ACIN
ASOCIACIÓN COLOMBIANA DE INFECTOLOGÍA

infectio

ORIGINAL ARTICLE

Evaluation of diminazene aceturate treatment efficacy in *Trypanosoma* spp. infected water buffaloes in Colombia: absence of drug resistance

Ingrid Lorena Jaramillo-Delgado[1,*], Carolina Ríos-Úsuga[2], Andrés Felipe Pérez-Rojo[3], Piedad Matilde Agudelo-Flórez[4], Jhon Didier Ruíz-Buitrago[5]

Abstract

Introduction: Hemotropic microorganisms, such as *Trypanosoma* spp., affect many animals, including water buffaloes. Some developing countries have reported trypanocide resistance to Diminazene Aceturate. This study aimed to describe the presence of resistance associated with trypanocide treatment in Colombian water buffaloes.

Materials and methods: In this cross-sectional study, 202 individuals were sampled from an endemic area in Colombia. The molecular technique quantitative polymerase chain reaction (qPCR) was performed targeting the *18S* gene for *Trypanosoma* spp., and phylogenetic analysis based on the ITS1 region was performed.

Results: Sixty-one animals (30.2%) were positive for *Trypanosoma* spp. and fifty-two sequences were completely analyzed. Of the sequences, 76.9% (40/ 52) were compatible with *T. vivax* and 23.1% (12/ 52) with *T. theileri*; the obtained results did not show homologous sequences for *T. evansi*. Subsequently, positive animals were treated with Diminazene Aceturate at the recommended dose (7 mg/kg), and a final qPCR was performed one month after the end of the treatment. All animals were negative for *Trypanosoma* spp. after treatment with the drug.

Discussion: Although no resistance to the drug was detected in the animals included in the study, it is necessary to implement continuous surveillance of trypanocidal drug resistance to verify the circulation of resistant strains in Colombia and endemic areas in other countries.

Keywords: Bovine; drug resistance; blood cell count; trypanosomiasis; qPCR

Evaluación de la eficacia del tratamiento con aceturato de diminazeno en búfalos de agua infectados por *Trypanosoma* spp. en Colombia: ausencia de resistencia al fármaco

Resumen

Introducción: Los microorganismos hemotrópicos como *Trypanosoma* spp. afectan a muchos animales, incluidos bovinos como los búfalos de agua. Algunos países en desarrollo han reportado la aparición del fenómeno de resistencia a los tripanocidas con Aceturato de Diminazeno. El objetivo de este estudio fue describir la presencia del fenómeno de resistencia asociado al tratamiento tripanocida en búfalos de agua colombianos.

Materiales y métodos: En este estudio transversal se muestrearon 202 individuos en una zona endémica de Colombia. Se realizó prueba molecular de qPCR (reacción en cadena de la polimerasa cuantitativa) dirigida al gen 18S para *Trypanosoma* spp. y se llevó a cabo un análisis filogenético basado en la región ITS1.

Resultados: Sesenta y un animales (30,2%) fueron positivos por qPCR para *Trypanosoma* spp. y se analizaron completamente cincuenta y dos secuencias. De las secuencias, 76,9% (40/ 52) fueron compatibles con *T. vivax* y 23,1% (12/ 52) con *T. theileri*, los resultados obtenidos no mostraron homología de secuencias para *T. evansi*. Posteriormente, los animales positivos fueron tratados con Aceturato de Diminazeno con la dosis recomendada (7mg/kg) y se realizó una qPCR final un mes después de haber finalizado el tratamiento. Todos los animales fueron negativos para *Trypanosoma* spp. después del tratamiento con el medicamento.

Discusión: Aunque no se detectó resistencia al medicamento en los animales incluidos en el estudio, es necesario realizar una vigilancia continua del fenómeno de resistencia a los medicamentos tripanocidas, para verificar la circulación de cepas resistentes en Colombia y zonas endémicas de otros países.

Palabras clave: Bovinos; resistencia a fármacos; recuento de células sanguíneas; tripanosomiasis; qPCR

1 Grupo de Investigación de Infectología, Zoonosis y Medio ambiente laboratorio TestMol (GIZMOL), Laboratorio TestMol S.A.S, Centro de Diagnóstico Especializado, Medellín, Antioquia, Colombia. Grupo de Ciencias Básicas, Escuela de Graduados, Universidad CES, Antioquia, Medellín, Colombia. https://orcid.org/0000-0001-9110-1242

2 Grupo de Investigación de Infectología, Zoonosis y Medio ambiente laboratorio TestMol (GIZMOL), Laboratorio TestMol S.A.S, Centro de Diagnóstico Especializado, Medellín, Antioquia, Colombia. https://orcid.org/0000-0001-7043-1656

3 Grupo de Investigación de Infectología, Zoonosis y Medio ambiente laboratorio TestMol (GIZMOL), Laboratorio TestMol S.A.S, Centro de Diagnóstico Especializado, Medellín, Antioquia, Colombia. Fundación Nuestra Señora de los Desamparados - Cría ganado bovino y bufalino, Montelíbano, Córdoba, Colombia. https://orcid.org/0000-0002-0574-4602

4 Grupo de Ciencias Básicas, Escuela de Graduados, Universidad CES, Antioquia, Medellín, Colombia. https://orcid.org/0000-0002-0002-776X

