



Short Communication

High mortality and lesions of the central nervous system in Trypanosomosis by *Trypanosoma vivax* in Brazilian hair sheep

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ABSTRACT

Here, we report an outbreak of *Trypanosoma vivax*-induced trypanosomosis in Brazilian hair sheep on a farm in Paraíba state, a non-endemic region in northeastern Brazilian. Of 306 total sheep, 240 showed clinical signs and 216 died. Clinical signs included anorexia, lethargy, anemia, rough hair coat, weight loss, submandibular edema, abortion, and in some cases, neurological signs such as head pressing, lateral recumbence, paddling movements and muscle tremors. *T. vivax* was identified by blood smear analysis and polymerase chain reaction (PCR). At necropsy, animals exhibited watery blood, pale tissue coloring, and the presence of liquid in the peritoneal cavity and pericardial sac. Histologically, nonsuppurative myocarditis and meningoencephalitis with areas of malacia were observed. After treatment, no parasites were detected by blood smear analysis or PCR. Cattle and buffalo that remained in the same pasture were also infected but presented with asymptomatic infections. Epidemiological data suggest that *T. vivax* was introduced to the farm and the susceptible flock by buffalos that were asymptomatic carriers of the infection; *T. vivax* was most likely transmitted by *Tabanus* spp. bites and also iatrogenically.

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1. Introduction

In Africa, animal trypanosomes are widely distributed and are transmitted by several species of their biological vector, the tsetse fly (*Glossina* spp.); further, mechanical transmission of trypanosomes occurs in areas free of tsetse flies. Among the trypanosomes that infect livestock, *Trypanosoma vivax* is one of the most important pathogenic species for bovines, equines, sheep and goats (Anosa, 1983; Desquesnes, 2004). In South America, three species of *Trypanosoma* have veterinary importance because they are the etiologic agents of important livestock diseases. *T. evansi* causes trypanosomosis in horses and dogs; further, this pathogen can also infect wild mammals (Ventura

et al., 2001; Rodrigues et al., 2005). *Trypanosoma equiperdum* causes dourine in horses and other equines such as donkeys and mules. Although this species was earlier reported in Brazil and other South American countries, it has more recently been observed only in Bolivia and Paraguay (Desquesnes, 2004). In cattle, buffalo, goats and sheep, trypanosomosis is caused by *T. vivax* (Silva et al., 1996; Batista et al., 2007, 2009; Cuglovici et al., 2010). *T. evansi* and *T. vivax* have adapted to mechanical transmission by bloodsucking insects, such as *Tabanus* spp. and *Stomoxys* spp., allowing for the occurrence of trypanosomosis in regions where *Glossina* spp. are not present (Anosa, 1983; Desquesnes, 2004).

In Latin America, the first case of trypanosomosis caused by *T. vivax* was reported in cattle in French Guiana in 1919, and in Brazil *T. vivax* was first observed in 1944 by Floch and Lajudie (Desquesnes, 2004), and reported again by Shaw and Lainson (1972) in buffalo in the state of Pará. Later,

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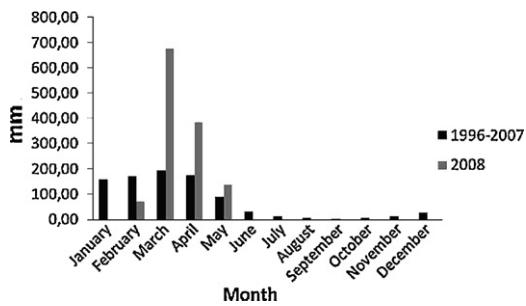


Fig. 1. Rainfall amounts during 2008 and from 1996 to 2007 in São João do Rio do Peixe, Paraíba. <http://www.aesa.pb.gov.br/> (accessed 10.10.10).

Silva et al. (1996) identified *T. vivax* in cattle in the Pantanal (a tropical wetland that lies mostly within the states of Mato Grosso do Sul and Mato Grosso). More recently, *T. vivax* has been reported in cattle throughout Brazil (Batista et al., 2007; Guerra et al., 2008; Da Silva et al., 2009; Cuglovici et al., 2010). In northeastern Brazil, the first outbreak of *T. vivax*-induced trypanosomosis occurred in cattle in the municipality of Catolé do Rocha, a semiarid region of Paraíba state (Batista et al., 2007).

