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EDITED BY

Francesca Conte,
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REVIEWED BY

Armando Reyna-Bello,
Universidad De las Fuerzas Armadas ESPE,
Ecuador
Gilderlandio Rodrigues,
Doctor Leão Sampaio University Center, Brazil

*CORRESPONDENCE

Vinícius Longo Ribeiro Vilela
✉ vinicius.vilela@ifpb.edu.br

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Epidemiological assessment and outbreak report of *Trypanosoma vivax* in dairy cattle from the Brazilian Semiarid region

Basílio Felizardo Lima Neto¹, Jordania Oliveira Silva²,
Felipe Boniedj Ventura Alvares¹, Samira Pereira Batista¹,
Geraldo Moreira Silva Filho¹, Gláucia Djojânia Azevedo Medeiros²,
Carla Monadeli Filgueira Rodrigues², Bruno Rafael Fermينو³,
Vinícius Longo Ribeiro Vilela^{1,2*} and Thais Ferreira Feitosa^{1,2}

¹Postgraduate Program in Science and Animal Health, Federal University of Campina Grande, Patos, Brazil, ²Department of Veterinary Medicine, Federal Institute of Paraíba, Sousa, Brazil,

³Department of Veterinary Medicine, Federal Institute of Rondônia, Jaru, Brazil

This study aimed to estimate the prevalence and identify risk factors associated with *Trypanosoma vivax* infection in dairy cattle herds in the Brazilian Semiarid region, as well as report an outbreak of trypanosomiasis. A total of 390 cattle blood samples were collected from 61 farms in the states of Ceará e Paraíba, and a conventional PCR was performed for the diagnosis of *T. vivax*. Positive samples were bidirectionally sequenced. An epidemiological questionnaire was applied on each farm, and the resulting data were subjected to univariate and multivariate analyses to identify potential risk factors. An outbreak was detected in the municipality of Orós, Ceará, where cases of abortion and estrus repetition were reported among cows. Of the 390 samples analyzed, 1.02% (4/390) tested positive for *T. vivax*. No statistically significant risk factors were identified. All positive samples originated from the farm in Orós, where 80% (4/5) of the tested animals were infected. Similarity analysis using BLAST revealed 100% identity with *T. vivax* isolates previously reported in donkeys, sheep, and cattle from the Brazilian semi-arid region, and high similarity with isolates from South American countries and West Africa. In the phylogenetic analysis, sequences TviBrCe13 and TviBrCe14 clustered with isolates from the Brazilian Semiarid region, as well as with South American and West African strains. It was concluded that the prevalence of *T. vivax* in the Brazilian Semiarid region is low, although the area remains susceptible to outbreak events.

KEYWORDS

trypanosomiasis, Brazilian northeast, epidemiological survey, molecular diagnosis, phylogenetic analysis

1 Introduction

Trypanosoma spp. is a genus of pathogenic protozoa of significant economic importance to the African continent, particularly in regions where the tsetse fly (*Glossina* spp.), its biological vector, is present (Gardiner, 1989). Within this genus, *Trypanosoma* (*Duttonella*) *vivax* (Ziemann, 1905, as cited in Silva, 1996) stands out as a major species affecting cattle, causing substantial impacts on herd health and both productive and reproductive performance (Couto et al., 2024). Through its adaptation to mechanical transmission by other hematophagous insects such as tabanids, *Stomoxys calcitrans*, and via contaminated fomites like needles and syringes, it has spread to South America (Levine, 1973; Melo Junior et al., 2022). Trypanosomiasis caused by *T. vivax* has been expanding and is currently responsible for significant economic losses in dairy farms across Brazil (Reis, 2021).

