PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 43:e07119, 2023.
DOI: 10.1590/1678-5150-PVB-7119

Original Article
Livestock Diseases

Experimental infection by *Trypanosoma vivax* in goats in the Brazilian semiarid: detection of *T. vivax* DNA in colostrum and assessment of lactogenic transmission[1]

Nicholas M. Bezerra[2], Tiago S. Teófilo[2]*, Hélio N. Araújo Júnior[2], Jardel B. Silva[2], Gabriela H.F. Moura[2], Kizzy M.F.M. Costa[2], Kaliane A.R. Paiva[2] and Jael S. Batista[2]

ABSTRACT.- Bezerra N.M., Teófilo T.S., Araújo Júnior H.N., Silva J.B, Moura G.H.F., Costa K.M.F.M., Paiva K.A.R. & Batista J.S. 2023.. **Experimental infection by *Trypanosoma vivax* in goats in the Brazilian semiarid: detection of *T. vivax* DNA in colostrum and assessment of lactogenic transmission.** *Pesquisa Veterinária Brasileira 43:e07119,* 2023. Departamento de Ciência Animal, Universidade Federal Rural do Semi-Árido. Av. Francisco Mota 572, Bairro Costa e Silva, Mossoró, RN 59625-900, Brazil. E-mail: tiago.teofilo@ufersa.edu.br

This study aimed to identify the presence of *Trypanosoma vivax* DNA in the colostrum of infected goats and to explore the possibility of transmission for neonates fed using colostrum collected from infected goats. We used twelve goats in the final third of gestation with an age of approximately 24 months. Six goats were inoculated intravenously with 0.5mL of blood containing approximately $1.25x10^5$ trypomastigotes of *T. vivax*, and six remained uninfected. The presence of *T. vivax* in colostrum was evaluated by Polymerase Chain Reaction (PCR). The possibility of *T. vivax* transmission by colostrum was assessed by feeding six neonates born of serologically negative goats using colostrum from infected goats. Peripheral blood from neonates was collected daily for thirty days to assess the *T. vivax* presence through the examination of Giemsa-stained smears of leukocyte layers with the buffy coat technique (BCT) and by PCR. The results of a direct examination of colostrum were negative, but PCR confirmed the presence of *T. vivax* DNA in all infected goats. Additionally, lactogenic transmission by colostrum was not demonstrated once both BCT and PCR of neonate peripheral blood were negative.

INDEX TERMS: Experimental infection, *Trypanosoma vivax*, goats, DNA, lactogenic transmission, PCR, colostrum, small ruminants, trypanosomosis, Brazil.

RESUMO.- [**Infecção experimental por *Trypanosoma vivax* em caprinos no semiárido brasileiro: detecção de DNA de *Trypanosoma vivax* no colostro e avaliação da transmissão lactogênica**.] Este estudo teve como objetivo identificar a presença de DNA de *Trypanosoma vivax* no colostro de cabras infectadas experimentalmente e verificar a possibilidade de transmissão para neonatos alimentados com colostro coletado de cabras infectadas. Foram utilizadas doze cabras no terço final de gestação com idade aproximada de 24 meses. Seis cabras foram inoculadas intravenosamente com 0,5mL de sangue contendo aproximadamente $1,25x10^5$ tripomastigotas de *T. vivax*, e seis permaneceram não infectadas. A presença de *T. vivax* no colostro foi avaliada por Reação em Cadeia da Polimerase (PCR). A possibilidade de transmissão de *T. vivax* pelo colostro foi avaliada através da alimentação de seis neonatos nascidos de cabras sorologicamente negativas com colostro de cabras infectadas. Foi coletado diariamente o sangue periférico dos neonatos, por trinta dias para avaliar a presença de *T. vivax* através do exame de esfregaços de camadas leucocitárias coradas por giemsa, pela técnica BCT e por PCR. Os resultados do exame direto do colostro foram negativos, mas a PCR confirmou a presença de DNA de *T. vivax* no colostro em todas as cabras infectadas. Além disso, a transmissão lactogênica pelo colostro não foi demonstrada, uma vez que tanto a BCT quanto a PCR do sangue periférico do neonato foram negativas.

