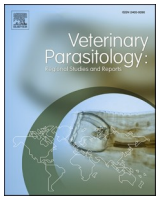
Veterinary Parasitology: Regional Studies and Reports 25 (2021) 100602

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Short Communication

First report of *Trypanosoma vivax* infection in sheep from Nicaragua

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ARTICLE INFO

Keywords:
Trypanosoma vivax
Sheep
Diagnosis
Epidemiology
Nicaragua

ABSTRACT

In Central America, outbreaks of trypanosomiasis by *Trypanosoma vivax* have been recorded only in cattle. This is the first report of an outbreak of trypanosomiasis by *T. vivax* in 30 Pelibuey sheep (2 to 7 years old, male and female) from Nicaragua, which occurred in 2009. Clinical signs included fever, apathy, pale mucous membranes, weakness, progressive weight loss, and sudden death. Infection by *T. vivax* was detected in 22 (73.3%) sheep by blood smear analysis and/or PCR. Trypanosomes were morphologically identified in 11 (36.7%) blood smear samples, whereas 17 (85%) of the 20 samples subjected to PCR were positive for *T. vivax*. Eighteen (81.8%) of the 22 infected sheep presented a packed red cell volume (PCV) lower than 25%. Upon diagnosis, the treated animals were clinically recovered and no parasites could be observed in subsequent examinations. Tabanids were potential mechanical vectors of *T. vivax* in the farm. This is the first report of *T. vivax* in Nicaragua and for the first time this haemoparasite is recorded in sheep in Central America.

1. Introduction

Trypanosoma (*Dutonella*) *vivax* is a protozoan parasite widespread in sub-Saharan, Africa and South and Central America. This haemoparasite is pathogenic to domestic ruminant livestock as well as wild ruminants (Osório et al., 2008). In Africa, *T. vivax* is predominantly transmitted cyclically by tsetse flies, while in Latin America, the parasite has the ability to be transmitted mechanically by hematophagous flies such as *Tabanus* spp., *Stomoxys calcitrans* and *Haematobia irritans*, which are responsible for the spread of *T. vivax* in tsetse-free areas (Fetene et al., 2021).

Natural infections have been reported in cattle and water buffalo from several countries of South America (Chávez-Larrea et al., 2020; Garcia Pérez et al., 2020) and Central America (Johnson, 1941; Oliveira et al., 2009). Natural infections in sheep have been recorded in Ecuador and Paraguay (Coello-Peralta et al., 2021; Tomassi et al., 2018). Naturally and experimentally infected sheep present with fever, anemia, weakness, progressive weight loss, hematological and carbohydrate metabolism alterations, myocarditis, and severe testicular degeneration (Batista et al., 2019; Parra-Gimenez and Reyna-Bello, 2019).

According to Batista et al. (2009), sheep develop more severe trypanosomiasis than goats. However, little is known about *T. vivax* reservoirs and which vectors are responsible for spreading the disease in Central America. This study aimed to describe the natural infection by *T. vivax* in sheep from Nicaragua.

2. Materials and methods

The case study was done with data from a farm with approximately 400 sheep of Pelibuey breed. From August to October 2009, 30 male and female animals of 2–7 years old presented clinical signs such as fever, pale mucous membranes, weakness, weight loss, and sudden death. The animals were bred semi-extensively, fed on native pasture, and shared the same pastures with other animals, including cattle and equines. The farm is located in San.

Antonio (136°56′37″ latitude, 59°34′95″ longitude), municipality of León, Nicaragua, which is characterized by a dry tropical climate, temperature ranging from 27.0 to 40.0 °C, and a relative humidity between 65% and 75%. The annual precipitation is 1000–1800 mm.

Whole blood samples were collected from the jugular vein into

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https://doi.org/10.1016/j.vprsr.2021.100602
Received 23 November 2020; Received in revised form 17 June 2021; Accepted 20 June 2021
Available online 22 June 2021

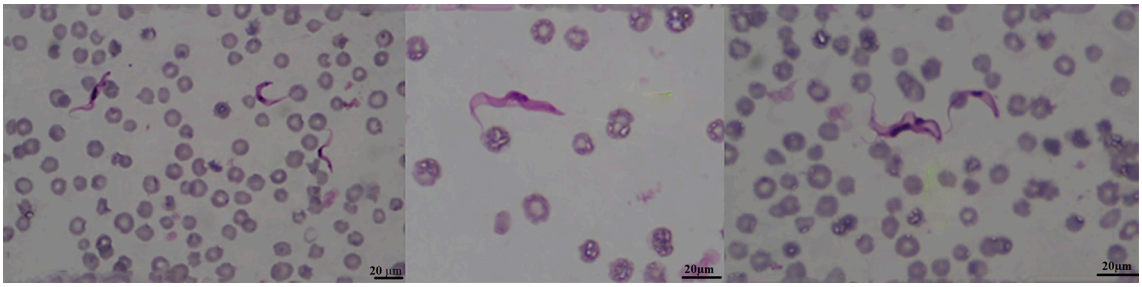

Fig. 1. *Trypanosoma vivax* from sheep of Nicaragua.

Table 1
T. vivax detection in 30 Blood Smear and 20 PCR from sheep of Nicaragua.

	PCR			
		Positive	Unrealized or Negative (total)	Total
Blood smears	Positive	6	Un = 5	11
	Negative or Unrealized	Ne = 11	Ne = 3, Un = 5 (8)	19
	Total	17	Ne = 3, Un = 10 (13)	30

Ne: Negative.
Un: Unrealized.

vacutainer tubes with EDTA and were kept on ice while transported to the laboratory. After complete blood count (CBC) and parasitological evaluations, the samples were stored at −20 °C for further PCR analysis.

