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Short communication

First report and molecular characterization of *Trypanosoma vivax* in cattle from state of Pernambuco, Brazil

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ABSTRACT

This paper describes an outbreak of *Trypanosoma vivax* for the first time in the state of Pernambuco, Brazil, affecting dairy cattle in the municipality of Itambé in the northern coastal zone of the state. Clinical signs compatible with infection by blood protozoa and epidemic miscarriages were observed. The diagnosis of *T. vivax* was confirmed through biometric microscopy and molecular analysis with PCR and DNA sequencing. The *T. vivax* isolate detected in the present study proved to be genetically very close to other Brazilian isolates of the protozoan despite being geographically distant.

1. Introduction

The protozoan *Trypanosoma* (Duttonella) *vivax* Ziemann (1905) infects a large variety of wild and domestic ungulates, among which cattle are the most susceptible (D'Ávila et al., 1997). *T. vivax* is believed to have been introduced in the Americas in the mid 19th century, when Zebu cattle from Senegal were imported to French Guyana and the Antilles. Diffusion throughout the region likely occurred due to the adaptation of the parasite to mechanical transmission carried out by hematophagous dipterans, such as *Tabanus* spp. and *Stomoxys* spp.; however, the biological vectors in these regions remain unknown (Shaw and Lainson, 1972; Clarkson, 1976; Gardiner, 1989; Paiva et al., 2000; Osório et al., 2008).

In Brazil, *T. vivax* was first described in the northern and central western regions (Shaw and Lainson, 1972; Serra-Freire, 1981; Silva et al., 1996; Paiva et al., 1997) and also has recently been described infecting cattle, buffalo and sheep in the northeastern region of Brazil (Batista et al., 2007; Guerra et al., 2008; Galiza et al., 2011; Melo et al., 2011), cattle in the southeastern (Carvalho et al., 2008) regions as well as horses in the southern region of the country (Silva et al., 2011).

Cattle infected by *T. vivax* may be completely asymptomatic or may exhibit severe hematological alterations leading to pale mucous membranes, progressive weight loss and miscarriage (Silva et al., 1999). Although this protozoan lives in balance with its vertebrate host throughout most of Brazil, clinical and hematological alterations have occurred in a sporadic, localized fashion (Barbosa et al., 2001; Linhares et al., 2006; Batista et al., 2007).

Despite the geographic proximity to the state of Paraíba (northeastern Brazil), where *T. vivax* has recently been identified (Batista et al., 2007; Galiza et al., 2011), this protozoan has not previously been reported for the state of Pernambuco. Thus, this paper reports the occurrence of *T. vivax* in cattle for the first time in the state of

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Pernambuco and offers the molecular characterization of the isolate found.

2. Materials and methods

An outbreak of trypanosomiasis by *T. vivax* occurred in the month of March 2010, which is the beginning of the rainy season in the region, on a dairy cattle farm located in the municipality of Itambé (07°26′40″S and 35°14′17″W) in the state of Pernambuco (northeastern Brazil).

From a total of 80 cattle, 22 (27.5%) showed hyperthermia, anorexia, pale mucous membranes, aqueous diarrhea of dark coloration, reduction in milk production, the occurrence of premature fetuses and a higher than average number of miscarriages for the farm. Blood samples of jugular vein from these 22 animals with hyperthermia were collected with EDTA. For parasitological exam blood smears were prepared and stained with Giemsa stain.

For the molecular diagnosis, DNA was extracted from 350 μL of blood using the method described by Araújo et al. (2009). The diagnosis was carried out in two steps. The first step was polymerase chain reaction (PCR) using the procedure described by Geysen et al. (2003), using the generic primers 18STnF2 (5′-CAACGATGACACCCATGAATTGGGGA-3′) and 18STnR3 (5′-TGCGCGACCAATAATTGCAATAC-3′), which delimit a fragment of 659 bp of the 18S rRNA gene in Brazilian isolates of *T. vivax* (Madruga et al., 2003). The second step was carried out through the sequencing of the amplicons obtained from the PCR.