TERMOS DE INDEXAÇÃO: Infecção experimental, *Trypanosoma vivax*, caprinos, DNA, transmissão lactogênica, PCR, colostro, pequenos ruminantes, tripanossomíase, Brasil.

[1] Received on August 12, 2022.
Accepted for publication on September 1, 2022.
[2] Departamento de Ciências Animais (DCAn), Centro de Ciências Agrárias, Universidade Federal Rural do Semi-Árido (UFERSA), Av. Francisco Mota 572, Bairro Costa e Silva, Mossoró, RN 59625-900, Brazil. *Corresponding author: tiago.teofilo@ufersa.edu.br

INTRODUCTION

Trypanosomosis is a disease caused by the pathogenic hemoparasite of African origin *Trypanosoma vivax*, which affects domestic and wild ruminants. Natural infections by *T. vivax* have already been described in several countries outside Africa, and currently, this trypanosome presents a wide geographic distribution, affecting herds in South and Central America. The disease causes serious economic losses, as the parasite causes reduced milk production, abortion, perinatal mortality, growth retardation, progressive weight loss, decreased fertility, infertility and death (Andrade Neto et al. 2019).

The main mode of transmission described for *T. vivax* in Africa is through the biological vector, the tsetse fly, which during hematophagia, inoculates the parasite along with saliva. In places outside Africa where there is no biological vector that transmits trypanosomosis, such as in Central and South American countries, *T. vivax* is transmitted mechanically by hematophagous insects, e.g., Tabanidae and Stomoxydae (Bastos et al. 2020, Dyonisio et al. 2020). Besides transmission by hematophagous Diptera, the mechanical transmission of trypanosomes may also occur iatrogenically through the misuse of needles contaminated with infected blood in several animals during the application of drugs or vaccinations (Schmith et al. 2020).

Despite *T. vivax* being known in Latin America since 1919 (Leger & Vienne 1919), many doubts remain about its transmission routes. In the semiarid region of the Brazilian Northeast, *T. vivax* transplacental transmission and perinatal infection seem to be epidemiologically relevant. Despite the high temperatures and low air humidity in the dry season not favoring the development of the vectors responsible for trypanosomosis transmission, a high trypanosomosis prevalence is reported with high mortality and lethality rates in the calves and neonates born of cows infected by *T. vivax* in the same dry season (Batista et al. 2012). Therefore, to confirm the transplacental transmission of *T. vivax*, a study using experimentally infected pregnant ewes was conducted (Silva et al. 2013).

The sexual transmission of *T. vivax* has not yet been proven. However, Bezerra et al. (2008) identified, through the PCR technique, the presence of *T. vivax* DNA in the testicles of experimentally infected sheep. Bezerra et al. (2018) demonstrated the presence of *T. vivax* DNA in the semen of infected goats, strengthening the hypothesis of sexual transmission of the parasite.

Previous studies have demonstrated that some trypanosomatids (*Trypanosoma cruzi* and *Trypanosoma evansi*) are transmitted by colostrum and milk (Amato Neto et al. 1992, Jörg 1992, Dias et al. 2011, Campigotto et al. 2015). However, there is no description of *T. vivax* in the colostrum of infected animals or lactogenic transmission. Only one study has demonstrated the possibility of *T. vivax* oral transmission, where blood with *T. vivax* was administered orally to nine goats, which became infected and died (Diaz-Ungria 1971). There is thus an urgent need to elucidate the possibility of ruminant neonates contracting trypanosomosis by the lactogenic route. In this paper, our group aimed to clarify whether *T. vivax* is present in the colostrum of experimentally infected goats and the possibility of *T. vivax* transmission when neonates are fed with colostrum from infected goats.

MATERIALS AND METHODS

Experimental groups. Twelve pregnant Saanen goats (in the final third of gestation), housed in individual enclosures, were used to conduct this experiment. The gestational age was determined through ultrasonographical exams. We used an ultrasound GE Logiq Pro 100 (GE Medical System, Wisconsin, USA) connected to a 3.5MHz abdominal transducer for diagnosis. The stage of pregnancy was estimated by the cephalo-coccygeal length of fetuses, and goats selected for the experiment were, on average 120th days of gestation.