5 Grupo de Ciencias Básicas, Grupo de Investigación en Veterinaria (INCACES), Facultad de Medicina Veterinaria y Zootecnia, Universidad CES, Medellín, Antioquia, Colombia. https://orcid.org/0000-0003-4410-4349

* Autor para correspondencia:
Correo electrónico: gerencia@testmol.com

Recibido: 21/11/2024; Aceptado: 25/11/2025

Cómo citar este artículo: I.L. Jaramillo-Delgado, *et al*. Evaluation of diminazene aceturate treatment efficacy in *Trypanosoma* spp. infected water buffaloes in Colombia: absence of drug resistance. Infectio 2026; 30(1): 41-47

Introduction

Colombia, like many other countries worldwide, has been using two leading trypanocide compounds for many years without significant changes: Diminazene Aceturate and Isometamidium[1]. However, verbal reports of trypanocide resistance have been reported in Colombia. Furthermore, it has been observed through personal experience in the daily practice of veterinary medicine that the misuse of trypanocides is a prevalent issue in the country, as many farmers lack the necessary theoretical knowledge about drug mechanisms of action, proper administration and dosage. Often, they are unable to interpret pharmaceutical information provided on leaflets; consequently, farmers heavily rely on visual clues and body condition scoring for diagnoses and base their treatment decisions on past experiences with related diseases.

Hemotropic agents such as *Trypanosoma* spp. pose a significant threat to various animals, including water buffaloes. This type of parasite can be transmitted to mammals (humans and animals) by organ transplantation, blood products, vertical transmission, through vectors (i.e. hemipteran insects belonging to the subfamily Triatominae and dipteran insects such as flies of different genera like *Glossina*, *Tabanus*, *Heamatopota*, *Stomoxys*, *Hippobosca*) as one of the main routes, as well as one of the emergent forms of transmission in endemic countries through contaminated food, representing the major cause of acute illness in several countries[2–4]. Parasites of the genus *Trypanosoma* are protozoans of the order Kinetoplastida and family Trypanosomatidae. This hemoflagellate lives freely in the blood of animals, causing particularly in the case of ruminants, great economic losses in cattle, sheep, goats and buffalo herds in endemic tropical countries such as Africa, parts of Asia, and South America[5], *Trypanosoma vivax* and *Trypanosoma theileri* are the most prevalent trypanosomes affecting livestock in Latin America, including Colombia. These species differ markedly in pathogenicity, with *T. vivax* being associated with severe clinical signs and significant economic losses[6,7]. Although references to *T. cruzi* and *T. congolense* exist in broader trypanosomiasis literature, these species are not central to livestock management in Colombia and are therefore excluded from the present discussion where blood-sucking insects such as horseflies have been reported as one of the main vectors of the microorganism[8]. In young or pregnant female animals, diseases caused by *Trypanosoma* spp. are characterized by acute presentations, leading to death within a few weeks. In endemic areas, chronic presentations are common, resulting in anemia, renal and liver platelet disorders, poor body condition, cachexia, and multiorgan damage, eventually leading to death[9]. While some animals may not exhibit symptoms during their lifetime in regions where the infection is endemic, a stressful environment can trigger clinical disease[10]. Additionally, trypanosome infection may predispose infected animals to other infections, and concurrent and opportunistic bacterial infections in wildlife may accelerate the onset of clinical trypanosomiasis[11].

Bovine trypanosomiasis can be caused mainly by *T. vivax*, and there are cattle that can be infected by both *T. evansi* and *T. theileri*, although these do not seem to seriously affect the health of the animals, unlike *T. vivax*, which has been described as pathogenic for individuals causing death in 50% of untreated animals[6,7,12]. The diagnosis of the disease can be made clinically based on the observation of the symptoms; however, due to how ambiguous it can be, it is inefficient. Therefore, it is recommended to use serological diagnosis by Indirect Immunoenzymatic Assay (ELISA)[13,] which is commonly used for screening or detecting chronic infections through antibody response or molecular techniques such as Polymerase Chain Reaction (PCR) test[14,15] due to the high sensitivity and specificity in detection during all phases of the disease[16].

Trypanosomiasis is a disease that threatens the health and productivity of livestock, mainly in Africa and Latin America. A global analysis of databases, in this case of *T. vivax*, based on 245 studies in 41 countries in Africa and Latin America, including different mammal hosts and a wide range of wild animals, estimated a pooled prevalence of 30.6% in domestic buffalo and 6.4% in cattle[17]. Specifically, in African countries, the prevalence data of bovine trypanosomiasis based on a random-effects model resulted in an overall mean prevalence of 15.1%, considering previous studies of approximately 180 reports[23]. In South America, studies based on molecular detection, in countries such as Peru and Bolivia have shown prevalences of bovine trypanosomiasis by *T. evansi* and *T. vivax* of 5.8% and 3.8% and 11.5% and 1.0%, respectively[18]. In Brazil, *T. vivax* prevalence has been reported as 0.7–56.8% in the southeastern regions, approximately 34% in the northern and central-western regions, and 0.7–41.7% in the northeastern regions of the country[19].

