeba*, *Parascaris*, *Strongylus*, *Trichostrongylus* and *Triodontophorus* and of the rickettsia *Anaplasma*. This limitation may have precluded the identification of a larger number of species, but which will hopefully be possible in the near future.

Declarations

Acknowledgments

The authors greatly appreciate the cooperation of the owners of the 24 farms included in this study, who allowed them to collect samples of parasites from their horses. Also, to the Ministerio de Ciencia, Tecnología e Innovación de Colombia (MINCIENCIAS) (Process No. 885/2020) for its financial support of postgraduate students.

Funding

The authors gratefully acknowledge to Ministerio de Ciencia, Tecnología e Innovación de

Colombia (MINCIENCIAS) (Process No. 885/2020) for its financial support of postgraduate students.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to the work presented in this article.

Author contributions

AR, DB, OB, DP, JT, LQ, GB, and JB conceived and designed the study. DB, OB, DP, JT, LQ, GB, and JB performed sample and data collection. AR, DB, OB, DP, JT, LQ, GB, JC and JC processed and analyzed the samples. AR, JC, and JC designed, reviewed, and edited the manuscript.

Data Availability

The data supporting the findings of this study are available upon reasonable request to the corresponding author.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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Supplementary Table 1. Analysis of risk factors according to the frequency of occurrence of parasite species identified infecting or infesting Creole horses Colombia's flooded savanna region of Orinoquia.