Several techniques can be used for the diagnosis of *Trypanosoma vivax*, classified into direct and indirect methods. Among the direct methods, the most notable are blood smear examination, the microhematocrit centrifugation technique (Woo's technique), and Polymerase Chain Reaction (PCR). As an indirect method, the Enzyme-Linked Immunosorbent Assay (ELISA) stands. Direct parasitological examinations exhibit low sensitivity in cases of chronic infection due to the reduced number of circulating parasites in the bloodstream out (Alcindo et al., 2022). Serological tests, such as IFA and ELISA, are useful as screening tools to identify herd exposure to infection but are unable to distinguish active infections from post-treatment immune responses. On the other hand, molecular methods, such as conventional PCR (cPCR), are considered highly sensitive and specific tools, especially during the patent phase of the disease. The combination of molecular and serological tests allows for more effective diagnosis, particularly in animals that remain infected even after treatment (Fidelis Junior et al., 2019). The use of highly sensitive diagnostic methods is crucial, as *T. vivax* often induces low parasitemia (Cadioli et al., 2015). In this context, the PCR assay based on a *T. vivax*-like CatL-specific sequence (TviCatL-PCR) has been shown to be species-specific, highly sensitive, and suitable for the diagnosis of *T. vivax* (Cortez et al., 2009; Rodrigues et al., 2015).

In the Brazilian Semiarid region, *T. vivax* is not endemic. Due to prolonged periods of drought and high temperatures, mechanical vectors are unable to develop properly throughout the entire year, resulting in limited and inconsistent exposure of animals to the parasite and, consequently, the lack of active immunity development. When the rainy season begins, vector populations resume development, and outbreaks are observed with greater frequency (Batista et al., 2007, 2008; Rodrigues et al., 2015). The main clinical signs observed in animals infected with *T. vivax* during outbreaks in Brazil include progressive weight loss, a significant drop in milk production, anemia, abortion, anestrus, and perinatal mortality (Batista et al., 2022).

In addition to climatic factors, anthropogenic aspects—such as the movement of animals without sanitary control, the shared use of piercing and cutting instruments (such as needles and syringes), and deficiencies in sanitary surveillance programs—also contribute

significantly to the emergence and spread of cattle trypanosomiasis outbreaks (Bastos et al., 2020). The high density of mechanical vectors during the rainy season, combined with inadequate sanitary management practices in dairy herds, facilitates the maintenance of the *T. vivax* epidemiological cycle (Batista et al., 2008), resulting in substantial economic losses for producers and posing a threat to animal health and survival (Couto et al., 2024). Given the scarcity of updated data on the epidemiology of this parasite in the Brazilian Semiarid region, this study aimed to estimate the prevalence and identify risk factors associated with *T. vivax* infection in cattle from dairy farms in the region, as well as to describe the occurrence of a trypanosomiasis outbreak in an affected property.

2 Method

2.1 Sampling

To determine the number of samples to be evaluated, a simple random sampling method was used, according to Thrusfield (2007).

$$N = \frac{Z^2 \cdot p \cdot (1 - p)}{d^2}$$

Where:

N: required sample size;

Z: standard normal distribution value for a 95% confidence level;

p: expected prevalence of 50%;

d: sampling error of 5%.

The local population was adjusted using the following formula:

$$n_{ajus} = \frac{N \times n}{N + n}$$

Where:

n_{ajus}: final number of selected farms;

n: initial number of selected farms;

N: total number of farms in the region.

The minimum number of samples to be evaluated, as determined by the sample size calculation, was 385. However, due to producer participation, a total of 390 samples were collected. To enhance spatial distribution, a maximum of six animals per farm were selected.