In Colombia, animal trypanosomiasis is one of the most important problems in the livestock industry, reducing production by approximately 30%[20]. According to the last livestock census (2025), the buffalo population in the country is approximately 538.574 animals, and most buffaloes are principally located in the departments of Córdoba (17.1%), belonging to the Caribbean region of Colombia[21]. Molecular analysis in cattle has shown a bovine trypanosomiasis prevalence of 39.2% (*T. theileri* 38.6%, *T. evansi* 6.7%, and *T. vivax* 0.2%), with higher values during the wet and late wet seasons, while in buffaloes, the prevalence was 28.2% (*T. theileri* 28.2% and *T. evansi* 1.3%), with higher values during the dry season. Additionally, it has been found that variables such as tabanid abundance, vector control, breeding system, age, and anemia signs are significantly associated with disease prevalence[22].

The determinants of trypanosomiasis prevalence in cattle hosts are varied and complex, as the population density of vectors, ecology of an area, livestock management system, and cattle trypanotolerance status could play an important role in influencing prevalence outcomes[23], also considering the sensitivity and limitations of the respective diagnostic tools being used for epidemiological studies can distort the real stage of prevalence of bovine trypanosomiasis[24,25].

Given the abuse of trypanocides and the reported resistance to treatments[1], this study aims to contribute to begin with the monitoring and the evaluating of drug resistance in *Trypanosoma* spp. among water buffaloes in Colombia. Understanding the phenomenon of drug resistance and identifying which agents are resistant can provide the basis for the formulation and implementation of effective prevention and control measures that allow the preservation of the health of large populations of species, which particularly have an economic impact on the country[26], as is the case of water buffaloes.

Materials and methods

Ethical considerations

The present study was approved by the CES University Ethics Committee number 28 and was performed in November 2017.

Study design and area

The study area was located at a latitude of 8°00'14.5"N and longitude of 75°24'09.2"W, an average of 20 m above MASL, with a range of temperature–26-34°C, precipitation of 1500 to 2000 mm, and humidity of 80-95%. The buffaloes sampled for evaluation came from an area of the department of Córdoba, Colombia, considering that, according to the distribution of animals in the country, it is the region where the largest population of buffaloes is found. The choice of study area was based on convenience, and all animals included in the study lived under the same environmental conditions.

Reference population

The farm from which the samples were collected had 2277 water buffaloes. Based on this study population, the project was performed using convenience sampling, randomly selecting 245 animals of different ages and sexes. The sampling process took approximately five days. Animals that had lethargy, were gestating, had poor body condition and/or externally visible lesions, or had been treated with any medicament, antibiotic, or antiparasitic in the last three months were excluded from the study. Blood samples were collected from 245 animals with a mean age of four years (range: 1-16 years). Clotted samples were removed owing to the extraction method used and the specifications of the equipment used for the process.

Preanalytical procedures

About seven milliliters of blood was aseptically collected from the coccygeal vein of the animals using vacutainer tubes with the anticoagulant ethylene diamine tetra acetic acid (EDTA) (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK) and a 20-gauge needle. All samples were collected on day zero (0) before treatment initiation. After collection, the blood samples were homogenized with inverted movements 8-10 times, and a part of the sample was taken for one microhematocrit tube and subsequently centrifuged. A drop of the buffy coat blood smear was placed on one end of a clean (wax-free) microscopic glass slide, and a thin film was drawn out using another fresh clean slide. The buffy coat blood smear was air-dried and packed separately and carefully for laboratory staining and analysis. The blood sample tubes (EDTA) were frozen in dry ice and transported to the laboratory TestMol© (Center of Molecular Diagnosis) located in Medellín (Antioquia, Colombia) for molecular analysis by quantitative Polymerase Chain Reaction (qPCR).

Buffy coat blood smear

The slides were stained (HemaColor, Merck) in the laboratory according to the manufacturer's instructions and examined under a microscope at magnification 100x (oil immersion) to detect the presence of parasites (*Trypanosoma* spp.).

DNA extraction method

The samples were extracted using an automatic method with the Kingfisher™ Duo open extraction equipment (Thermo Fisher Scientific Inc.) and the purification kit of nucleic acid MagMAX™ CORE M Express-96 system (Thermo Fisher Scientific, Waltham, MA, USA) according to the established conditions of the manufacturer for blood samples with frozen blood in EDTA (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK) tubes. The extracted DNA was stored at -80°C until processing. DNA purity and quantity were analyzed using a NanoDrop spectrophotometer (Fisher Scientific, Madrid, Spain). Samples with DNA concentrations greater than 10 ng/µL and purity ratios of 260/280 between 2.0 and 2.2 were considered suitable for processing by qPCR. Samples were re-extracted if they did not meet the criteria until the quality parameters were reached.

Molecular analysis by qPCR method

The samples were processed with specific primers (Macrogen, Korea) for each evaluated agent, targeting the *18S* gene for *Trypanosoma* spp. and a gene for phylogenetic analysis based on the Internal Transcribed Spacer ITS1, using primers described in a previous study[28]. The real-time qPCR assay was performed in a Mic 4 channels (Biomolecular Systems, Australia) using the laboratory's own protocols. For the reaction, MasterMix for real-time PCR (SYBR Green, Thermo Fisher Scientific®) was used with a final volume of 19 µL consisting of 10 µL of SYBR Green, 2 µL of each primer at a final concentration of 100 nM and 5 µL of DNA. The thermal profile was as follows: initial denaturation for 3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 1 min at 57°C, and 1 min at 72°C, followed by a final extension for 5 min at 72°C. The positive controls were provided by TestMol©, laboratory of specialized molecular diagnostics and research, which is a government-registered entity for testing large species of animals in Colombia. DNase-RNase-free sterile water (Cat No.:129114, Qiagen, Germany) was used as a negative control. For the internal controls of DNA extraction and qPCR, specific primers for Cytochrome B genes in mammals were employed[27].