The main clinical signs of *T. vivax* infection are fever, anemia, decreased milk production, anorexia, progressive weakness, emaciation, corneal opacity, abortion, diarrhea, hemorrhage (Batista et al., 2007) and neurological signs (Batista et al., 2007, 2008). In Africa, cattle and goat breeds range from being trypanotolerant to highly susceptible to *T. vivax* infection (Anosa, 1983; Desquesnes, 2004). In small ruminants from Africa and South America, *T. vivax* infection can result in a variety of clinical outcomes ranging from acute to chronic or subclinical disease; the course of infection varies depending on the parasite strain and the species and breed of the ruminant host (Anosa, 1983; Desquesnes, 2004; Batista et al., 2008, 2009).

In Brazil, the first recorded outbreak of *T. vivax*-induced trypanosomosis in sheep and goats occurred in 2007 in northeastern Brazil and resulted in anemia, weight loss and abortion (Batista et al., 2009); morbidity and mortality were low, neurological signs were not reported and necropsies were not performed. Here, this study reports the clinical signs and the epidemiological, hematological and pathological findings of an outbreak of *T. vivax*-induced trypanosomosis with a high mortality in Santa Inês hair sheep that also occurred in northeastern Brazil.

2. Outbreak report

The outbreak occurred in a flock of Santa Inês sheep in the municipality of São João do Rio do Peixe in Paraíba state in northeastern Brazil. The farm had approximately 1200 hectares of pastures containing both native grasses and *Brachiaria decumbens*. Of 306 young and adult hair sheep, 240 showed severe anemia and weight loss, and 216 (70.58%) died between April and July 2008. During this time, regional precipitation levels were increased compared to previous years (Fig. 1). The sheep were grazed extensively, grazing in the same pastures as 14 cattle and 6 buffalo; each afternoon all animals were placed into the

same corral. The buffalo, which were introduced on January 2008, were from the municipality of Recife in the state of Pernambuco in northeastern Brazil, in a coastal region with a humid, tropical climate. The farmer reported that from March to May, during the rainy season, the populations of *Tabanus* spp. were high. After the start of the outbreak, all sheep in the flock were treated with anthelmintics and vaccinated against clostridial diseases. Sheep with clinical signs were treated with oxytetracycline, sulfonamides, iron dextran, mineral supplements, vitamins and amino acids. No results were observed, and new cases of the disease still occurred. To perform these treatments, the farmer used the same needle for all animals treated in the same day.

Affected animals exhibited pale mucous membranes, poor appetite, weight loss, rough hair coat, submandibular edema, diarrhea, and recumbence. Perinatal mortality, due to abortions and neonatal deaths, was nearly 75%. Additionally, several animals were found dead in the stable or in the paddock without presenting with any clinical signs. Several sheep with progressive weight loss and anemia showed areas of alopecia with crust formation; skin smears from these animals stained with methylene blue were positive for *Dermatophilus*. When exercised, several sheep with severe anemia exhibited muscle tremors, head pressing, nystagmus and difficulty remaining standing. However, when left in repose, these animals recovered from their neurological signs but remained weak and anemic. Several sheep with progressive weight loss died after showing neurological signs such as depression, slight muscle tremor, head pressing (Fig. 2A) lateral torsion of the head, loss of facial sensation, and permanent lateral recumbence with paddling for 2–5 days.