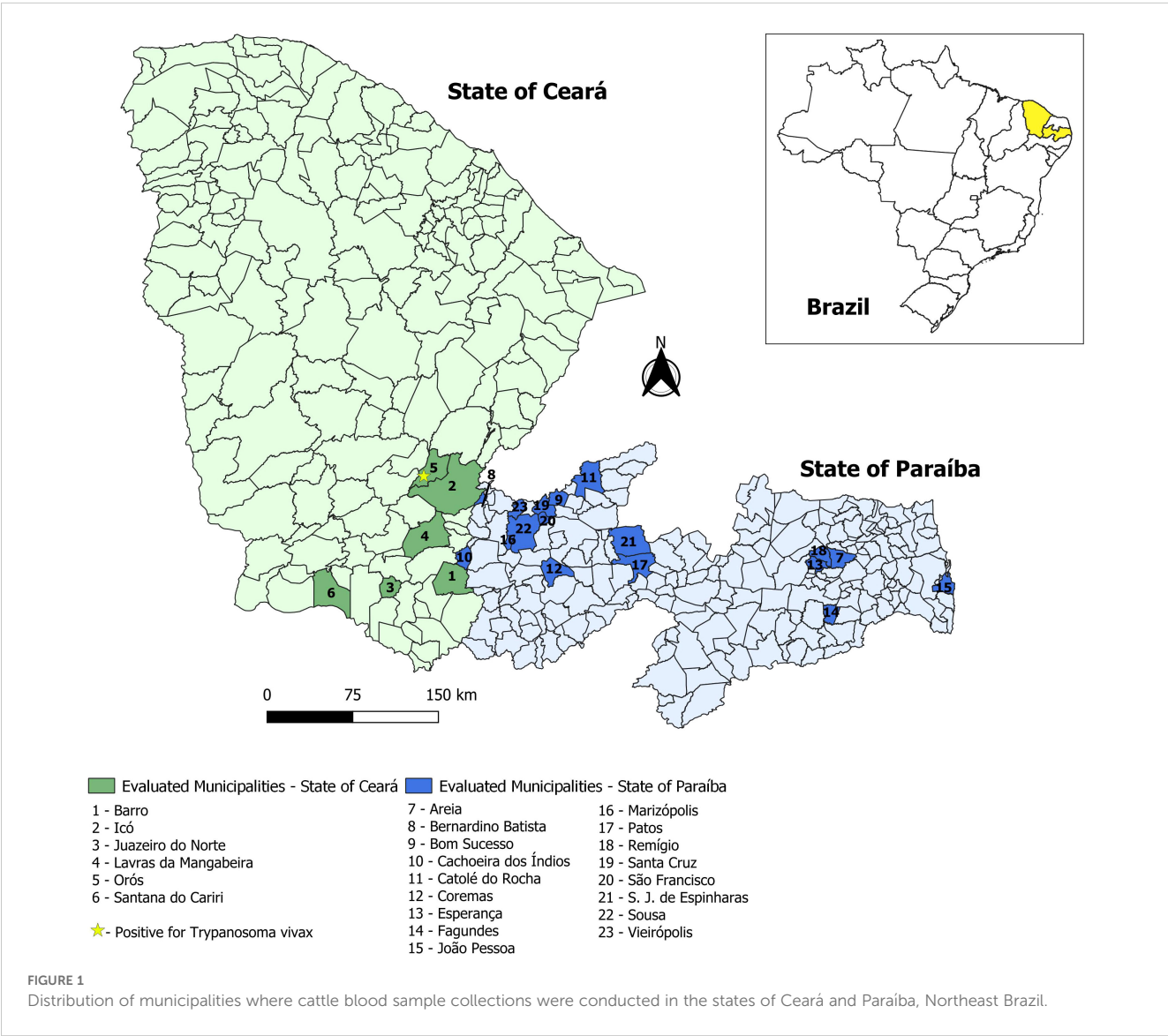
2.2 Study area

The state of Ceará has a herd of 2,772,173 cattle, while the state of Paraíba has a herd of 1,412,480 (IBGE 2023). A total of 390 blood samples were collected from the median caudal vein of cattle in six municipalities of the microregions Cariri, Iguatu, Lavras da Mangabeira and Sul Cearense of the state of Ceará and 17

municipalities of the microregions Brejo Paraibano, Cajazeiras, Campina Grande, Catolé do Rocha, Curimataú Ocidental, Esperança, João Pessoa, Patos, Piancó and Sousa of the state of Paraíba, located in the semi-arid region of Brazil (Figure 1). In the state of Ceará, 125 samples were collected from 17 farms, while in the state of Paraíba, 265 samples were collected from 44 farms. The samples were collected between September 2023 and December 2024 using Vacutainer® tubes containing EDTA as an anticoagulant, through reusable holders. Samples were collected from farms with semi-intensive and extensive dairy production systems, comprising male and female animals aged 0 to over 5 years. All samples were properly labeled and transported in isothermal boxes for processing at the Laboratory of Molecular Biology (LabMol) of the Federal Institute of Paraíba.