Approximately 15 days before the inoculation of *Trypanosoma vivax*, all animals were submitted to clinical, hematological and parasitological examination followed by the detection of anti-IgG antibodies to *T. vivax* by indirect immunofluorescence assay (IFA) per the methodology developed by Silva et al. (2002). Clinically healthy and serology-negative goats for *T. vivax* were randomly distributed into two groups: an infected group composed of six goats experimentally infected with *T. vivax* (Goats from 1 to 6) and a control group composed of six non-infected goats (Goats from 7 to 12). All the animals were submitted to identical management conditions, fed with water and Tifton (*Cynodon* spp.) *ad libitum*, and supplemented with commercial concentrate (1.5 % BW/day/goat) and a source of salt and minerals.

This study was conducted under the approval of the Ethics Committee on Animal Use (CEUA) of the "Universidade Federal Rural do Semi-Árido" (UFERSA) - number 41/2011. All procedures performed on the animals were carried out strictly according to the standards of the "Colégio Brasileiro de Experimentação Animal" (Brazilian School of Animal Experimentation – COBEA) and the National Institute of Health Guide for Care and Use of Laboratory Animals.

Inoculum and experimental infection of goats. *T. vivax* strain used to infect experimentally pregnant goats was derived from a natural outbreak in cows described in Catolé do Rocha city, Brazilian Northeast. The strain isolates were frozen in liquid nitrogen at -196°C. Each animal of the infected group was inoculated intravenously with 0.5mL of blood containing approximately $1.25x10^5$ trypomastigotes, a method previously described by Batista et al. (2007).

Clinical exams, hematological and *T. vivax* diagnosis. The animals of the infected and control groups were submitted to a rigorous clinical examination, in which the rectal temperature, respiratory rate, cardiac auscultation, mucosal exams, lymph nodes exams and degree of dehydration were verified.

Hemograms were performed on all animals one day before infection and every five days after infection for 30 days. For analysis of the hemograms, 2.0mL of blood was obtained by puncturing the jugular. The samples were packed in sterile tubes containing 1mg/mL EDTA, and the erythrogram and leukogram were performed according to the methodology described Pimentel et al. (2012).

The parasitemia was evaluated daily by searching for trypanosomes in the collected blood using a slide and coverslip under microscopy, as reported previously by Batista et al. (2007).

Colostrum collection and detection of *T. vivax*. Goats from the infected group had their colostrum collected between 12h00, 24h00 and 48h00 after the delivery. Colostrum was stored in sterile containers labeled with the animal's identification. Microhematocrit tubes were filled with the colostrum of each goat and submitted to centrifugation at 10,000rpm for five minutes. A drop of the interface between cream and whey was deposited on a glass slide and then observed under an optical microscope with a magnification of 100x.

For *T. vivax* DNA research, a sample of 1mL of colostrum from each animal was collected, mixed with 1 mL of alcohol at 99 % and stored

in sterile microtubes until PCR. For DNA extraction, a commercial kit was used (Qiagen DNeasy blood and tissue), according to the manufacturer's recommendations, and a highly sensitive PCR assay specific for *T. vivax* (TviCatL-PCR) was performed, as standardized by Cortez et al. (2009). This method targets repeated gene sequences encoding cysteine proteases (Cathepsin L) and was carried out using the oligonucleotides Tvi2 (forward: 5'GCCATCGCCAAGTACGCCCTCAG3') and DTO156 (reverse 5'TTAGAATTCCCAGGAGTTCTTGATGATCCAGTA3'). Through the amplification of a DNA fragment containing 177bp, PCR was used to confirm the diagnosis. *T. vivax* isolates from a natural outbreak in cows in the Pantanal Region of Brazil were used as a positive control. As a negative control, blood samples from uninfected goats were used.

Aliquots of 25µl of PCR products were submitted to electrophoresis on a 2% agarose gel and stained with ethidium bromide, and the image was captured by a documentation system under ultraviolet light. For the molecular-weight size marker, a 100bp DNA ladder was used (Thermo Scientific®, USA).