Parasites/rickettsia	Total	Risk factors/Positive (% ± C.I.)												
		Sex		P	Season		P	Control		P	Location			P
		Female	Male		Rainy	Dry		Control	No control		Arauca	Cravo Norte	Puerto Rondón	
Gastrointestinal parasites														
Strongylus edentatus	12	4 (33.3 ± 0.33)	8 (66.7 ± 0.34)	0.89	9 (75.0 ± 1.22)	3 (25.0 ± 1.22)	0.18	5 (41.7 ± 0.49)	7 (58.3 ± 0.44)	0.03*	5 (41.7 ± 0.74)	3 (25.0 ± 0.55)	4 (33.3 ± 0.82)	0.76
Strongylus equinus	23	8 (34.8 ± 0.44)	15 (65.2 ± 0.43)	0.97	15 (65.2 ± 1.52)	8 (34.8 ± 1.03)	0.15	7 (34.8 ± 1.06)	13 (65.2 ± 1.32)	0.03*	7 (30.4 ± 0.64)	10 (43.5 ± 1.22)	6 (26.1 ± 1.29)	0.29
Strongylus spp.	40	9 (22.5 ± 0.45)	31 (77.5 ± 0.50)	0.02*	34 (85.0 ± 4.63)	6 (15.0 ± 0.89)	0.03*	20 (50.0 ± 2.23)	20 (50.0 ± 1.64)	0.31	15 (37.5 ± 1.64)	18 (45.0 ± 4.22)	7 (17.5 ± 1.71)	0.52
Trichostrongylus spp.	9	3 (33.3 ± 0.45)	6 (66.7 ± 0.30)	0.90	3 (33.3 ± 0.55)	6 (66.7 ± 1.10)	0.34	1 (77.8 ± 0.64)	2 (22.2 ± 0.44)	0.32	6 (66.7 ± 0.71)	3 (33.3 ± 0.55)	0 (00.0 ± 0.00)	0.00*
Oxyuris equi	9	6 (66.7 ± 0.39)	3 (33.3 ± 0.22)	0.03*	7 (77.8 ± 1.17)	2 (22.2 ± 0.52)	0.14	3 (33.3 ± 0.46)	6 (66.7 ± 0.73)	0.24	5 (55.6 ± 0.92)	3 (33.3 ± 0.55)	1 (11.1 ± 0.50)	0.31
Parascaris spp.	6	4 (66.7 ± 0.33)	2 (33.3 ± 0.18)	0.09	1 (16.7 ± 0.41)	5 (83.3 ± 0.41)	0.01*	5 (83.3 ± 0.49)	1 (16.7 ± 0.33)	0.24	3 (50.0 ± 0.52)	3 (50.0 ± 0.55)	0 (00.0 ± 0.00)	0.14
Dictyocaulus arnfieldi	6	2 (33.3 ± 0.24)	4 (66.7 ± 0.25)	0.92	2 (33.3 ± 0.82)	4 (66.7 ± 1.21)	0.58	1 (16.7 ± 0.26)	5 (83.3 ± 0.73)	0.02*	3 (50.0 ± 0.52)	2 (33.3 ± 0.55)	1 (16.7 ± 0.50)	0.54
Triodontophorus spp.	14	5 (35.7 ± 0.36)	9 (64.3 ± 0.36)	0.95	8 (57.1 ± 0.82)	6 (42.9 ± 0.89)	0.51	5 (35.7 ± 0.49)	9 (64.3 ± 0.71)	0.01*	4 (28.6 ± 0.53)	6 (42.9 ± 0.84)	4 (28.6 ± 1.41)	0.79
Anoplocephala spp.	10	4 (40.0 ± 0.33)	6 (60.0 ± 0.30)	0.73	6 (60.0 ± 1.26)	4 (40.0 ± 0.82)	0.59	4 (40.0 ± 0.46)	6 (60.0 ± 1.12)	0.22	7 (70.0 ± 0.83)	1 (10.0 ± 0.45)	2 (20.0 ± 0.58)	0.04*
Eimeria leuckarti	12	4 (33.3 ± 0.33)	8 (66.7 ± 0.34)	0.89	10 (83.3 ± 1.21)	2 (16.7 ± 0.52)	0.03*	8 (66.7 ± 0.52)	4 (33.3 ± 0.53)	0.68	5 (41.7 ± 0.52)	3 (25.0 ± 0.55)	4 (33.3 ± 1.15)	0.76
Entamoeba spp.	8	3 (37.5 ± 0.29)	5 (62.5 ± 0.28)	0.88	4 (50.0 ± 0.82)	4 (50.0 ± 0.52)	1.00	4 (50.0 ± 0.46)	4 (50.0 ± 53)	0.39	3 (37.5 ± 0.74)	2 (25.0 ± 0.55)	3 (37.5 ± 0.50)	0.88
Neobalantidium coli	2	2 (100 ± 0.24)	0 (0.0 ± 0.00)	0.05*	2 (100.0 ± 0.52)	0 (00.0 ± 0.00)	0.14	0 (00.0 ± 0.00)	2 (100.0 ± 0.44)	0.06	0 (00.0 ± 0.00)	1 (50.0 ± 0.45)	1 (50.0 ± 0.50)	0.61
Cycloposthium compressum	19	6 (31.6 ± 0.39)	13 (68.4 ± 0.41)	0.72	7 (36.8 ± 1.94)	12 (63.2 ± 1.55)	0.43	7 (36.8 ± 0.52)	12 (63.2 ± 1.12)	0.02*	10 (52.6 ± 1.67)	6 (31.6 ± 1.10)	3 (15.8 ± 0.96)	0.36

