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Soroprevalência de anticorpos anti- *Trypanosoma vivax* em bovinos em Tapira-MG, Brasil*

Serum prevalence of anti- *Trypanosoma vivax* antibodies in cattle in Tapira-MG, Brazil

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Resumo

O *Trypanosoma vivax* é considerado o mais importante trypanosoma patogênico para bovinos e causa grandes prejuízos na pecuária de corte e leite . Este estudo teve como objetivo avaliar a prevalência anticorpos de anti-*Trypanosoma vivax* em bovinos leiteiros do município de Tapira, localizado na região do Alto Paranaíba, Minas Gerais, Brasil. As 74 amostras de soro sanguíneo de bovinos leiteiros foram analisadas por meio de reação de imunofluorescência indireta. A soroprevalência foi de 82,4% (61/74), que pode estar relacionada ao trânsito de animais não testados, presença de vetores e compartilhamento de agulhas pelos proprietários. Os dados permitiram definir Tapira como uma área de expansão das infecções epizoóticas por *Trypanosoma vivax* no estado de Minas Gerais.

Palavras-chave: *Trypanosoma vivax*, imunofluorescência, Tapira, bovinos.

Abstract

Trypanosoma vivax is considered the most important pathogenic *Trypanosoma* for cattle and causes great damage to the dairy and beef cattle industries. This study aimed to evaluate the prevalence of anti-*T. vivax* antibodies in dairy cattle from the municipality of Tapira, located in the Alto Paranaíba region, Minas Gerais, Brazil. The 74 blood serum samples from dairy cattle were analyzed using an indirect immunofluorescence reaction. The seroprevalence was 82.4 % (61/74), and the highest incidence observed can be correlated with the transit of untested animals, the presence of vectors, and needle sharing by owners. The data allowed defining Tapira as an area of expansion of *T. vivax* epizootic infections in the state of Minas Gerais.

Keywords: *Trypanosoma vivax*, immunofluorescence, Tapira, cattle.

Introduction

Trypanosoma vivax (*T. vivax*) is considered the most important bovine trypanosome in Sub-Saharan Africa, where it is mainly transmitted by tsetse flies (Diptera: Glossinidae) (Dagnachew and Bezie, 2015). The adaptation to mechanical transmission by hematophagous insects such as *Tabanids* sp. and *Stomoxys* spp. allowed the expansion of *T. vivax* to Central America, South America, and the Caribbean (Jones and Dávila, 2001). Trypanosomosis has played an important role by causing acute anemia, weight loss, decreased milk production, and other clinical signs that may lead to cattle death (Osório et al., 2008; Dagnachew and Bezie, 2015).

In Brazil, the first report of *T. vivax* occurred in the 1970s in the state of Pará in buffaloes (Boulhosa, 1946, apud Shaw and Lainson, 1972). Since then, the disease has spread, and cases have been reported in the North (Guedes-Junior et al., 2008), Northeast (Batista et al., 2007, Sousa et al., 2021), Midwest (Paiva et al., 2000), South (Silva et al., 2009), and Southeast (Cadioli et al., 2012) regions. In the state of Minas Gerais (Southeast Brazil), the occurrence of the parasite was first described in Igarapé (Carvalho et al., 2008), with expansion to other areas of the state (Reis et al., 2019).

Trypanosoma vivax infections may be diagnosed by parasitological, immunological, and molecular methods. Both indirect immunofluorescence (IFA) and indirect ELISA are useful techniques for epidemiological investigations, especially for the determination of *T. vivax* distribution (Osório et al., 2008).

In this context, this study aimed to evaluate the prevalence of anti-*T. vivax* antibodies by indirect immunofluorescence in dairy cattle from the municipality of Tapira, Minas Gerais, Brazil.

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Material and methods

The municipality of Tapira (19° 55′ 9″ S, 46° 49′ 22″ W) is located in the Alto Paranaíba region, Minas Gerais, with a total area of 1,180.229 km² and a population of 4,102 inhabitants (IBGE, 2010).