Laboratory examinations performed in samples collected during five visits to the farm are presented in Table 1. The feces of six sheep with clinical signs collected during the first visit had 1800–3500 *Eimeria* spp. oocysts and 100–350 *Trichostrongyloidea* eggs per gram of feces. At the same time, blood smears from the same sheep revealed trypanosomes with the characteristics of *T. vivax*. In a second visit, one week after the first visit, 26 blood samples from sheep with clinical signs and two from asymptomatic sheep were collected. In affected sheep, the mean hematocrit was $21.92\% \pm 3.95\%$, ranging from 11% to 28%; the mean red cell count was $3 \pm 0.28 \times 10^6/\text{mm}^3$, ranging from 2.7 to $7.8 \times 10^6/\text{mm}^3$; and the mean hemoglobin was 7.38 ± 0.75 g/dl, ranging from 3.5 to 9.4 g/dl. In the asymptomatic sheep, the hematocrit was 31% in both animals, red cell counts were 4 and $4.3 \times 10^6/\text{mm}^3$, and hemoglobin concentrations were 10.4 and 10.7 g/dl, respectively. Trypanosomes, morphologically similar to *T. vivax* (Fig. 2B), were observed in 18 (69.2%) out of the 26 blood smears taken from sheep with clinical signs and stained by the quick Panoptic method. No trypanosomes were found in blood smears from 3 asymptomatic sheep. Blood samples (1.5 ml) from 2 asymptomatic and 12 symptomatic sheep were collected and mixed with ethanol (v/v) for PCR diagnosis according to Cortez et al. (2009). All 14 blood samples were positive for *T. vivax* by PCR.

In a third visit, one week after the second visit, blood samples for PCR examination were collected from 11 sheep that had been treated with 3.5 mg/kg body weight of dim-

Table 1
Results of laboratory examinations performed in different dates in sheep, cattle and buffalo in an outbreak of trypanosomosis by *T. vivax*.

Date of the visit	Samples collected	Species	Number of samples	Diagnostic test	Result
July 17, 2008	Feces	Sheep	6	Fecal examination	Low number of helminths eggs and coccidial oocysts
	Skin	Sheep	3	Methylene blue stain	Positive for <i>Dermatophylus</i>
	Blood	Sheep	6	Hemogram	Anemia, positive for <i>T. vivax</i> on blood smears
July 24, 2008	Blood	Sheep	12 symptomatic	PCR	All positive
		Sheep	2 asymptomatic	PCR	All positive
	Blood	Sheep	26 symptomatic	Hemogram	Anemia
		Sheep	2 asymptomatic	Direct examination Hemogram	18 were positive* Normal values
July 31, 2008	Blood	Sheep	11 treated	Direct examination PCR	All negative
		Sheep	11 non treated	PCR	10 were positive
August 24, 2008	Blood	Cattle	4 asymptomatic	PCR	All positive
		Buffalo	4 asymptomatic	Direct examination PCR	All were negative
			4 asymptomatic	Direct examination PCR	3 out of 4 positive
August 28, 2009	Blood	Sheep	62 asymptomatic	Direct examination PCR	All were negative
		Cattle	5 asymptomatic	PCR	23/62 positive
		Buffalo	15 asymptomatic	PCR	1/5 positive
	Blood	Sheep from a neighbor farm	29 asymptomatic	PCR	5/15 positive All negative

* Positive for *T. vivax*.

inazene aceturate and from 11 non-treated, asymptomatic sheep. All samples from previously treated sheep were negative, but 10 out of 11 of the non-treated asymptomatic sheep were positive by PCR analysis for *T. vivax* infection. In a fourth visit, one month after the end of the outbreak, blood samples were obtained from 4 asymptomatic cattle and 4 asymptomatic buffalo. Although all samples were negative for trypanosomes by blood smear staining, the 4

cattle samples and 3 buffalo samples were positive by PCR analysis.

Blood samples of sheep, cattle and buffalo of the affected farm were examined by PCR one-year after the outbreak. In the PCR, 23 (37.09%) of 62 sheep samples tested positive for *T. vivax*. For non-sheep samples, 2 out of 5 buffalo samples and one out of 5 cattle samples were also positive. Additionally, blood samples were obtained also from 11 buffalo

