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Short communication

Follicular degeneration in the ovaries of goats experimentally infected with *Trypanosoma vivax* from the Brazilian semi-arid region

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ABSTRACT

Infection by *Trypanosoma vivax* and other African trypanosomes plays an important role in reproductive disorders in male and female livestock. Outbreaks of *T. vivax* in the semi-arid region of northeastern Brazil are characterized by wasting disease in cattle, sheep and goats with hematological, cardiac and nervous compromises in addition to reproductive failures. Similar to reports from Africa, we previously observed a reduction in fertility rates and severe testicular degeneration and epididymitis in male sheep infected with *T. vivax* from this region. Although anestrus is frequently reported in goats and sheep infected with *T. vivax*, the effects of this infection on the female reproductive organs need clarification. In this study, we addressed this issue through a histopathological evaluation of ovarian follicular morphology and classification in goats experimentally infected with a *T. vivax* isolate from the Brazilian semi-arid region. The infected animals presented typical clinical signs of trypanosomosis by *T. vivax*, including anemia, hyperthermia, pallor of the mucous membranes, enlarged lymph nodes, and progressive loss of weight. All the infected goats remained anestrus throughout the experimental period and exhibited important disturbances in the ovaries, evidenced by reduced size and a smooth surface without follicles or corpora lutea, and abnormal follicular development. In addition, through PCR, we detected *T. vivax* DNA in the ovarian tissues of the infected goats. Our findings contributed to understand the female reproductive failure associated with trypanosomosis caused by *T. vivax*.

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1. Introduction

The African trypanosomes *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax* are the agents of an important livestock disease known as Nagana in Africa, where these species are cyclically transmitted by the tsetse fly. Nagana strongly compromises the productive and reproductive performance of livestock. Trypanosomosis caused by *T. vivax* can be a highly debilitating and fatal disease in domestic ruminants, mainly due to the hematological disturbances that induce severe anemia and inflammatory foci in the central nervous system (CNS), heart, liver, spleen and lymph nodes (Gardiner et al., 1989; Desquesnes, 2004; Chamond et al., 2010).

Infection by *T. vivax* plays an important role in the reproduction failures of both male and female livestock (Masake, 1980; Gardiner and Mahmoud, 1992). Reproductive disorders in males include delayed puberty, loss of libido, and severe degenerative changes of the genitalia. Testicular atrophy, degeneration and calcification have been

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documented in sheep and goats infected with *T. vivax* resulting in very poor quality semen, and may lead to a total lack of spermatogenesis. Pathological changes in *T. vivax*-infected males include epididymitis and orchitis (Isoun and Anosa, 1974; Anosa and Isoun, 1980; Sekoni et al., 1988, 1990a,b; Okech et al., 1996; Bezerra et al., 2008; Mbaya et al., 2011).

In females, trypanosomosis causes temporary or permanent anestrus, abnormal estrus cycles (Ogwu et al., 1984) and rapid decline in milk production (Batista et al., 2007). Additionally, *T. vivax* infection has induced abnormal pregnancy, dystocia, abortion, premature and low birth weights, stillbirths, transplacental fetal infection, neonatal death and other pathogenic effects on fetuses and offspring (Ogwu et al., 1986; Okech et al., 1996; Batista et al., 2012).

T. vivax was introduced into South America by cattle imported from Africa. Outside Africa, this species is only mechanically transmitted by hematophagous flies such as *Tabanus* spp. and *Stomoxys* spp. The parasite is now endemic in some regions of Brazil, Venezuela and Bolivia (Jones and Dávila, 2001; Desquesnes, 2004; Gardiner and Mahmoud, 1992; Garcia et al., 2005; Silva et al., 1999; Osório et al., 2008). In Brazil, *T. vivax* has been reported in cattle, sheep, goat and buffalo herds from the northern to the southern regions (Silva et al., 1996; Batista et al., 2007, 2009, 2012; Cuglovici et al., 2010; de Araujo Melo et al., 2011; Galiza et al., 2011). Recently, horses infected with *T. vivax* were found for the first time in Brazil (Da Silva et al., 2010).