2.3 Initial sample processing and DNA extraction

The samples were centrifuged, and 200 µL of the buffy coat was collected and transferred to sterile, 1.5 mL microtubes that were free of DNases, RNases, pyrogens, dyes, and heavy metals. DNA was extracted using the Cellco® Blood-Animal-Plant DNA Preparation Kit (Cellco®, São Carlos, SP, Brazil), following the manufacturer's instructions. After extraction, DNA and RNA quantification was performed using a microvolume spectrophotometer (L-Quant III, Loccus, Brazil) to assess extraction efficiency. 50 µL of extracted DNA was stored in 1.5 mL microtubes and kept at -20°C until use in Polymerase Chain Reaction (PCR) assays.



2.4 Polymerase chain reaction

For a final volume of 20 μL , 2 μL of DNA (the DNA concentration range from 50 to 92 ng/ μL) were added to 3.15 μL of PlatinumTM Taq DNA Polymerase mix (cat. 10966-030, Invitrogen, Carlsbad, CA, USA), 12.85 μL of ultrapure water (dH_2O), and 1.0 μL of each primer (10 μM). The PCR assay employed the primers TviCatL1-F (5' GCCATCGCCAGTACCTCGCGA 3') and DTO155-R (5' TTAAAGCTCCAGGAGATTCTTGATGATCCAGTA 3'), using the thermal cycling conditions described by Cortez et al. (2009).

The PCR products were subjected to electrophoresis in 1.5% agarose gel at 80 V for 40 minutes and visualized under UV light. Positive samples amplified a 177 base pairs (bp) fragment, consistent with the band size described for *T. vivax* (Cortez et al., 2009). Amplicon sizes were determined using a 100 bp molecular weight marker (GeneRuler DNA Ladder MixTM, Invitrogen®, Carlsbad, CA, USA).

2.5 Sequencing and phylogenetic analysis

The CATL-amplified DNA sequences were analyzed using Geneious v4.8.5 (<http://www.geneious.com/>). The sequences were aligned using the MUSCLE algorithm (Edgar, 2004), implemented in MEGA X (Kumar et al., 2018). The alignment, which included *Trypanosoma vivax* sequences from Brazil, Venezuela, Ecuador, The Gambia, Burkina Faso, Nigeria, Mozambique, Kenya, and Zambia, was used to construct a Maximum Likelihood (ML) phylogenetic tree.

The ML analysis was performed with RAxML v8.2 (Stamatakis, 2014), employing the GTRGAMMA model with a proportion of invariable sites. Model parameters were estimated during tree searches, and branch support values were obtained with 500 bootstrap replicates, following the methodology previously described by Cortez et al. (2009). Importantly, the use of the TviCatL-PCR assay (Cortez et al., 2009), which targets repeated gene sequences encoding the cathepsin L-like enzyme, was intended exclusively for species confirmation of *T. vivax* through genetic sequencing.

2.6 Epidemiological questionnaire

At the time of sampling, an epidemiological questionnaire was administered at each farm, addressing the following items: herd size, production system, breed, type of farming activity, grazing area, main health problems, use of the same needle on multiple animals, presence of flies, pasture sharing with other farms, bull rotation, and contact of cows with bulls from other properties.