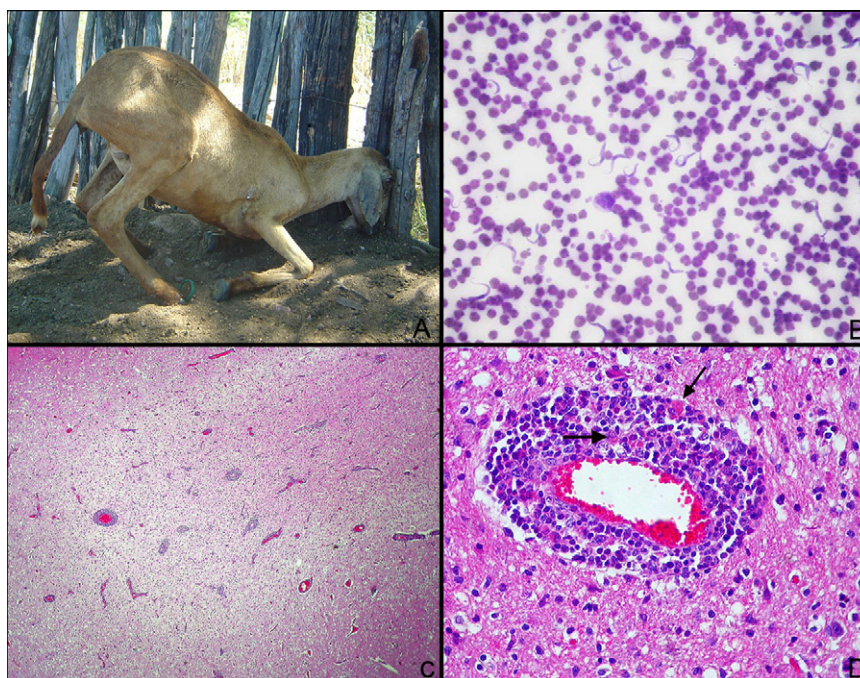


Fig. 2. (A) Sheep with clinical trypanosomosis showing head pressing. (B) Blood smears containing numerous *T. vivax* parasites. Quick Panoptic, 400 \times . (C and D) Sheep brain showing marked lymphocytic infiltrate around vessels, malacia of the white matter (C) and vasculitis with up to eight layers of mononuclear cells and the presence of rare Mott cells (arrows) (D). HE (A100 \times) and (B400 \times).

introduced on the farm after the outbreak; of these, 3 were positive. At this time, 29 samples were also collected and analyzed from sheep from an adjoining neighboring farm; all tested negative.

One sheep that died spontaneously (Sheep 1), two that were euthanized (Sheep 2 and 3), and one aborted fetus were necropsied. At the time of death, Sheep 1 was recumbent and had a red cell count of $2.8 \times 10^6/\text{mm}^3$, a 19% hematocrit, and 6.4 g/dl of hemoglobin; fecal analysis revealed 1800 *Eimeria* spp. oocysts and 100 *Trichostrongyloidea* eggs per gram of feces. Sheep 2 was emaciated and had a red cell count of $5.7 \times 10^6/\text{mm}^3$, a 19% hematocrit, and 6.0 g/dl of hemoglobin; further, 2500 *Eimeria* spp. oocysts and 100 eggs of *Trichostrongyloidea* per gram of feces were also observed. Trypanosomes similar to *T. vivax* were observed in blood smears from Sheep 2 and 3. Macroscopic changes observed in the three sheep were similar and included poor body condition, pale mucous membranes, pale tissue coloring, aqueous blood, slightly enlarged lymph nodes and spleen, atrophy of pericardial fat, and the presence of a translucent liquid in the thoracic and abdominal cavities and in the pericardial sac.

Histologic examination revealed multifocal myocarditis with mononuclear cell infiltrates comprised of lymphocytes and plasma cells that were associated with areas of fibrosis in each of the sheep. Additionally, moderate mononuclear infiltrates in the portal spaces with few Mott cells were observed in the liver tissues of the animals. Mott cells were also observed in the lymph nodes of Sheep 1. In Sheep 2 and 3, the spleens exhibited moderate hyperplasia of the white pulp. Severe nonsuppurative meningoencephalitis with marked perivascular mononuclear inflammatory infiltrate comprised of lymphocytes and plasma cells with up to eight layers of cells was observed in the central nervous system (CNS) of Sheep 3. Vasculitis and the presence of rare Mott cells were also observed. Inflammatory lesions were also associated with malacia of the white matter (Fig. 2C and D). The lesions were more accentuated in the frontal cortex and basal nuclei of the brain. In the parietal, occipital and temporal cortex and in the thalamus, there were only mononuclear inflammatory infiltrates in the leptomeninges. In the rostral colliculus, cerebellum, obex and pons, a discrete mononuclear leptomeningitis was observed. In the CNS of Sheep 2, a discrete mononuclear infiltrate was observed in the leptomeninges of the basal nuclei, hippocampus, thalamus, occipital cortex and cerebellum. No inflammatory changes were observed in the CNS of Sheep 1. There were no significant lesions in the aborted fetus examined from a sheep that exhibited clinical signs and aborted after diminazene aceturate treatment.