Clinical changes in newborns and lactogenic transmission of *T. vivax.* Six neonates born of the goats from the control group were proven serologically negative for anti-*T. vivax* antibodies by IFA were used for this experiment. Only neonates delivered to negative goats were chosen to avoid contamination of the fetus by the transplacental route. The newborns were fed 48 hours after birth, with the help of bottles containing colostrum obtained from goats of the infected group. Each newborn received colostrum from the same goat. Colostrum was administered eight times a day to neonates at a quantity of 20% BW, with an interval of three hours between the administrations. After being fed with colostrum, the neonates began receiving milk replacers.

The neonates were submitted daily for thirty days to clinical and hematological exams. Five *milliliters* (5mL) of blood were collected from each animal by a jugular puncture to diagnose *T. vivax* infection. The blood samples were used to make smears of the buffy coat by the buffy coat technique (BCT), stained with Giemsa and analyzed under an optical microscope with 100x magnification. To confirm the diagnosis of *T. vivax* infection in neonates, PCR was performed on blood samples collected on the 30th day after colostrum ingestion.

RESULTS

Clinical examination and parasitemia evaluation

From the 15th dpi, three goats in the infected group showed a reduction in the number of red blood cells, a reduction in the hematocrit value, and the hemoglobin levels, besides leukopenia with lymphocytosis. The hematological parameters of the control group remained within the reference values for the species during the experimental period.

All animals from the infected group had *T. vivax* trypomastigotes in the blood from the 4th day post-infection (DPI) until the end of the experiment. Parasitemia increased progressively, achieving peaks at the 13th and 22nd DPI. After that, the average values of parasitemia presented a slight decrease, despite remaining high during the entire experiment. Throughout the experimental period, the infected goats showed intermittent fever, enlarged lymph nodes, reduced body condition score, pale mucous membranes and apathy. Five goats gave birth to goats, small and weak. The goats in the control group showed no clinical signs of trypanosomosis.

Diagnosis of *Trypanosoma vivax* in colostrum

T. vivax trypomastigotes were not detected upon direct examination of the colostrum under microscopy or with an analysis of the interface collected between the solid and liquid part after colostrum centrifugation.

The detection of *T. vivax* DNA in colostrum by PCR demonstrated an amplification of 177bp in the colostrum of five experimentally infected animals collected 12 hours after delivery. In the colostrum collected 24h after delivery, only the previously negative animal showed a positive result. As for the samples analyzed after 48 hours, they all turned out to be negative. No DNA amplification was detected in the colostrum from the control group (Fig.1).

Clinical changes in newborns and lactogenic transmission of *T. vivax*

No changes in hematological parameters were observed in newborns during the observation period. In the daily evaluation carried out during 30 days in the newborns fed with colostrum of infected goats, it was found that *T. vivax* was absent in the smears of the leukocyte layer stained with Giemsa. The PCR results on these animals' blood were also negative.

DISCUSSION

Vector transmission is considered the most epidemiologically relevant transmission route of trypanosomosis (Sánchez & Ramírez 2013). The climatic conditions of high temperature and low humidity that occur in the semiarid region of the Brazilian Northeast are unfavorable for the development of trypanosomosis vector insects, being considered an area of enzootic instability and, therefore, trypanosomosis often manifests as outbreaks with high morbidity and mortality rates. Other secondary routes of horizontal transmissions, such as lactogenic, have not yet been proven. Considering that some infectious agents can be transmitted from mother to child through colostrum, we raised the hypothesis of the possibility of lactogenic transmission of *Trypanosoma vivax*.

The clinical signs observed in the animals (parasitemia, reduction in the hematocrit value, reduction of the body condition score and pale mucous membranes) were quite characteristic and, therefore, similar to those reported in most research with *T. vivax*, in which they report that the infection is characterized by high parasitism, hyperthermia, and hematological changes evidenced mainly by anemia (Bezerra et al. 2008, Batista et al. 2012, Silva et al. 2013).