2.7 Statistical analysis

To identify factors associated with the prevalence of *T. vivax*, data from the epidemiological questionnaires were analyzed in two steps: univariate and multivariate analyses. In the univariate analysis, each independent variable was correlated with the

dependent variable (positivity), and those with a p-value ≤ 0.2 , according to the chi-square test (Zar, 1999), were included in a multivariate analysis using multiple logistic regression (Hosmer and Lemeshow, 2000), with a significance level of 5%. To detect possible collinearity among variables, a correlation test was applied; if the correlation coefficient exceeded 0.9, one of the variables was excluded based on biological plausibility. Model fit was assessed using the chi-square statistic and the Omnibus test. The results were analyzed using GraphPad Prism software, version 8.0.1.

2.8 Monitoring of a trypanosomiasis outbreak in the State of Ceará

During the study, an outbreak was identified on a farm located in the municipality of Orós, State of Ceará, where successive abortions and repeated estrus cycles were being observed among breeding cows.

3 Results

3.1 Prevalence and risk factors associated with *T. vivax* infection

Among the 390 blood samples collected in the states of Ceará and Paraíba, 1.02% (4/390) tested positive for *T. vivax* (Table 1). No statistically significant risk factors were associated with *T. vivax* infection.

3.2 Outbreak of trypanosomiasis in the municipality of Orós, State of Ceará

All positive samples in the present study originated from a single farm located in the municipality of Orós, in the state of Ceará, where *T. vivax* infection was detected in 80% (4/5) of the sampled animals from the herd (Table 1). The outbreak occurred among the dairy cows on the property, which exhibited cases of successive abortions and repeated estrus cycles. The outbreak took place in September 2023, during the dry season, in the absence of mechanical vectors. The animals were raised under a semi-extensive production system and had no contact with animals from other farms. It was reported that, in the months prior to sampling, animals had been acquired from another property in the region. There was no history of reproductive problems in the herd before the outbreak. Additionally, it was reported that the farm practiced sharing of syringes and needles among animals for the administration of medications and exogenous oxytocin.

3.3 Sequencing and phylogenetic analysis

The samples positive for *T. vivax* were subjected to Cathepsin L gene sequencing, resulting in two sequences from cattle, named

TABLE 1 Distribution of farms and cattle blood samples collected by municipality in the states of Ceará and Paraíba, Northeast Brazil.

Municipalities of Ceará	N° of farms	N° of animals on the farms	N° of animals collected (%)	Positive samples (%)
Barro	3	123	19 (15.4)	0 (0)
Icó	10	1017	86 (8.4)	0 (0)
Juazeiro do Norte	1	49	5 (10.2)	0 (0)
Lavras da Mangabeira	1	10	5 (50)	0 (0)
Orós	1	20	5 (25)	4 (80)
Santana do Cariri	1	150	5 (3.3)	0 (0)
Municipalities of Paraíba				
Areia	5	552	27 (4.8)	0 (0)
Bernardino Batista	3	27	15 (55.5)	0 (0)
Bom Sucesso	1	83	6 (7.2)	0 (0)
Cachoeira dos Índios	1	100	6 (6)	0 (0)
Catolé do Rocha	5	365	30 (8.2)	0 (0)
Coremas	1	21	13 (61.9)	0 (0)
Esperança	3	112	17 (15.1)	0 (0)
Fagundes	3	138	15 (10.8)	0 (0)
João Pessoa	5	133	25 (18.7)	0 (0)
Marizópolis	1	110	8 (7.2)	0 (0)
Patos	3	89	25 (28)	0 (0)
Santa Cruz	1	25	6 (24)	0 (0)
Remígio	6	151	31 (20.5)	0 (0)
São Francisco	1	14	6 (42.8)	0 (0)
São José de Espinharas	1	650	5 (0.7)	0 (0)
Sousa	3	125	15 (12)	0 (0)
Vieirópolis	1	200	15 (7.5)	0 (0)
Total	61	4.264	390 (9.1)	4 (1.02)

TviBrCe13 (PX262614) and TviBrCe14 (PX262615). The similarity analysis conducted using BLAST revealed that both sequences shared 100% identity with previously reported isolates from the Brazilian Semiarid region, obtained from donkeys, sheep, and cattle, and also showed high similarity with isolates from other South American countries and West Africa.