Before the second visit to the farm, 210 out of 306 sheep died with signs of severe anemia and weight loss. On the second visit to the farm, 29 sheep with clinical signs were treated with three applications of diminazene aceturate at a dose of 3.5 mg/kg body weight at eight day intervals. After the first treatment, 24 animals recovered, 5 died and one sheep that presented neurological signs (Sheep 3) did not recover and was euthanized. Following diminazene aceturate treatment, three untreated and one treated sheep aborted. One month after diminazene aceturate treatment,

no new cases were observed and the affected sheep were totally recovered. Further, no new cases of symptomatic trypanosomosis have been observed at the farm up to the time of article publication (May 2011).

3. Discussion

The diagnosis of trypanosomosis by *T. vivax* was based on epidemiological data, hematology, clinical signs, and pathologic findings; further, the cases were confirmed by the identification of the parasite in blood smears and specific PCR diagnostics. The presence of low numbers of helminth eggs in the feces and the lack of response to anthelmintic treatment was important to differentiate trypanosomosis from hemonchosis, which is the most common disease of sheep in semiarid regions of Brazil (Costa et al., 2009). In Brazil, in areas such as the Pantanal, *T. vivax* is endemic and asymptomatic infections in animals in good nutritional conditions or in animals affected by other diseases can occur (Paiva et al., 2000).

It has been suggested that the semiarid region of Brazil is non-endemic for *T. vivax*-induced trypanosomosis and that the disease is likely introduced into susceptible populations following an increase in the populations of *Tabanus* spp. (Batista et al., 2007, 2008). In a recent serologic survey comprised of 485 cattle blood samples from 38 farms in different regions of the semiarid state of Paraíba, all samples tested negative for antibodies against *T. vivax*, demonstrating that the region is non-endemic for *T. vivax*-induced trypanosomosis (Valéria Costa 2010, unpublished data). Also, after a previous outbreak of trypanosomosis in cattle, only 2 out of 85 serum samples had *T. vivax*-specific antibodies two years after the outbreak (Batista et al., 2007).

In the outbreak reported in this paper, the epidemiological data and the absence of *T. vivax* infections in a neighboring farm one year after the outbreak, suggest that *T. vivax* was introduced into the flock by asymptomatic animals; in this situation, it was most likely the buffalo introduced into the herd prior to the outbreak. Because the sheep, cattle and buffalo were grazed in the same pastures and were housed in the same corral each evening, it is possible that the initial transmission occurred through the bites of mechanical vectors, such as *Tabanus* spp. Furthermore, the outbreak occurred during the rainy season, with higher amounts of precipitation compared to previous years (Fig. 1); the increased rainfall was associated, according to the farmer, with an increase in the numbers of *Tabanus* spp. An important factor for the occurrence of trypanosomosis in hair sheep is likely the characteristic of their coat, which can be suckled by tabanids over the whole body surface. In contrast, the body surface of woolled sheep is protected from tabanids by the wool. Another factor that may have contributed to the spread of *T. vivax* among the flock was the use of the same needle for the administration of various drugs to sheep during the initial phase of the disease. A similar situation occurred in an outbreak of *T. evansi*-induced trypanosomosis in horses in Rio Grande do Sul (Rodrigues et al., 2005).

In addition to the high frequency of disease caused by *T. vivax* infection in sheep, this trypanosome was detected by PCR in apparently healthy sheep, cattle and buffalo,

demonstrating the occurrence of asymptomatic cases; these asymptomatic infections likely result from individual resistance. Also, although several animals were still carrying *T. vivax*, as detected by PCR, one year after the resolution of the outbreak, no new cases of symptomatic disease were observed. Another feature suggesting that *T. vivax* was introduced in a highly susceptible population, without previous parasite exposure, was the high disease morbidity (76.7%) and mortality (70.58%) rates. Prior outbreaks of *T. vivax* infection in sheep and goats (Batista et al., 2009), with low mortality rates, occurred in a region where *T. vivax* had been previously introduced 2 years earlier during a severe disease outbreak in cattle (Batista et al., 2007, 2008), suggesting a different epidemiological condition in the region: the reactivation of asymptomatic infections due to a failure in immunity caused by concomitant infections and physical and nutritional stress (Batista et al., 2009).