The visualization of the *T. vivax* parasite in the colostrum through optical microscopy was negative, even when submitted to previous centrifugation of the colostrum in microhematocrit tubes to concentrate the parasites. In this respect, studies have shown that when a negative, direct parasitological examination is insufficient to ensure the absence of parasites (Bastos et al. 2015, Bittar et al. 2015). Colostrum samples that showed negative results in the direct parasitological test, when submitted to the molecular test by PCR, tested positive. The results indicated that the molecular analysis showed greater reliability for the detection of *T. vivax* than those obtained in the analysis of colostrum. This test is more sensitive and detects minute amounts of *T. vivax* DNA (Ventura et al. 2001). This seems to be the first study to demonstrate the presence of the DNA of *T. vivax* in the colostrum.

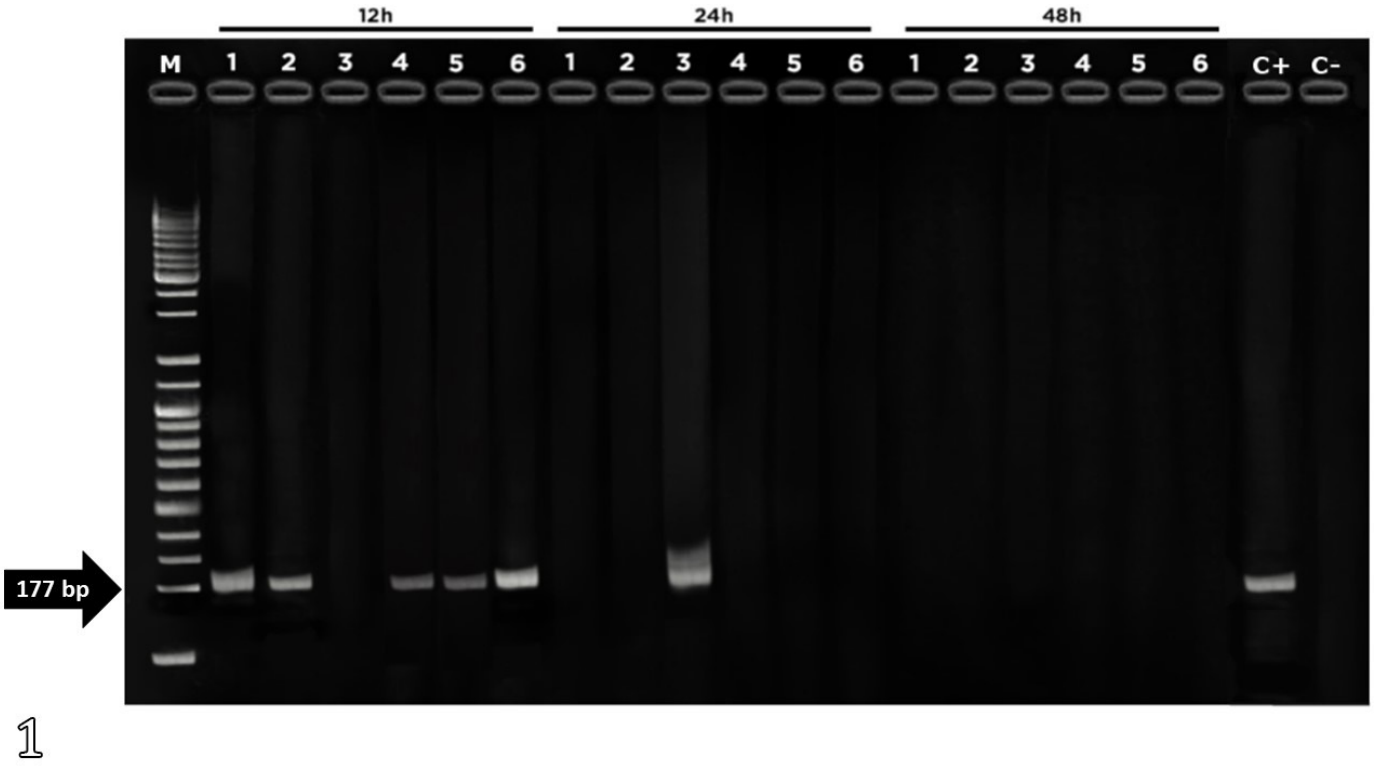

Fig.1. Polymerase Chain Reaction results for fragments of DNA with 177 bp, specific for *Trypanosoma vivax* (TviCatL-PCR), withdrawn from the Cathepsin L gene domain, in colostrum of contaminated goats, collected 12, 24 and 48 hours after birth. M = molecular marker, Numbers 1-6 = animal identification, C+ = positive control, C– = negative control.