Phylogenetic analysis

The qPCR products were sequenced at the National Center for Genomic Sequencing (Macrogen®, Korea). To edit the sequences, DNA Sequence Assembler MEGA X[28] was

used for manual editing and subsequent identification and analysis was performed with Blast® data bank[29]. Some sequences, selected according to their integrity and type of isolation related to animal trypanosomiasis, were obtained from the GeneBank® database for comparison and alignment: MW364077.1 *Trypanosoma theileri*; MT605805.1, MT605807.1, *Trypanosoma evansi* and MH247152.2, MT586222.1, MH247152.2, MK880189.1, *Trypanosoma vivax*, were downloaded. *Leishmania major* strain MHOM/Ir/02/PIIDT1 was used as an outgroup. Alignment and phylogenetic analyses were performed using MEGA X software [28], using the Maximum Likelihood method and the Tamura-Nei model [30]. The phylogenetic tree with the highest logarithmic probability (-1893.98) is shown in the Results section. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a maximum likelihood log value. This analysis involved 49 nucleotide sequences. The included codon positions were 1st+2nd+3rd+No coding. The final dataset contained 445 positions. Evolutionary analyses were performed using MEGA X[28].

Diminazene Aceturate treatment

Animals that amplified the *18S* gene using qPCR were treated with the compound described below. The animals remained in the barn for the entire month for treatment and analysis. Each week they were supplied with Agita® (Based TIAMETOXAM) to remove the risk of reinfections with circulating vectors. The temperature was between 27-35°C and humidity of 80-95%. The buffaloes were fed hay, silage, supplemental milk (calves) and water *ad libitum*. For the treatment, the animals were medicated with Pirofort® (Diminazene Aceturate) (Ourofino, Brazil), with a dose of 7 mg/kg, according to manufactured instructions. The weights of the animals were measured on a scale to calculate the correct dose. On day one (1), the first dose of the compound was administered to each individual, and the second dose was administered on day fifteen (15) after the first dose. The rationale for administering a second DA dose on Day 15 was based on the literature describing *T. vivax's* capacity to evade immune clearance by sequestering in adipose tissue, where trypanocidal concentrations may be insufficient [31]. Additionally, similar dual-dose protocols have been explored to ensure parasite clearance in high-endemic areas[32].

After treatment, the animals remained without any medication for eight (8) days and the final blood samples were collected, repeating the protocol from day zero (0).

Statistical analysis

Data of the animals in the study were georeferenced, and variables such as sex, age, qPCR, and treatment results were registered using Excel software and analyzed using SPSS statistical software V.22 (IBM) for descriptive and bivariable analysis.

Results

Description of the study population

The results of 202 individuals were obtained for analysis (8.9%, 202/2277). Among these samples, 32.2% (65/202) were from female buffaloes and 67.8% (137/202) were from male buffaloes. The average age was 13.2 months, with a maximum age of 27.5 months and a minimum of 3.5 months.

Buffy coat blood smear examination

The stained blood films examined microscopically under an oil immersion lens at 100x revealed some trypanosomes in a long slender form. Some abnormalities in erythrocyte structure, such as hypochromia and acanthocytosis, were observed in Trypanosoma spp.-positive smears. Based on the examination of the buffy coat blood smear, 22 positive samples (10.9%, 22/202) were found to contain the parasite.

DNA extraction and qPCR (quantitative Polymerase Chain Reaction)

Genetic material was extracted from 202 blood samples. Within the quality parameters, a minimum of 20 ng/dl was obtained in the processed samples, with a purity range between 1.5 and 2.0 on the relation 260/280.

Amplification of the *18S* gene fragment was obtained in 30.2% (61/202) of the animals tested, of which 24.6% (15/61) of the positive buffaloes were female and the remaining 75.4% (46/61) were male.

After treatment with Pirofort® (Diminazene Aceturate), none of the animals were positive for *Trypanosoma* spp. infection by the final qPCR test.

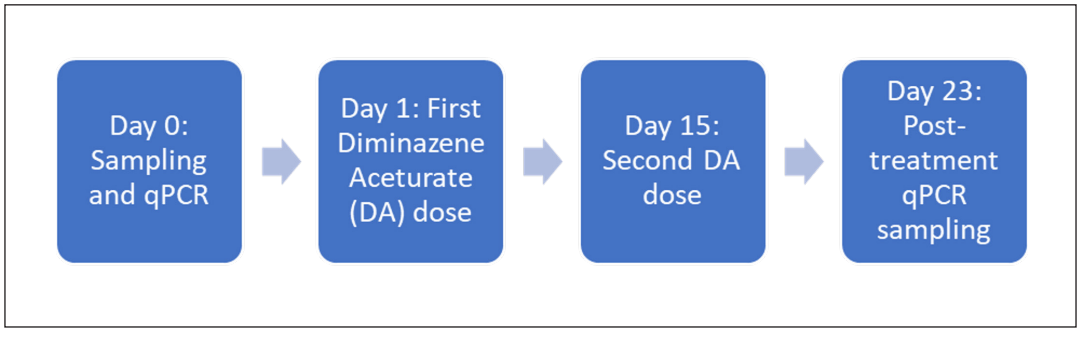

Figure 1. Visual timeline of the research.