Clinical signs of anemia, lethargy, submandibular edema, abortion, high neonatal mortality, and weight loss were similar to those reported by other authors in natural and experimental *T. vivax* infections in sheep (Losos and Ikede, 1972; Batista et al., 2006, 2009). However, the neurological signs and the inflammatory and degenerative CNS lesions observed in this outbreak had not been previously reported in *T. vivax*-induced trypanosomosis in sheep. The lesions observed in this study were similar to those reported in *T. vivax*-infected cattle (Batista et al., 2007, 2008). Recently, in addition to neurological signs, inflammatory lesions of the central nervous system were observed in sheep experimentally infected with a *T. vivax* strain isolated from an outbreak in cattle in the state of Paraíba (Almeida et al., 2010). The transient neurological signs observed during the movement of some sheep could have been caused by cerebral hypoxia due to severe anemia. Neurological clinical manifestations without the presence of microscopic brain lesions were reported in goats and sheep experimentally infected with *T. vivax* (Losos and Ikede, 1972; Whitelaw et al., 1988).

In the outbreak reported in the present study, although disease control through the treatment of clinical cases was efficient, several non-treated, asymptomatic animals still carried the parasite one year after the outbreak. In the future, to avoid other outbreaks in the farm and the spread of *T. vivax* to other farms, it is recommended all animals be treated with 7 mg/kg of diminazene aceturate to eliminate the parasite from the flock.

In conclusion, *T. vivax*-induced trypanosomosis in sheep is a disease of rare occurrence in the semiarid regions of Brazil that can result in high mortality rates accompanied by anemia, weight loss, abortion and neurological signs if not treated. The disease can be controlled by diminazene aceturate treatment; further, diagnosis should be made based on epidemiological data, clinical signs, complete blood cell counts, microhematocrits, PCR, blood smears and histopathology. Also, serological surveys should be used to follow trypanosomosis in affected farms.