In the phylogenetic analysis, sequences TviBrCe13 and TviBrCe14 consistently clustered within the same clade as isolates from the Brazilian Semiarid region and were closely related to other Brazilian isolates, as well as those from South American countries and West Africa (Figure 2). This clustering confirms the low genetic diversity observed among South American isolates and reinforces the evolutionary connection between these genotypes and those originally described in West Africa.

4 Discussion

This is the first report in the state of Ceará that includes not only molecular detection but also sequencing-based characterization and phylogenetic analysis of *T. vivax*, complementing previous reports that were limited to molecular diagnosis without in-depth genetic characterization (Rodrigues et al., 2024). This finding expands the understanding of the genetic diversity of the parasite in the Northeast region of Brazil and provides essential data to support control strategies and epidemiological surveillance in the region.

Studies estimating the prevalence of *T. vivax* in the Brazilian Semiarid region are scarce, with most research focused on isolated outbreaks. Four animals tested positive for *T. vivax*, and these cases were associated with an outbreak of trypanosomiasis in the

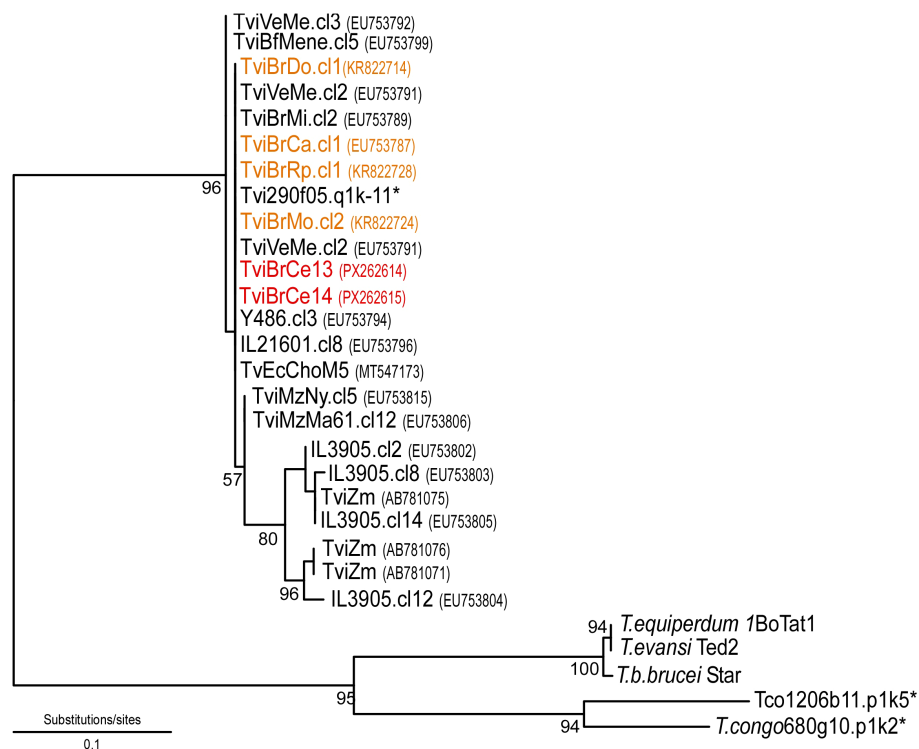


FIGURE 2

Neighbor-joining phylogenetic tree displaying the evolutionary relationships among the sequences obtained in this study and related GenBank entries, constructed using MEGA software (version 11). Bootstrap percentages are shown next to the main branches, indicating the level of statistical confidence for each cluster. The isolates from this research (TviBrCe13 and TviBrCe14) are highlighted in red and positioned within a strongly supported clade. Black sequences, derived from various hosts and geographic regions, were incorporated to demonstrate the genetic variability and evolutionary connections among the isolates. The sequences highlighted in orange represent isolates previously reported in the Semiarid region of Northeast Brazil. Sequences marked with an asterisk were retrieved from genome projects (Sanger Institute; <https://www.sanger.ac.uk/science/data>).