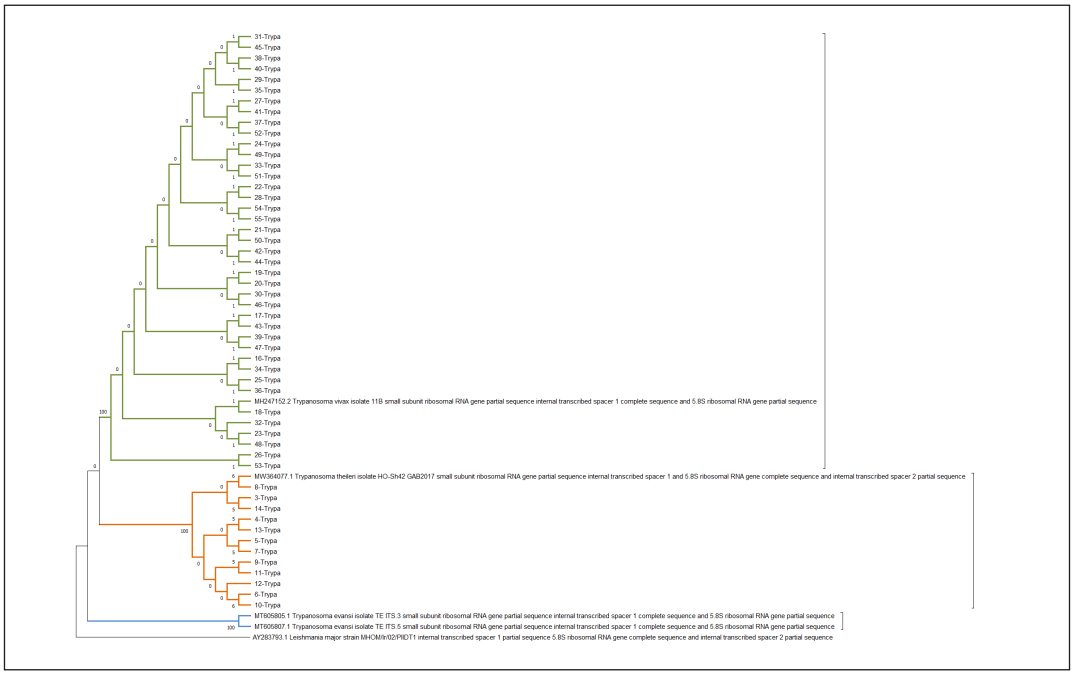

Figure 2. Phylogenetic tree of the positive nucleotide sequences for *Trypanosoma* spp.[52], using the Maximum Likelihood method and the Tamura-Nei model[30], selecting the topology with a maximum likelihood log value. Evolutionary analysis was performed using MEGA X software[28].

Phylogenetic analysis

Owing to the quality of the sequences reported by the National Center for Genomic Sequencing (Macrogen®, Korea) only 52 samples were included for sequence analysis, alignment and phylogenetic tree. According to sequence identification, 76.9% (40/ 52) were compatible with *T. vivax* and 23.1% (12/ 52) with *T. theileri*, no sequences compatible with *T. evansi* were observed.

The phylogenetic tree obtained from this analysis is shown in Figure 2.

Statistical analysis

The data were analyzed using descriptive statistics, and Pearson's chi-square ($\chi 2$) test was performed to explore the association between demographic variables and the presence of parasites. Considering significant associations with a p-value < 0.05, there were no significant differences between age or sex and Trypanosoma spp. infections.

Discussion

Trypanosomiasis is treated with trypanocidal drugs, such as Diminazene Aceturate (DA) and Isometamidium Chloride (ISM), which are the most effective methods to combat the disease in veterinary medicine[33]. In human medicine, other trypanocidal compounds have been reported in the early stages, such as Sodium Suramine and Pentamidine, while Melasoprol and Eflornithine have been recommended in the late stages of *T. brucei* infection[34]. In the present study, 100% efficacy was evidenced in all individuals treated with Diminazene Aceturate, presenting negative results when performing qPCR control test eight (8) days post-treatment and demonstrating a clearance of the infectious agents (*T. vivax* and *T. theileri*). In 2020, Bastos and collaborators[32] had already performed a study where they compared the efficacy of three different treatments, with Diminazene Aceturate, Imidocard Dipropionate and Sometamidium Chloride, in experimentally infected bovines with *T. vivax* in this particular case, and although all the groups evaluated showed a 100% of efficacy, patients treated with Imidocard Dipropionate relapsed 10–14 days after treatment. Despite the implemented research, it is important, and it is suggested that other studies should be executed to evaluate the presence of the parasite at different post-treatment times, which will allow the evaluation of the long-term effectiveness of the drugs, bearing in mind that possible reinfections should be considered if there is no comprehensive control of vectors. Likewise, it is worth mentioning that the success of a treatment depends largely on a good diagnosis, and accurate and highly sensitive diagnostic methods such as PCR are very useful tools for evaluating different treatments[35,36].