References

- Almeida, K.S., Freitas, F.L.C., Tebaldi, J.H., Alessi, A.C., Machado, R.Z., Nascimento, A.A., 2010. Alterações clínicas, histopatológicas e enzimáticas em ovinos infectados experimentalmente por *Trypanosoma vivax*. Ci. Anim. Bras. 11, 669–676.
- Anosa, V.O., 1983. Diseases produced by *Trypanosoma vivax* in ruminants, horses and rodents. Vet. Med. 30, 717–741.
- Batista, J.S., Riet-Correa, F., Barbosa, R.C., Guerra, J.L., 2006. Infecção experimental por *Trypanosoma vivax* em ovinos. Pesq. Vet. Bras. 26, 31–37.
- Batista, J.S., Riet-Correa, F., Teixeira, M.M.G., Madruga, C.R., Simões, S.V.D., Maia, F., 2007. Trypanosomosis by *Trypanosoma vivax* in cattle in the Brazilian semiarid: description of an outbreak and lesions in the nervous system. Vet. Parasitol. 143, 174–181.
- Batista, J.S., Bezerra, F.S., Lira, R.A., Carvalho, J.R.G., Neto, A.M.R., Petri, A.A., Teixeira, M.M.G., 2008. Aspectos clínicos, epidemiológicos e patológicos da infecção natural em bovinos por *Trypanosoma vivax* na Paraíba. Pesq. Vet. Bras. 28, 63–69.
- Batista, J.S., Oliveira, A.F., Rodrigues, C.M.F., Damasceno, C.A.R., Oliveira, I.R.S., Alves, H.M., Paiva, E.S., Brito, P.D., Medeiros, J.M.F., Rodrigues, A.C., Teixeira, M.M.G., 2009. Infection by *Trypanosoma vivax* in goats and sheep in the Brazilian semiarid region: from acute disease outbreak to chronic cryptic infection. Vet. Parasitol. 165, 131–135.
- Cortez, A.P., Rodrigues, A.C., Garcia, H.A., Neves, L., Batista, J.S., Bengaly, Z., Paiva, F., Teixeira, M.M.G., 2009. Cathepsin L-like genes of *Trypanosoma vivax* from Africa and South America—characterisation, relationships and diagnostic implications. Mol. Cell. Probes 23, 44–51.
- Costa, V.M.M., Simões, S.V.D., Riet-Correa, F., 2009. Doenças parasitárias em ruminantes no semi-árido brasileiro. Pesq. Vet. Bras. 29, 563–568.
- Cuglović, D.A., Bartholomeu, D.C., Reis-Cunha, J.L., Carvalho, A.U., Ribeiro, M.F., 2010. Epidemiologic aspects of an outbreak of *Trypanosoma vivax* in a dairy cattle herd in Minas Gerais state, Brazil. Vet. Parasitol. 169, 320–326.
- Da Silva, A.S., Costa, M.M., Polenz, M.F., Polenz, C.H., Teixeira, M.M.G., Lopes, S.T.A., Monteiro, S.G., 2009. Primeiro registro de *Trypanosoma vivax* em bovinos no Estado do Rio Grande do Sul, Brasil. Ciênc. Rural 39, 2550–2554.
- Desquesnes, M., 2004. Livestock Trypanosomoses and Their Vectors in Latin America. CIRAD-EMVT Publication, OIE, Paris, p. 174. ISBN 92-9044-634-X <http://www.oie.int/doc/ged/D9818.PDF>.
- Guerra, R.M.S.N.C., Junior, A.B.F., Santos, H.P., Abreu-Silva, A.L., Santos, A.C.G., 2008. Biometry of *Trypanosoma vivax* found in a calf in the state of Maranhão, Brasil. Ciênc. Rural 38, 833–835.
- Losos, G.J., Ikede, B.O., 1972. Review of pathology of diseases in domestic and laboratory animals caused by *T. congolense*, *T. vivax*, *T. brucei*, *T. rhodediense* and *T. congolense*. Vet. Pathol. 9, 267–274.
- Paiva, F., Lemos, R.A.A., Nakato, L., Mori, A.E., Brum, K.B., Bernardo, K.C., 2000. *Trypanosoma vivax* em bovinos no Pantanal do Estado do Mato Grosso do Sul, Brasil: I Acompanhamento clínico, laboratorial e anatomopatológico de rebanhos infectados. Rev. Bras. Parasitol. Vet. 9, 135–141.
- Rodrigues, A., Figuera, R.A., Souza, T.M., Schild, A.L., Soares, M.P., Milano, J., Barros, C.S.L., 2005. Surtos de tripanossomíase por *Trypanosoma evansi* em equinos no Rio Grande do Sul: aspectos epidemiológicos, clínicos, hematológicos e patológicos. Pesq. Vet. Bras. 25, 239–249.
- Shaw, J.J., Lainson, R., 1972. *Trypanosoma vivax* in Brazil. Ann. Trop. Med. Parasitol. 66, 25–32.
- Silva, R.A.M.S., Silva, J.A., Schneider, R.C., Freitas, J., Mesquita, D., Mesquita, T., Ramirez, L., Dávila, A.M.R., Pereira, M.E.B., 1996. Outbreak of trypanosomosis due to *Trypanosoma vivax* (Ziemann, 1905) in bovines of the Pantanal, Brazil. Mem. Inst. Oswaldo Cruz 91, 561–562.
- Ventura, R.M., Paiva, F., Silva, R.A.M.S., Takeda, G.F., Buck, G.A., Teixeira, M.M.G., 2001. *Trypanosoma vivax*: characterization of the spliced-leader gene for a Brazilian stock and species-specific detection by PCR amplification of an intergenic space sequence. Exp. Parasitol. 99, 37–48.
- Whitelaw, D.D., Gardiner, P.R., Murray, M., 1988. Extravascular foci of *Trypanosoma vivax* in goats: the central nervous system and aqueous humor of the eye as potential sources of relapse infections after chemotherapy. Parasitology 97, 51–61.