municipality of Orós, State of Ceará. The low prevalence found in the present study is consistent with other reports from the Brazilian Semiarid. Rodrigues et al. (2024) reported a prevalence of 0.4% (1/246) for *T. vivax* in cattle in the state of Ceará. However, their research was limited to a single microregion, the Sertão Central, with five municipalities, whereas the present study was conducted across 23 municipalities of different microregions of the states of Ceará and Paraíba, covering a broader geographical area. Seroprevalence surveys carried out in the state of Paraíba similarly found no animals positive for *T. vivax*, as observed in the present work (Costa et al., 2013). In contrast, other regions of Brazil that are endemic for *T. vivax* exhibit higher prevalence rates. Bastos et al. (2020) reported a prevalence of 8.4% in cattle from 24 dairy farms in the state of Goiás, a known endemic area for *T. vivax*, which differs from the findings of the present study.

The absence of significant risk factors in this study may be attributed to the low prevalence observed. Nevertheless, the introduction of *T. vivax*-infected animals and the administration of exogenous oxytocin using shared syringes and needles in dairy cows are identified as causes of trypanosomiasis transmission. Bastos et al. (2020) has previously highlighted the importance of this risk factors and transmission method in Brazil. On this farm, the lack of contact with animals from other herds and the absence of mechanical vectors at the time of the outbreak indicate that the

introduction of cattle not subjected to quarantine was the most plausible source of infection. The unrecorded history of previous reproductive problems reinforces the hypothesis that *T. vivax* was introduced with the newly arrived animals. Supporting this finding, Rodrigues et al. (2024) reported that the only *T. vivax*-positive animal identified in their study originated from a farm with a known history of trypanosomiasis, low incidence of *Stomoxys calcitrans* and tabanids, and that six months prior to sampling, new animals from external sources had been introduced into the herd. In areas of enzootic instability, where animals are not regularly exposed to the parasite, the introduction of animals infected may lead to trypanosomiasis outbreaks (Batista et al., 2008). Therefore, the absence of quarantine in dairy farms, combined with the introduction of new animals, represents an important risk factor for the occurrence of *T. vivax* outbreaks, particularly in areas of enzootic instability, reinforcing the need for preventive management practices and sanitary surveillance measures.

The occurrence of the outbreak in the absence of mechanical vectors suggests that management practices, such as the shared use of needles and syringes, may play a considerable role in the transmission of *T. vivax* in dairy herds. This underscores the importance of considering iatrogenic routes of transmission, particularly when newly introduced animals may serve as

asymptomatic carriers. Studies have demonstrated the viability of *T. vivax* in 109 injectable veterinary products following contamination during administration, revealing that in 44% (48/109) of the samples, *T. vivax* remained viable regardless of time. Among the drug classes evaluated, the parasite was found to be viable in 100% (7/7) of oxytocin-based hormonal products (Melo Junior et al., 2022). The survival of the parasite in injectable fomites enables the transmission of *T. vivax* from an infected animal to a healthy one during the administration of medications or hormones. The persistence of *T. vivax* in injectable products, raises significant concerns regarding routine veterinary practices, emphasizing the urgent need for training and awareness among farmers and veterinarians to mitigate iatrogenic transmission.