In several published studies from different parts of the world, In Brazil, field studies have reported treatment failure of Diminazene Aceturate (DA) in cattle infected with *T. vivax,* confirmed by follow-up PCR diagnostics[37]. In French Guiana, a *T. vivax* stock showed complete resistance to DA at the maximum recommended dose but remained sensitive to Isometamidium Chloride[38]. While no molecular markers for resistance are standardized, overuse and unregulated access to trypanocides have fostered underdosing and incomplete

treatments, exacerbating this phenomenon[3] resistance to the treatments used for different species of trypanosomes has been reported[39]. For instance, animal resistance to trypanocides has been reported in 17 African countries[8]. Nevertheless, reports from the field in Brazil have described the ineffectiveness of DA treatment against *T. vivax* by performing control PCR tests in patients with previous treatments[37]. Similarly, another study in French Guiana evaluated drug resistance of *T. vivax* in cattle and sheep, and the study population showed resistance at the maximum recommended dose of DA, but sensitivity to ISM at a dose of 0.5 mg/kg of body weight[38].

The resistance of infectious agents to conventional therapies used in veterinary medicine is currently a topic of clinical and research importance, as incomplete treatments or underdoses have influenced this worrying phenomenon[35]. In addition, these drugs are available for sale to farmers without a prescription, who treat animals if they suspect trypanosome infection based on criteria such as low weight gain or reduced milk production[31]. Unfortunately, there are few studies in this regard, especially about a frequent agent in cattle and buffaloes, *Trypanosoma* spp., of which, in Colombia, a prevalence of 39.2% has been estimated, generating a decrease in bovine production by 30%[21]. It is important to mention that one of the biggest problems in the control of *Trypanosoma* spp. in South America is the unregulated transport of infected animals across national and international borders and the absence of a clearly defined vector in the transmission of the disease to focus on vectorial prevention and control strategies [31]. Therefore, the control that is currently made of the disease in South America is based mainly on host treatment, and it is necessary to continue research that evaluates the resistance and sensitivity to different compounds used to treat trypanosomiasis in the country.

In this study, it was possible to demonstrate that there was no resistance to the trypanocidal drug (DA) by negative results through control diagnosis molecular techniques (qPCR). For future studies, emphasis should be placed on the need to evaluate the effect of long-term treatment and consider analyzing genes associated with drug resistance in pathogens, hosts, and vectors, which will allow understanding the mechanisms of resistance in microorganisms. Evaluating the phenomenon of resistance to other compounds used for treatment in different areas of the country where other *Trypanosoma* species may circulate is another important issue, since some species have shown more resistance than others. Thus, this could allow the generation of control and treatment strategies according to the type of trypanosome identified and the area in which it is diagnosed, to avoid zoonotic dissemination and the direct and indirect impacts that vector-borne diseases, such as trypanosomiasis, have on animal production.

The efficacy of a drug against an infectious agent can be reliably assessed by the identification of DNA using molecular diagnosis. It is a tool that also makes it possible to identify genes associated with resistance in different organisms, allowing a more appropriate approach to generate effective treatment and prevention strategies for infectious diseases transmitted by vectors.

One limitation of this study is the short follow-up interval after the second dose of DA. As relapse parasitemia under resistance conditions is commonly observed 10–21 days post-treatment, an 8-day window may be insufficient to rule out recrudescence[32,39]. Future studies should incorporate serial qPCR over a 30-day period or longer. Additionally, HemaColor was used for buffy coat smears, which is less sensitive than Giemsa for *Trypanosoma* detection[40]. This may have affected the blood smear detection rate. Future studies should include resistance gene profiling in *Trypanosoma* spp. and their hosts to understand the molecular mechanisms and allow the development of better treatment and control strategies. Including PCR-based detection of resistance-associated mutations or efflux mechanisms could provide insights into the dynamics of emerging resistance.

Ethical considerations

Protection of persons. Not applicable.

Protection of vulnerable populations. Not applicable.

Confidentiality. The use of each patient's information for research was supported by signing an informed consent by the Veterinary Doctors. All data provided were authorized by the laboratory. A confidentiality agreement was established and signed for the information provided by TESTMOL© S.A.S. Veterinary Doctors performed the sampling in compliance with all the professional ethics protocols in Colombia for the handling of animals in the veterinary medical practice under law 576 of 2000 and law 84 of 1989. This approach adheres to ethical principles, ensuring that the privacy and confidentiality of individuals are respected.

Privacy. The names, initials, medical record numbers of any individual, or any other type of information that violates the right to privacy of the animals included in the study (protecting their identity), are not used in the writing of the article or in the images.

Financing. This research received no external funding. The grants were provided by Fundación of Nuestra Señora de los Desamparados (FNSD) and TestMol© laboratory.

Conflict of interests. The authors have no conflict of interest to declare

Acknowledgements. The authors would like to thank all the veterinarians, technicians and the team of laboratory analysts, for their kind help during sample collection and processing and the information of the evaluated cases.

Authors´ contribution. Conceptualization: I.L.J., C.R., A.F.P., P.M.A., J.D.R.; Data curation: I.L.J., C.R., A.F.P., P.M.A., J.D.R.; Formal analysis: I.L.J., C.R., A.F.P., P.M.A., J.D.R.; Investigation:

I.L.J., C.R., A.F.P., P.M.A., J.D.R.; Methodology: I.L.J., C.R., A.F.P., P.M.A., J.D.R.; Visualization: I.L.J., C.R., A.F.P., P.M.A., J.D.R.; Writing – Review & Editing: I.L.J., C.R., A.F.P., P.M.A., J.D.R. All authors contributed to read and approved the version of the submitted manuscript.