It is an important discovery because it suggests the possibility of lactogenic transmission of the parasite. The presence of *T. vivax* DNA in the colostrum suggests that the protozoan can cross the maternal bloodstream to migrate through the connective tissue of the mammary gland to the alveolar lumen during the first hours of lactation. The presence of *T. vixax* DNA in colostrum corresponds to the period in which macromolecules, such as immunoglobulins, pass from maternal blood to this milk secretion. Thus, with the change from colostrum to milk during breastfeeding, the concentrations of these components are decreasing, which is why *T. vivax* DNA was not found in the colostrum after 24 hours.

In the present study, although the presence of *T. vivax* DNA in colostrum has been confirmed here by PCR, it was not possible to detect trypomastigotes in the blood of newborns through direct parasitological examinations or the presence of the parasite DNA by PCR. The non-transmission of *T. vivax* infection by colostrum to newborns may possibly be associated with the low number of trypomastigotes in this milk secretion. Although the experimental infection is the ideal model for observational studies of the possibility of transmission of an infectious agent, the amount of the *Trypanosoma inoculum* is crucial for the success of the infection, similar to that observed by Dias (2006). On the other hand, on some occasions, PCR can detect the presence of DNA and not the viability of an infectious agent and can amplify genetic material due to contamination with the host (Fikru et al. 2014). In addition, the absence of infection in newborns may have occurred due to the sensitivity of the trypomastigotes in colostrum to stomach acidity. According to Cortez et al. (2006), isolated from *T. cruzi* differ widely in their efficiency in infecting the gastric mucosa epithelium when administered orally to rats because host factors as components present in gastric juice can act on parasites, modulating their infectivity.

CONCLUSION

The *Trypanosoma vivax* DNA recorded for the first time in the colostrum of infected goats may suggest that the secretion of the mother's milk may be an alternative route of transmission of the parasite. Also, it indicates the need for further studies to define in which situations mother-colostrum-newborn transmission may occur, as well as the epidemiological importance of this transmission route in the dissemination and maintenance of the disease in herds.

Acknowledgements.- The authors thank the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq).

Conflict of Interest statement.- The authors declare no conflict of interests for this article.

REFERENCES

Amato Neto V., Matsubara L., Campos R., Moreira A.A., Pinto P.L., Faccioli R. & Zugaib M. 1992. *Trypanosoma cruzi* in the milk of women with chronic chagas disease. Revta Hosp. Clín. Fac. Med. São Paulo. 47(1):10-11. <PMid:1307397>

Andrade Neto A.Q., Mendonça C.L., Souto R.J.C., Sampaio P.H., Junior Fidelis O.L., André M.R., Machado R.Z. & Afonso J.A.B. 2019. Diagnostic, Clinical and Epidemiological aspects of dairy cows naturally infected by *Trypanosoma*

vivax in the states of Pernambuco and Alagoas, Brazil. Braz. J. Vet. Med. 41(1):e094319. <https://dx.doi.org/10.29374/2527-2179.bjvm094319>

Bastos T.S.A., Faria A.M., Couto L.F.M., Nicaretta J.E., Cavalcante A.S.A., Zapa D.M.B., Ferreira L.L., Heller L.M., Madrid D.M.C., Cruvinel L.B., Rossi G.A.M., Soares V.E., Cadioli F.A. & Lopes W.D.Z. 2020. Epidemiological and molecular identification of *Trypanosoma vivax* diagnosed in cattle during outbreaks in central Brazil. Parasitol. 147(12):1313-1319. <https://dx.doi.org/10.1017/S0031182020001006> <PMid:32624014>