The phylogenetic analysis of the isolates from this study reveals a pattern consistent with that previously observed in *T. vivax* outbreaks in South America, including the Brazilian Semiarid region. The sequences TviBrCe13 and TviBrCe14, obtained from cattle during an outbreak in the municipality of Orós, Ceará, showed 100% identity with isolates previously described in the Semiarid region, originating from donkeys, sheep, and cattle (Batista et al., 2007; Rodrigues et al., 2015). This finding demonstrates the low genetic diversity of the CatL gen in the *T. vivax* population circulating in the region, in accordance with previous studies, and supports the hypothesis that *T. vivax* was introduced into South America from West Africa, possibly through the transatlantic movement of animals during the colonial period (Cortez et al., 2009; Rodrigues et al., 2015). Rodrigues et al. (2015) previously identified that Brazilian isolates of *T. vivax*, similar to those in the present study, are genetically related to isolates from other regions of South America and West Africa, while differing from isolates from East and South-Central Africa.

Studies conducted in the Brazilian Semiarid region have demonstrated that the circulating genotypes of *T. vivax* are highly conserved and are shared among different host species such as donkeys, cattle, and sheep, regardless of clinical severity (Batista et al., 2007; Rodrigues et al., 2015). Previous studies have shown that sequences from isolates obtained from donkeys (TviBrDo) were identical or highly similar to sequences from isolates associated with acute outbreaks in cattle (TviBrCa and TviBrMo) and sheep (TviBrRp) in the same region, as well as similar to those from asymptomatic animals in endemic areas. The severity of clinical manifestations is influenced by previous contact with *T. vivax* and by the tolerance levels of different cattle species and breeds, rather than being associated with a specific parasite genotype (Rodrigues et al., 2015). This same pattern was observed in the present study, in which the genotypes of *T. vivax* isolates obtained from cattle affected by the outbreak were indistinguishable from those previously described in the Brazilian Semiarid region. Furthermore, outbreaks of trypanosomiasis in the Brazilian semiarid region are not restricted to cattle, having also been reported in goats and sheep (Batista et al., 2008, 2022), species of great importance for livestock production in this area. The monitoring of the disease's occurrence in cattle, the preferred hosts of *T. vivax*, serves as an important indicator for

epidemiological monitoring, enabling the implementation of measures to prevent the spread of the parasite among different species and to minimize the economic impacts of the disease on herds in the semiarid region.

Pereira et al. (2018) stated that trypanosomiasis presents clinical signs similar to other diseases, such as anaplasmosis and babesiosis, which are widely distributed throughout the Semiarid region. Furthermore, several studies conducted in the Brazilian Semiarid have shown that the disease occurs in the form of sporadic outbreaks and is not endemic to the region. A study conducted in Uganda found higher positivity for *T. vivax* using rapid diagnostic tests compared to PCR, which was attributed to the fact that the rapid test detects antibodies, whereas PCR detects parasite DNA. The authors further emphasized that this limits the use of antibody detection methods in guiding treatment decisions, as compared to antigen-based methods. Therefore, antigen detection methods are considered more relevant for supporting field veterinarians in treatment decision-making (Kizza et al., 2024). Consequently, caution is advised when interpreting results from rapid tests as a diagnostic tool for trypanosomiasis in the Brazilian Semiarid, since these tests are highly specific but have low sensitivity, which may result in false positives and lead to unnecessary treatment expenditures.

Based on these findings, it can be concluded that the prevalence of *T. vivax* in cattle in the Brazilian semiarid is low and that the region is predisposed to experiencing trypanosomiasis as outbreak events. Therefore, the disease should not be underestimated in this area.

Data availability statement

The data presented in the study are deposited in the GenBank repository, accession numbers PX262614 and PX262615.

Ethics statement

The animal studies were approved by Comitê de Ética no Uso de Animais - CEUA/Instituto Federal da Paraíba - IFPB. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BL: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. JS: Investigation, Methodology, Writing – review & editing. FA: Investigation, Methodology, Writing – review & editing. SB: Investigation, Methodology, Writing – review & editing. GS: Investigation, Methodology, Writing – review & editing. GM: Investigation, Methodology, Writing – review & editing. CR:

Investigation, Methodology, Writing – review & editing. BF: Investigation, Methodology, Writing – review & editing. VV: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. TF: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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