Serum blood samples (n = 74) were analyzed from the serum bank kindly provided by the Municipal Department of Agriculture and Environment of Tapira- MG. Samples came from herds in the municipality participating in an agricultural exhibition. These samples were collected for diagnosing bovine brucellosis as a standard procedure of the city. The samples were taken from dairy cattle from the Girolando breed raised in an extensive system and aged over twenty-four months. The animals evaluated in the present study did not present clinical symptoms and were considered asymptomatic. The IFA test (IFAT) were performed by the Clinical Pathology Laboratory, located at the Veterinary Hospital of Uberaba (HVU).

Obtaining T. vivax and preparing the slides

Two *T. vivax* samples stored in liquid nitrogen at -196 ºC were used. These samples were thawed in a water bath at 37 ºC, and an aliquot (4 mL) was inoculated subcutaneously in a healthy splenectomized sheep from the Santa Inês breed, aged eight months and isolated in a stall with an anti-mosquito screen. Six tubes of blood with EDTA from the sheep were collected at the parasitemia peak of 63 parasites per field (40x objective) after twelve days of antigen inoculation. Blood samples were centrifuged for 5 min (2000 g) in a Celm® centrifuge. After centrifugation, all the leukocytes were removed, and the samples were transferred to a 50 ml centrifuge tube washed twice in 0.9 % NaCl. Thick blood smear slides were prepared with 30 µl of blood and fixed with cold acetone for 5 min. The smears were packed with paper towels, wrapped in aluminum foil, and frozen at -20 ºC.

Immunofluorescence test

Initially, the slides with fixed antigens were removed from the freezer and dried at room temperature. The positive and negative control sera were diluted in PBS at 1:80 dilutions, and bovine serum samples were diluted in PBS at 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280, with 20 µl being distributed on slides containing *T. vivax* antigens. The slides were incubated for 30 min in a humid chamber at 37 ºC and then washed with PBS for 5 min each. The FITC-labeled anti-bovine conjugate was added at a 1:300 dilution (Sigma®). The slides were incubated for another 30 min and washed with PBS as previously described. After drying the slides, the readings were performed using a Nikon® epifluorescence microscope.

The samples were considered reagent (positive) at a titration of 1:80 according to the methodology described by Cuglovici et al. (2010). The positive control was from a bovine from the Dutch breed, aged over 24 months, and inoculated with 4 mL of blood containing *T. vivax.* The inoculations were made every 21 days until completing three applications, and the titer for the control antibody was 1:80. For the negative control, bovine serum negative for *T. vivax* was used in the same dilution.

Results

Of the 74 samples tested, 82.4 % (61/74) were positive for *T. vivax* (Table 1).

Table 1: Absolute Frequency (FA) and Relative Frequency (RF) of samples that are reactive to anti-*Trypanosoma vivax* by the indirect immunofluorescence test in different titers.

Degrees	Positives		Negatives	
	FA	FR%	FA	FR%
1:80	20/74	27.0		
1:160	18/74	24.3		
1:320	9/74	12.2	13/74	17.6
1:640	2/74	2.7		
1:1.280	12/74	16.2		

Discussion

Serological diagnostic methods such as IFAT can show if the animals are positive even if they are negative according to parasitological tests or are in the chronic or subclinical phases of the disease. The chronic phase of the disease is associated with low and fluctuating parasitemia, and the serological study is important to complement the parasitological test since the animals can control the parasitemia after exposure to the parasite (Cuglovici et al., 2010). This may be linked to the immune response capacity of some animals (innate or acquired resistance) or even to the low virulence of the *T. vivax* isolate (Uzoigwe, 1986). The IFAT test has high sensitivity and genus specificity, but the species specificity is generally low. At present, it can only be used for the presumptive diagnosis of trypanosomosis. Antibodies persist on average three to four months after curative treatment or self-cure but may last up to thirteen months (OIE, 2018). When possible, it is interesting to combine several diagnostic techniques in order to decrease the frequency of false negative results and contribute to better disease control (Fidelis- Junior et al, 2019).

Of the 74 animals, 82.4 % had anti-*T. vivax* antibodies, thus being in the chronic or subclinical phase of the disease. This prevalence is higher than that described by Cuglovici et al. (2010) when clinical cases of the disease began in Igarapé, MG, in 2007, and a gradual increase in the prevalence of antibody titers in the same municipality has occurred.