Bastos T.S.A., Madrid D.M.C., Faria A.M., Bessa C.L., Souza M.A. & Linhares G.F.C. 2015. Detecção de *Trypanosoma vivax* por diferentes técnicas de diagnóstico parasitológico realizadas à campo. Ars Vet. 31(2):40. <https://dx.doi.org/10.15361/2175-0106.2015v31n2p40>

Batista J.S., Riet-Correa F., Teixeira M.M.G., Madruga C.R., Simões S.D.V. & Maia T.F. 2007. Trypanosomiasis by *Trypanosoma vivax* in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system. Vet. Parasitol. 143(2):174-181. <https://dx.doi.org/10.1016/j.vetpar.2006.08.017> <PMid:16965857>

Batista J.S., Rodrigues C.M.F., Olinda R.G., Silva T.M.F., Vale R.G., Câmara A.C.L., Rebouças R.E.S., Bezerra F.S.B., García H.A. & Teixeira M.M.G. 2012. Highly debilitating natural *Trypanosoma vivax* infections in Brazilian calves: epidemiology, pathology, and probable transplacental transmission. Parasitol. Res. 110(1):73-80. <https://dx.doi.org/10.1007/s00436-011-2452-y> <PMid:21626156>

Bezerra F.S.B., Garcia H.A., Alves H.M., Oliveira I.R.S., Silva A.E., Teixeira M.M.G. & Batista J.S. 2008. Trypanosoma vivax nos tecidos testicular e epididimário de ovinos experimentalmente infectados. Pesq. Vet. Bras. 28(12):575-582. <https://dx.doi.org/10.1590/S0100-736X2008001200002>

Bezerra N.M., Moura G.H.F., Araújo Jr H.N., Bezerra F.S.B., de Paiva K.A.R., Costa K.M.F.M., Costa W.P., Medeiros D.A.S. & Batista J.S. 2018. Detection of T*rypanosoma vivax* DNA in semen from experimentally infected goats. Vet. Res. Commun. 42(2):131-135. <https://dx.doi.org/10.1007/s11259-018-9715-3> <PMid:29464589>

Bittar J.F.F., Bassi P.B., Moura D.M., Garcia G.C., Martins-Filho O.A., Vasconcelos A.B., Costa-Silva M.F., Barbosa C.P., Araújo M.S.S. & Bittar E.R. 2015. Evaluation of parameters related to libido and semen quality in Zebu bulls naturally infected with *Trypanosoma vivax*. BMC Vet. Res. 11:261. <https://dx.doi.org/10.1186/s12917-015-0571-x> <PMid:26467756>

Campigotto G., Da Silva A.S., Volpato A., Balzan A., Radavelli W.M., Soldá N.M., Grosskopf H.M., Stefani L.M., Bianchi A.E., Monteiro S.G., Tonin A.A., Weiss P.H.E., Miletti L.C. & Lopes S.T.A. 2015. Experimental infection by *Trypanosoma evansi* in sheep: occurrence of transplacental transmission and mice infection by parasite presenting the colostrum and milk of infected ewes. Vet. Parasitol. 212(3/4):123-129. <https://dx.doi.org/10.1016/j.vetpar.2015.07.007> <PMid:26223153>

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. <https://dx.doi.org/10.1016/j.mcp.2008.11.003> <PMid:19063960>

Cortez M., Silva M.R., Neira I., Ferreira D., Sasso G.R.S., Luquetti A.O., Rassi A. & Yoshida N. 2006. *Trypanosoma cruzi* surface molecule gp90 down regulates invasion of gastric mucosal epithelium in orally infected mice. Microb. Infect. 8(1):36-44. <https://dx.doi.org/10.1016/j.micinf.2005.05.016> <PMid:16153873>

Dias J.C.P. 2006. Notas sobre o *Trypanosoma cruzi* e suas características bio-ecológicas, como agente de enfermidades transmitidas por alimentos. Revta Soc. Bras. Med. Trop. 39(4):370-375. <https://dx.doi.org/10.1590/S0037-86822006000400010>

Dias J.C.P., Amato Neto V. & Luna E.J.A. 2011. Mecanismos alternativos de transmissão do *Trypanosoma cruzi* no Brasil e sugestões para sua prevenção. Revta Soc. Bras. Med. Trop. 44(3):1–5. <https://dx.doi.org/10.1590/S0037-86822011005000032>

Diaz-Ungria C. 1971. Transmision bucal del *Trypanosoma vivax* en cabras, en condiciones experimentales. Bol. Soc. Venez. Cienc. Nat. 29:119-120.