A pool of the amplicons was used for binding to the pGem-T Easy plasmid (Promega). Five clones of the recombinant plasmid were sequenced using the method described by Sanger et al. (1977) in an ABI 3130 automatic sequencer (Applied Biosystems). The Sequencher 4.1.4 program (Gene Codes) was used for the editing and generation of the consensus sequence. A search by homology was then carried out using the BLASTn algorithm (http://www.ncbi.nlm.nih.gov/BLAST) (Altschul et al., 1990). The phylogenetic analysis was carried out using the MEGA 4.0 program (Tamura et al., 2007) and the phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987), involving a bootstrap procedure with 1000 replicates (Felsenstein, 1985).

3. Results

During the clinical exam, besides hyperthermia, 13.63% (3/22) of the animals were prostrate and had reduced milk production, premature offspring and miscarriages in the final third of the gestation. The protozoan was observed microscopically in 9.09% (2/22). No other blood parasites, such as *Babesia* sp. and *Anaplasma marginale,* were observed in the microscopic exam.

The morphometric analysis of the protozoan revealed the following dimensions: total size = 20 μm; distance from kinetoplast to posterior region = 1.0 μm; distance from kinetoplast to nucleus = 5.7 μm; distance from posterior region to nucleus = 6.0 μm; distance from nucleus to anterior region = 8.0 μm; and free flagellum = 6.0 μm. These measurements are in agreement with those obtained for other New World isolates of *T. vivax* (Johnson, 1941; Shaw and Lainson, 1972; Oliveira et al., 2009).

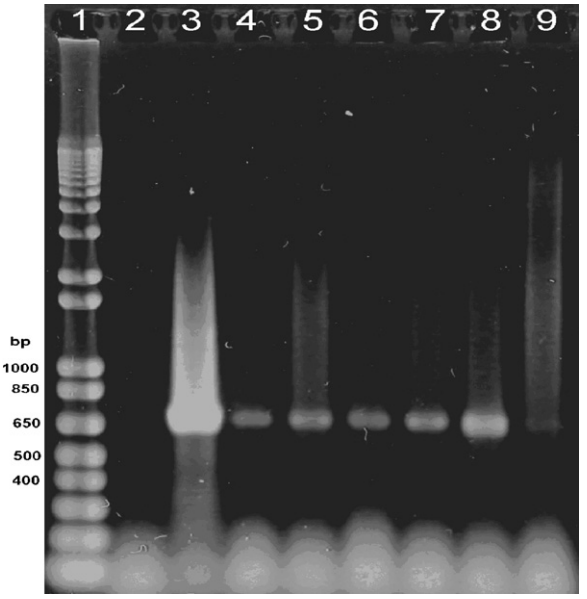

Fig. 1. Electrophoresis in 1% agarose gel of PCR amplification of 18S rRNA gene from *Trypanosoma vivax*; (1) base pair marker (1 kb plus Invitrogen); (2) negative control; (3) positive control; (4–9) amplifications from DNA extracted from blood of bovines suspected of trypanosomiasis.

In the molecular diagnosis (PCR), 100% (22/22) of the animals from which blood samples were collected were positive for *Trypanosoma* sp. following the viewing of amplicons of approximately 650 bp (Fig. 1).

In the homology search of the sequenced amplicons with the aid of the BLASTn algorithm, 99% identity was found between the sequences of the 18S rRNA gene in the present study and sequences from Brazilian isolates of *T. vivax* (Aquidauana AY362546, Belém AY363164 and Poconé AY363165) as well as an African isolate (Nigeria U22316).

The partial sequence of the 18S rRNA gene of the Itambé/PE isolate of *T. vivax* was deposited in the Genbank under accession number HM209400.

The phylogenetic analysis revealed that the Brazilian isolates are grouped together with an isolate from west Africa (Nigeria) and separated from an isolate found in antelopes (*Tragelaphus angasi*) in Mozambique (east Africa) (Fig. 2).