Recent studies of outbreaks of trypanosomosis by *T. vivax* in the semi-arid region of northeastern Brazil showed very devastating and often fatal disease that creates serious economic losses in cattle, goat and sheep breeding operations due to productive and reproductive problems (Batista et al., 2007, 2009, 2012; Galiza et al., 2011). We previously evaluated the pathogenicity for sheep of one *T. vivax* isolate from the Brazilian semi-arid region. In this study, in addition to severe haematological and neurological disorders, the infected males showed severe testicular degeneration and epididymitis, and DNA of the parasite was detected in testicular and epididymal tissues using a *T. vivax*-specific PCR (Bezerra et al., 2008).

There is a paucity of information on the effects of *T. vivax* infection on the female reproductive organs of ruminants in Africa and South America. A previous study reported numerous cysts and parasites in smears from the ovaries of sheep infected with *T. vivax* in Nigeria, West Africa (Isoun and Anosa, 1974). The main goal of this study was to evaluate the effects of *T. vivax* infection on the ovaries of goats experimentally infected with a virulent isolate from the Brazilian semi-arid region.

2. Materials and methods

2.1. Composition of the experimental groups and experimental infection

In this study, we used ten female mixed breed goats, approximately 15 months of age, housed in a properly screened stall at the Veterinary Hospital of the University of the Semi-Arid (UFERSA), Mossoró, Rio Grande do Norte, Brazil. For 14 days before the inoculation of *T. vivax*, the goats were evaluated by clinical and hematological examination with approval from the local ethics committee in the use of animals of UFERSA-CEUA (process n° 23091.1901/10-98). Blood samples from all the animals were tested using a *T. vivax*-specific diagnostic PCR (Cortez et al., 2009) before and during the experimental period conducted in April and May (winter season). The goats were treated with the anthelmintic Ivermectin (Ivomec®). Healthy animals were randomly distributed into two experimental groups: one group of six goats infected with *T. vivax* (goats 1–6) and a control group composed of four goats not infected by *T. vivax* (goats 7–10). All the animals were kept under identical management conditions and were fed with Tifton hay (*Cynodon* sp.) supplemented with commercial food at 1.5% of their body weight per day, with water *ad libitum*.

The isolate of *T. vivax* used for the experimental infections was obtained from a sheep during an outbreak in São João do Rio do Peixe, Paraíba, in the Brazilian semi-arid region, where severe hematological and nervous symptoms were reported (Galiza et al., 2011). Blood samples were collected from a sheep showing very high parasitemia using 10% EDTA (ethylenediaminetetraacetic acid disodium), mixed with 8% glycerol, distributed in aliquots and frozen in liquid nitrogen. Immediately before inoculation, the cryopreserved parasites were thawed, and each animal was inoculated intravenously with 1.25×10^5 trypomastigotes of *T. vivax* as described previously (Batista et al., 2007, 2012).

2.2. Clinical exams, PCV and parasitemia assessment

Daily for 60 days post infection (dpi), the animals from both the infected and the control groups were clinically examined to assess rectal temperature and status of mucous membrane and external lymph nodes. We also performed a daily inspection of the animals for signs that indicate the occurrence of estrus, such as restlessness, sexual receptivity, edema and hyperemia of the vulva, and the presence of vaginal discharge.

Parasitemia was determined daily by microscopic determination of the number of parasites in 5 μl of peripheral blood collected from the ear and dispersed between two glass slides as standardized previously (Batista et al., 2007). At the same time, blood was collected by puncture of the jugular vein into sterile tubes containing 1.0 mg/ml EDTA for the PCV analysis and DNA preparations (Cortez et al., 2009).

2.3. Collection, macroscopic and histologic evaluation of ovaries

Surgical collection of the ovaries and macroscopic evaluation were performed 60 days after infection. Several representative pieces of ovarian cortex were fixed in Bouin solution for 48 h and preserved in 99% ethanol. The fixed tissues were embedded in paraffin, sectioned at 4.0 μm thickness and stained with hematoxylin and eosin. For qualitative assessment, 30 follicles from each ovary (right and left) per animal were morphologically classified as

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