The packed red cell volume (PCV) was measured using the standard microhematocrit method, and the hemoglobin (Hb) concentration was determined colorimetrically. Blood smears were stained with Giemsa for differential white blood cell count (WBC) (Meyer and Harvey, 1999).

DNA extraction was performed in 20 of 30 randomly selected samples with Wizard® Genomic DNA purification kit (Promega), as described by manufacturer instructions. DNA amplification was carried out according to protocols of Gonzales et al. (2006), and primers used to amplify target DNA were TVW A (5′-GTG CTC CAT GTG CCA CGT TG-3′) and TVW B (5’-CAT ATG GTC TGG GAG CGG GT-3′). The amplification reaction was carried out in a final volume of 25 µl, which contained 12.5 µl of MasterMix 2× (Promega, U.S), 5.5 µl of nuclease-free water, 1 µl of each specific primer at 500 nM, and 5 µl of genomic DNA. The PCR amplification consisted of an initial denaturation of 94 °C, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min. The final extension was carried out at 72 °C for 7 min. The expected amplification product was 175 bp in length. Positive and negative controls were systematically run with the samples. PCR products were resolved in 2% agarose gels, stained with ethidium bromide and photographed under UV illumination.

3. Results and discussion

Trypanosoma vivax is a widespread haemoparasite in tropical areas and is pathogenic to domestic ruminant livestock. In Central America, outbreaks of trypanosomiasis by *T. vivax* have been recorded only in cattle (Oliveira et al., 2009). In this study, the diagnosis of trypanosomiasis was performed using blood smear analysis and confirmed by PCR. Overall, *T. vivax* was detected in 22 of 30 sheep (73.3%) by blood smears and/or PCR techniques. By blood smear analysis, *T. vivax* was detected in 11 (36.7%) samples. Trypanosomes were identified based on morphological data according to Hoare (1972) Fig. 1; out of 20 blood samples tested by PCR, *T. vivax* was detected in 17 (85%) samples (Table 1).

During August until October 2009 a case study about *T. vivax* in sheep was realized, being the first report of this infection in the Central American region. Currently, the parasite has spread and has now become endemic (Batista et al., 2009). It is possible that in other Central American countries underreporting may exist due to the fact that animal trypanosomiasis diagnostics is still problematic, because of the lack of the pathognomonic clinical signs. Also insufficient sensitivity of standard techniques in parasitology for detecting chronic diseases is still a problem (Hassan-Kadle et al., 2020). Diagnosis of *T. vivax* infections has historically been based on parasitological techniques such as hematocrit centrifuge technique (HCT) and stained blood smear examination. However, these methods lack adequate sensitivity as parasitemia levels in chronically infected animals are often very low (Alves et al., 2017). The reported blood smear sensitivity for the diagnosis of infections caused by *T. vivax* is low (31.5%), while HCT has a sensitivity of 44.4% (Fidelis Junior et al., 2019). In the present work, trypanosomes were found in stained blood smears from collected samples for hematological evaluation. Positive and negative samples were submitted to PCR and DNA from *T. vivax* was detected in 85% of samples. Molecular diagnostic techniques such as PCR have usually proven to be very specific and sensitive tools, capable of detecting low parasitemia levels of *T. vivax* infections (Cadioli et al., 2015; Fidelis Junior et al., 2019). PCR has also been proposed as a tool for retrospective studies to detect trypanosome DNA directly in serum (Desquesnes, 1997) and detect extravascular foci (Williams et al., 2009).

In this report, clinical signs in the sheep were similar to those reported for sheep from Brazil (Batista et al., 2009). According to Batista et al. (2009), sheep develop more severe trypanosomiasis than goats. Clinical signs caused by *T. vivax* are common to several etiologies, such as those of nutritional, toxic and infectious origin. Frequently, *T. vivax* occurs together with other infections, complicating the diagnosis and causing higher morbidity and mortality (Oliveira et al., 2009). In Nicaragua, the sheep breeding is extensive with deficient sanitary and nutritional management. This situation can contribute to the occurrence of trypanosomiasis outbreaks and economic losses.

The infected animals' PCV values were lower than the non-infected animals, similar to reported by Batista et al. (2006). Eighteen (81.8%) of the 22 infected sheep presented PCV values below 25%. Anemia is the principal sign of trypanosomiasis in livestock (Batista et al., 2009). In the case reported here, we cannot associate PCV values with *T. vivax* infection because other factors could be involved. According to the farmer, in the past, infection with *Fasciola hepatica* was found in some slaughtered sheep of the farm; this parasite also could cause anemia and contribute to decreased PCV values. Nevertheless, after diagnosis, the animals were treated with diminazene aceturate (Berenil®, MSD Animal Health), administered IM (7 mg/kg) and were clinically recovered (Clausen et al., 1999). After an acute outbreak, sheep can be asymptomatic carriers and consequently are an essential source of *T. vivax* for other ruminants (Dávila et al., 2003).

For the current work, tabanids could be considered as a potential mechanical vector since in South and Central America, *T. vivax* is mechanically transmitted by tabanids and *Stomoxys calcitrans* (Oliveira et al., 2009); although *Haematobia irritans* is also frequently found in tropical areas (Fetene et al., 2021).

The high percentage of sheep naturally infected by *T. vivax* highlights the need to study the epidemiology and economic impact of this haemoparasite in Nicaragua and other countries of Central America. Follow-up studies should be carried out to update the epidemiological situation that include vector population characterization in the outbreak area, which allow for the implementation of control measures.

Declaration of Competing Interest

None

Acknowledgments

This work was partially supported by grants from Cooperativa de Productores de Leche Dos Pinos R.L. We are very thankful with José L. Balcázar for providing language help, writing assistance and proof reading this article.

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