Dyonisio G.H.S., Batista H.R., Silva R.E., Azevedo R.C.F., Costa J.O.J., Manhães I.B.O., Tonhosolo R., Gennari S.M., Minervino A.H.H. & Marcili A. 2020. Molecular diagnosis and prevalence of *Trypanosoma vivax* (Trypanosomatida: Trypanosomatidae) in buffaloes and ectoparasites in the Brazilian Amazon region. J. Med. Entomol. 58(1):403-407. <https://dx.doi.org/10.1093/jme/tjaa145> <PMid:32696974>

Fikru R., Hagos A., Roge S., Reyna-Bello A., Gonzatti M.I., Merga B., Goddeeris B.M. & Büscher P. 2014. A proline racemase based PCR for identification of *Trypanosoma vivax* in cattle blood. PLoS One 9(1):e84819. <https://dx.doi.org/10.1371/journal.pone.0084819>

Jörg M.E. 1992. The transmission of *Trypanosoma cruzi* via human milk. Revta Soc. Bras. Med. Trop. 25(1):83. <https://dx.doi.org/10.1590/s0037-86821992000100014> <PMid:1308072>

Leger M. & Vienne M. 1919. Epizootie a trypanosomes chez lês bovines de la Guyane Française. Bull. Soc. Pathol. Exot. 12:258-266.

Pimentel D.S., Ramos C.A.N., Ramos R.A.N., Araújo F.R., Borba M.L., Faustino M.A.G. & Alves L.C. 2012. First report and molecular characterization of *Trypanosoma vivax* in cattle from state of Pernambuco, Brazil. Vet. Parasitol. 185(2/4):286-289. <https://dx.doi.org/10.1016/j.vetpar.2011.10.019> <PMid:22054681>

Sánchez L.V. & Ramírez J.D. 2013. Congenital and oral transmission of American trypanosomiasis: an overview of physiopathogenic aspects. Parasitology 140(2):147-159. <https://dx.doi.org/10.1017/S0031182012001394> <PMid:23010131>

Schmith R., Moreira W.S., Vassoler J.M., Vassoler J.C., Souza J.E.G.B., Faria A.G.A., Almeida L.C., Teixeira M.C. & Marcolongo-Pereira C. 2020. *Trypanosoma vivax* epizootic infection in cattle from Espírito Santo State, Brazil. Adv. Anim. Vet. Sci. 8(6):629-632. <https://dx.doi.org/10.17582/journal.aavs/2020/8.6.629.632>

Silva R.A.M.S., Seidl A., Ramirez L. & Dávila A.M.R. 2002. *Trypanosoma evansi* e *Trypanosoma vivax*: biologia, diagnóstico e controle. 1ª ed. Embrapa Pantanal, Corumbá, MS. 141p.

Silva T.M.F., Olinda R.G., Rodrigues C.M.F., Câmara A.C.L., Lopes F.C., Coelho W.A.C., Ribeiro M.F.B., Freitas C.I.A., Teixeira M.M.G. & Batista J.S. 2013. Pathogenesis of reproductive failure induced by *Trypanosoma vivax* in experimentally infected pregnant ewes. Vet. Res. 44:1-9. <https://dx.doi.org/10.1186/1297-9716-44-1>

Ventura R.M., Paiva F., Silva R.A., Takeda G.F., Buck G.A. & Teixeira M.M. 2001. *Trypanosoma vivax*: characterization of the spliced-leader gene of a Brazilian stock and species-specific detection by PCR amplification of an intergenic spacer sequence. Exp. Parasitol. 99(1):37-48. <https://dx.doi.org/10.1006/expr.2001.4641> <PMid:11708832>