References

1. Giordani F, Morrison LJ, Rowan TG, De Koning HP, Barrett MP. The animal trypanosomiases and their chemotherapy: a review. *Parasitology*. 2016;143(14):1862-1889. doi:10.1017/S0031182016001268
2. Hernández C, Salazar C, Brochero H, et al. Untangling the transmission dynamics of primary and secondary vectors of Trypanosoma cruzi in Colombia: parasite infection, feeding sources and discrete typing units. *Parasit Vectors*. 2016;9(1). doi:10.1186/s13071-016-1907-5
3. Diall O, Cecchi G, Wanda G, et al. Developing a Progressive Control Pathway for African Animal Trypanosomosis. *Trends Parasitol*. 2017;33(7):499-509. doi:10.1016/j.pt.2017.02.005
4. Kasozi KI, MacLeod ET, Ntulume I, Welburn SC. An Update on African Trypanocide Pharmaceutics and Resistance. *Front Vet Sci*. 2022;9:828111. doi:10.3389/fvets.2022.828111
5. Aregawi WG, Agga GE, Abdi RD, Büscher P. Systematic review and meta-analysis on the global distribution, host range, and prevalence of Trypanosoma evansi. *Parasit Vectors*. 2019;12(1). doi:10.1186/s13071-019-3311-4
6. Desquesnes M. *Livestock Trypanosomoses and Their Vectors in Latin America*. OIE; 2004
7. Osório ALAR, Madruga CR, Desquesnes M, Soares CO, Ribeiro LRR, Costa SCGD. Trypanosoma (Duttonella) vivax: its biology, epidemiology, pathogenesis, and introduction in the New World - a review. *Mem Inst Oswaldo Cruz*. 2008;103(1):1-13. doi:10.1590/s0074-02762008000100001
8. Svobodová M, Volf P, Votýpka J. Trypanosomatids in ornithophilic bloodsucking Diptera. *Med Vet Entomol*. 2015;29(4):444-447. doi:10.1111/mve.12130
9. Hilali M, Abdel-Gawad A, Nassar A, Abdel-Wahab A. 2006. Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*. *Vet Parasitol*. 2006;139(1-3):237-243. doi:10.1016/j.vetpar.2006.02.013
10. Sheppe WA, Adams JR. The Pathogenic Effect of Trypanosoma duttoni in Hosts under Stress Conditions. *J Parasitol*. 1957;43(1):55. doi:10.2307/3274757
11. Gupta & Kumar, Harrshal & Singla, Lachhman Das. Trypanosomosis concurrent to tuberculosis in black bucks. *Indian Vet J*. 2009;86: 727-728
12. Baldacchino F, Muenworn V, Desquesnes M, Desoli F, Charoenviriyaphap T, Duvallet G. Transmission of pathogens by*Stomoxys*flies (Diptera, Muscidae): a review. *Parasite*. 2013;20:26. doi:10.1051/parasite/2013026
13. Medina-Naranjo VL, Reyna-Bello A, Tavares-Marques LM, Campos AM, Ron-Román JW, Moyano JC, et al. Diagnóstico de los Hemotrópicos *Anaplasma marginale*, *Trypanosoma* spp. y *Babesia* spp. mediante las técnicas de ELISAi y PCR en tres fincas ganaderas de la provincia de Pastaza, Ecuador. *Revista Científica*. 2017; 27(3):162-171. Available from: https://www.redalyc.org/pdf/959/95952010005.pdf
14. Masake RA, Majiwa PAO, Moloo SK, et al. Sensitive and Specific Detection ofTrypanosoma vivaxUsing the Polymerase Chain Reaction. *Exp Parasitol*. 1997;85(2):193-205. doi:10.1006/expr.1996.4124
15. Garcia HA, Ramírez OJ, Rodrigues CMF, et al. Trypanosoma vivax in water buffalo of the Venezuelan Llanos: An unusual outbreak of wasting disease in an endemic area of typically asymptomatic infections. *Vet Parasitol*. 2016;230:49-55. doi:10.1016/j.vetpar.2016.10.013
16. Ramírez-Iglesias JR, Eleizalde MC, Gómez-Piñeres E, Mendoza M. Trypanosoma evansi: A comparative study of four diagnostic techniques for trypanosomosis using rabbit as an experimental model. *Exp Parasitol*. 2011;128(1):91-96. doi:10.1016/j.exppara.2011.02.010
17. Fetene E, Leta S, Regassa F, Büscher P. Global distribution, host range and prevalence of Trypanosoma vivax: a systematic review and meta-analysis. *Parasit Vectors*. 2021;14(1). doi:10.1186/s13071-021-04584-x
18. Mekata H, Konnai S, Witola WH, Inoue N, Onuma M, Ohashi K. Molecular detection of trypanosomes in cattle in South America and genetic diversity of *Trypanosoma evansi* based on expression-site-associated gene 6. *Infect Genet Evol*. 2009;9(6):1301-1305. doi:10.1016/j.meegid.2009.07.009
19. Oliveira WJ, Barbosa FC, Moraes FR. Tripanossomose bovina no Brasil. *Rev Acad Ciênc Anim*. 2019;17:1-11. doi:10.7213/1981-4178.2019.17104
20. Jaimes-Dueñez J, Mogollon E, Árias-Landazabal N, Rangel DE, Jimenez-Leaño A, Mejia-Jaramillo AM, et al. Molecular surveillance of *Trypanosoma* spp. reveals different clinical and epidemiological characteristics associated with the infection in three creole cattle breeds from Colombia. *Prev Vet Med*. 2021; 193(9):105414. doi:10.1016/j.prevetmed.2021.105414