4. Discussion

According to Geysen et al. (2003), the primers used in the present study delimit fragments between 700 and 800 bp, based on the species of Trypanosoma involved. Using these same primers, Madruga et al. (2003) found that the amplified fragment was 659 bp for Brazilian isolates of *Trypanosoma vivax*. Therefore, the amplicons obtained in the present study were compatible in size to those described for Brazilian isolates of *T. vivax* (Madruga et al., 2003).

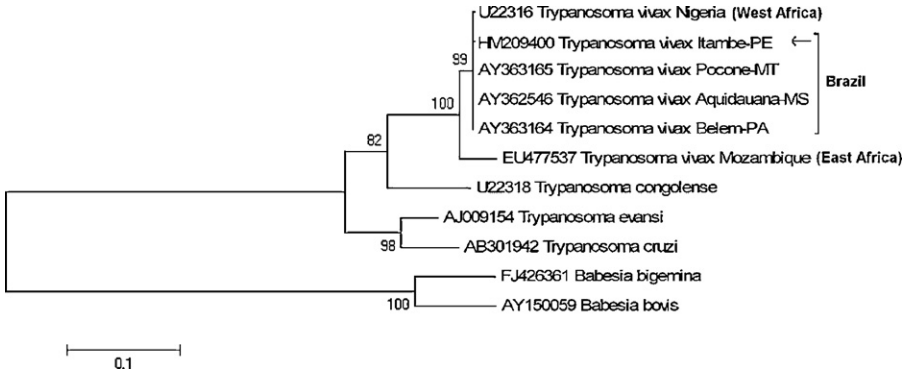

Fig. 2. Phylogenetic tree constructed using neighbor-joining method with sequences of 18S rRNA gene of *Trypanosoma vivax* isolates, other species of *Trypanosoma* sp., *Babesia bigemina* and *Babesia bovis*; number at each knot corresponds to bootstrap percentage derived from 1000 replicates; scale represents number of replacements per site of sequence.

Although genetic polymorphism is reported between isolates of *T. vivax* from the Pantanal wetlands of Brazil by RAPD, REP and ERIC-PCR (Osório, 2002), the genetic diversity of New World isolates of *T. vivax* is lesser than that of African isolates (Cortez et al., 2006), as geographically distant Brazilian isolates [Aquidauana and Poconé (central western region), Belém (northern region) and Itambé (northeastern region)] are genetically very close to one another. This low degree of diversity is likely due to the absence of genetic recombination in the parasite, which normally occurs in its biological vector (Gibson, 1995), thereby enabling the maintenance of the parasite/host balance in the New World bovine population, unlike what occurs in Africa (Osório et al., 2008).

The results of phylogenetic analysis corroborate with observations put forth by Cortez et al. (2006) and Rodrigues et al. (2008) and the hypothesis of a west African origin for South American isolates of *T. vivax*.

The pathway of *T. vivax* introduction in the State of Pernambuco remains unknown, as no animals were recently introduced on the farm of occurrence. However, it is likely that this introduction occurred through the transit of chronically infected animals (cattle, buffalo or small ruminants) from regions of occurrence to protozoan-free regions (Batista et al., 2007). Moreover, as the property studied has a high density of hematophagous flies, the dissemination of the parasite may have occurred through mechanical transmission by these insects, which are likely the transmitters of this protozoan in the Americas (Otte et al., 1994; Jones and Dávila, 2001).

5. Conclusion

The outbreak of trypanosomíasis by *T. vivax* described for the first time in the state of Pernambuco and previous descriptions of outbreaks in Brazil demonstrate that this protozoan is spreading throughout the country, which can cause serious problems in susceptible herds. Therefore, greater attention and control are needed regarding the transit of animals from regions in which the parasite is present to regions that remain free of this parasite in order to avoid economic losses stemming from the establishment of the parasite, especially in cattle herds.

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