Cycloposthium elongatum	6	4 (66.7 ± 0.33)	2 (33.3 ± 0.18)	0.09	2 (33.3 ± 0.52)	4 (66.7 ± 1.03)	0.49	3 (100.0 ± 0.51)	0 (00.0 ± 0.00)	0.03*	3 (50.0 ± 0.52)	1 (16.7 ± 0.45)	2 (33.3 ± 0.58)	0.54
Cycloposthium spp.	23	6 (26.1 ± 0.39)	17 (73.9 ± 0.45)	0.30	17 (73.9 ± 1.33)	6 (26.1 ± 1.26)	0.03*	14 (60.9 ± 0.80)	9 (39.1 ± 0.80)	0.83	13 (56.5 ± 0.92)	9 (39.1 ± 1.30)	1 (4.3 ± 0.50)	0.01*
Blepharocorys spp.	6	1 (16.7 ± 0.17)	5 (83.3 ± 0.28)	0.33	1 (16.7 ± 0.41)	5 (83.3 ± 0.41)	0.01*	1 (16.7 ± 0.26)	5 (83.3 ± 0.53)	0.00*	5 (83.3 ± 0.92)	0 (00.0 ± 0.00)	1 (16.7 ± 0.50)	0.08
Blepharocorys valvata	3	3 (100.0 ± 0.29)	0 (0.0 ± 0.00)	0.01*	3 (100.0 ± 0.55)	0 (00.0 ± 0.00)	0.04*	2 (66.7 ± 0.35)	1 (33.3 ± 0.33)	0.88	2 (66.7 ± 0.46)	1 (33.3 ± 0.45)	0 (00.0 ± 0.00)	0.34
Haemoparasites/rickettsias														
Trypanosoma evansi	4	0 (0.0 ± 0.00)	4 (100.0 ± 0.25)	0.13	4 (100.0 ± 0.52)	0 (00.0 ± 0.00)	0.01*	1 (25.0 ± 0.26)	3 (75.0 ± 0.50)	0.09	4 (100.0 ± 0.53)	0 (00.0 ± 0.00)	0 (00.0 ± 0.00)	0.00*
Anaplasma spp.	5	1 (20.0 ± 0.17)	4 (80.0 ± 0.25)	0.47	2 (40.0 ± 0.52)	3 (60.0 ± 0.55)	0.59	1 (20.0 ± 0.26)	4 (80.0 ± 0.53)	0.02*	0 (00.0 ± 0.00)	4 (80.0 ± 0.45)	1 (20.0 ± 0.50)	0.03*
Anaplasma phagocytophilum	8	0 (0.0 ± 0.00)	8 (100.0 ± 0.34)	0.02*	1 (12.8 ± 0.41)	7 (87.5 ± 0.75)	0.02*	2 (25.0 ± 0.35)	6 (75.0 ± 0.50)	0.00*	0 (00.0 ± 0.00)	8 (100.0 ± 1.34)	0 (00.0 ± 0.00)	0.02*
Ectoparasites														
Amblyomma mixtum	98	29 (29.6 ± 1.43)	69 (70.4 ± 1.87)	0.50	25 (25.5 ± 2.23)	73 (74.5 ± 3.49)	0.00*	34 (34.7 ± 3.33)	64 (65.3 ± 4.62)	0.01*	31 (31.6 ± 3.36)	19 (19.4 ± 1.92)	48 (49.0 ± 4.32)	0.70
Dermacentor nitens	1241	380 (30.6 ± 20.58)	861 (69.4 ± 27.12)	0.63	962 (77.5 ± 117.83)	279 (22.5 ± 46.15)	0.05*	534 (43.0 ± 44.85)	707 (57.0 ± 44.85)	0.02*	651 (52.5 ± 51.57)	324 (26.1 ± 31.19)	266 (21.4 ± 37.44)	0.71
Stomoxys calcitrans	783	124 (15.9 ± 8.42)	658 (84.1 ± 21.00)	0.07	615 (78.5 ± 96.41)	168 (21.5 ± 14.85)	0.09	560 (71.5 ± 30.68)	223 (28.5 ± 24.24)	0.31	342 (43.7 ± 30.81)	237 (30.3 ± 28.97)	204 (26.1 ± 9.59)	0.34
Haematobia irritans	317	22 (6.9 ± 1.99)	295 (93.1 ± 11.24)	0.04*	216 (68.1 ± 48.56)	101 (31.9 ± 12.46)	0.37	245 (77.3 ± 21.00)	72 (22.7 ± 13.35)	0.29	186 (58.7 ± 25.38)	53 (16.7 ± 10.16)	78 (24.6 ± 21.39)	0.71
Tabanus spp.	159	41 (25.8 ± 2.89)	118 (74.2 ± 3.47)	0.33	115 (73.7 ± 13.11)	41 (26.3 ± 3.87)	0.05*	74 (46.5 ± 5.79)	85 (53.5 ± 6.17)	0.08	73 (45.9 ± 6.64)	33 (20.8 ± 4.39)	53 (33.3 ± 6.70)	0.05*

Abbreviations: C.I.: 95% confidence interval, P: P value ($p < 0.05$), ∗: Statistically significant.