21. Instituto Colombiano Agropecuario- ICA. CENSO BUFALINO EN COLOMBIA. Censo pecuario. January 5, 2022. Accessed November 11, 2025. Available from: https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018
22. Jaimes-Dueñez J, Triana-Chávez O, Mejía-Jaramillo AM. Spatial-temporal and phylogeographic characterization of Trypanosoma spp. in cattle (Bos taurus) and buffaloes (Bubalus bubalis) reveals transmission dynamics of these parasites in Colombia. *Vet Parasitol*. 2018;249:30-42. doi:10.1016/j.vetpar.2017.11.004
23. Silbermayr K, Li F, Soudré A, Müller S, Sölkner J. A Novel qPCR Assay for the Detection of African Animal Trypanosomosis in Trypanotolerant and Trypanosusceptible Cattle Breeds. Solano P, ed. *PLoS Negl Trop Dis*. 2013;7(8):e2345. doi:10.1371/journal.pntd.0002345
24. Moti Y, Fikru R, Büscher P, Van Den Abbeele J, Duchateau L, Delespaux V. Detection of African animal trypanosomes: The haematocrit centrifugation technique compared to PCR with samples stored on filter paper or in DNA protecting buffer. *Vet Parasitol*. 2014;203(3-4):253-258. doi:10.1016/j.vetpar.2014.04.014
25. Abdi RD, Agga GE, Aregawi WG, et al. A systematic review and meta-analysis of trypanosome prevalence in tsetse flies. *BMC Vet Res*. 2017;13(1). doi:10.1186/s12917-017-1012-9
26. Tapasco J, LeCoq JF, Ruden A, Rivas JS, Ortiz J. The Livestock Sector in Colombia: Toward a Program to Facilitate Large-Scale Adoption of Mitigation and Adaptation Practices. *Front Sustain Food Syst*. 2019;3:61. doi:10.3389/fsufs.2019.00061
27. Pfeiffer I, Burger J, Brenig B. Diagnostic polymorphisms in the mitochondrial cytochrome b gene allow discrimination between cattle, sheep, goat, roe buck and deer by PCR-RFLP. *BMC Genet*. 2004;5(1):30. doi:10.1186/1471-2156-5-30
28. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Battistuzzi FU, ed. *Mol Biol Evol*. 2018;35(6):1547-1549. doi:10.1093/molbev/msy096
29. NCBI. NCBI-Taxonomy. https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=391902&lvl=3&lin=f&keep=1&srchmode=1&unlock
30. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol*. 2011;28(10):2731-2739. doi:10.1093/molbev/msr121
31. Jones TW, Dávila AMR. Trypanosoma vivax – out of Africa. *Trends Parasitol*. 2001;17(2):99-101. doi:10.1016/S1471-4922(00)01777-3
32. Bastos TSA, Faria AM, de Assis Cavalcante AS, et al. Comparison of therapeutic efficacy of different drugs against Trypanosoma vivax on experimentally infected cattle. *Prev Vet Med*. 2020;181:105040. doi:10.1016/j.prevetmed.2020.105040
33. Dagnachew, S B M. Review on Trypanosoma vivax. *Afr J Basic Appl Sci*. 7(1):41-64. doi:10.5829/idosi.ajbas.2015.7.1.92116
34. Efferth. Development of drug resistance in Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense. Treatment of human African trypanosomiasis with natural products (Review). *Int J Mol Med*. 1998;22(4). doi:10.3892/ijmm_00000037
35. Sivajothi S, Rayulu VC, Malakondaiah P, Sreenivasulu D. Diagnosis of Trypanosoma evansi in bovines by indirect ELISA. *J Parasit Dis Off Organ Indian Soc Parasitol*. 2016;40(1):141-144. doi:10.1007/s12639-014-0465-z
36. Maharana BR, Tewari AK, Saravanan BC, Sudhakar NR. Important hemoprotozoan diseases of livestock: Challenges in current diagnostics and therapeutics: An update. *Vet World*. 2016;9(5):487-495. doi:10.14202/vetworld.2016.487-495
37. Gall Y, Woitag T, Bauer B, et al. Trypanocidal failure suggested by PCR results in cattle field samples. *Acta Trop*. 2004;92(1):7-16. doi:10.1016/j.actatropica.2004.04.003
38. Desquesnes M, (de) La Rocque S, Peregrine AS. French guyanan stock of Trypanosoma vivax resistant to diminazene aceturate but sensitive to isometamidium chloride. *Acta Trop*. 1995;60(2):133-136. doi:10.1016/0001-706X(95)00117-W
39. Mdachi RE, Ogolla KO, Auma JE, et al. Variation of sensitivity of Trypanosoma evansi isolates from Isiolo and Marsabit counties of Kenya to locally available trypanocidal drugs. Aboelhadid SM, ed. *PLOS ONE*. 2023;18(2):e0281180. doi:10.1371/journal.pone.0281180
40. Sivajothi S, Reddy BS, Rayulu VC. Study on impression smears of hepatic coccidiosis in rabbits. *J Parasit Dis*. 2016;40(3):906-909. doi:10.1007/s12639-014-0602-8