Higher prevalences were described in the northern region of the state of Pará (83 % to 96.7 %) (Guedes-Júnior et al., 2008) and in Pernambuco (100 %) (Pimentel et al., 2012). In the northern region, Linhares et al. (2006) reported a lower prevalence (1.2 %) in cattle coming from the state of São Paulo that could be negative or in the subclinical or chronic phases of the disease since they came from areas free from the disease. According to Linhares et al. (2006), animals submitted to this epidemiological situation would be at risk of presenting the clinical disease as they are not immune to it and are entering an enzootic area.

The high seroprevalence described in Tapira may be related to three main factors: the transit of untested animals, the presence and increase of vectors, and the shared use of needles

(Nascimento, personal communication). The transit of animals untested for *T. vivax* is constant in agricultural exposures. Despite being described in several states in Brazil, trypanosomosis caused by *T. vivax* is still a neglected disease. In this way, animal circulation between areas of different epidemiological conditions may be a reservoir for transmission (Batista et al., 2007; Pimentel et al., 2012). With the increase in the number of mechanical vectors such as *Tabanids* sp. and *Stomoxys* sp. (Batista et al., 2007; Batista et al., 2008), exposures are stimulated by the high concentration of animals. Also, after the animals return to their properties, it is already established that the shared use of needles, mainly in the injection of oxytocin, is also a factor of the increase in the number of cases (Bastos et al., 2017), with a similar reason having been proposed by Carvalho et al. (2008) for an outbreak reported in Igarapé, MG.The study carried out had important limitations regarding its sampling, considering the small number of animals evaluated. The high prevalence observed demonstrates that *T.vivax* is well distributed in the region. The intense traffic of animals and the agglomerations carried out in livestock events becomes worrying with the spread of the disease to other regions, where the parasite is not found.

Conclusion

The shared use of syringes for the application of oxytocin, the absence of vector control and the non-testing of animals at agricultural fairs are attitudes that contribute to the increase in cases of *T. vivax* trypanosomosis.

References

BASTOS, T. S. A.; FARIA, A.M.; MADRID, D.M.C.; BESSA, L. C.; LINHARES, G. F. C.; JUNIOR, O. L. F.; SAMPAIO, P.H.; CRUZ, B.C.; CRUVINEL, L. B.; NICARETTA,J. E.; MACHADO, R.Z.; COSTA, A.J.; LOPES, W.D.Z. First outbreak and subsequent cases of *Trypanosoma vivax* in the state of Goiás, Brazil. *Revista Brasileira de Parasitologia Veteterinária*, v. 26, n 3, p. 366-371, 2017.

BATISTA, J.S.; BEZERRA, F.S.B.; LIRA, R.A.; CARVALHO , J. R. G. et. al. Aspectos clínicos, epidemiológicos e patológicos da infecção natural em bovinos por *Trypanosoma vivax* na Paraíba**.** *Pesquisa Veterinária Brasileira*, v. 28, n. 1, p. 63-69, 2008.

BATISTA, J.S.; RIET-CORREA, F.; TEIXEIRA, M.M.G.; MADRUGA, C.R; SIMÕES,S. D. V.; MAIA, T. F. Trypanosomiasis by *Trypanosoma vivax* in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system. *Veterinary Parasitology*, v.143, p.174-181, 2007.

CADIOLI, F.A.; BARNABÉ, P.A.; MACHADO, R. Z.; TEIXEIRA, M. C. A.; ANDRÉ, M.R.; SAMPAIO,O. L. F.; TEIVEIRA, M. M. G.; MARQUES, L. C. First report of *Trypanosoma vivax* outbreak in dairy cattle in São Paulo state, Brazil. *Revista Brasileira de Parasitologia Veterinária*, v.21, n.2, p.118-124, 2012.

CARVALHO, A.U.; ABRÃO, D.C.; FACURY FILHO, E.J.; PAES, P. R. O.; RIBEIRO, M. F. B. Ocorrência de *Trypanosoma vivax* no estado de Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* v. 60, n. 3, p. 769-771, 2008.

CUGLOVICI, D. A., BARTHOLOMEU, D.C.; REIS-CUNHA, J.L. CARVALHO, A.U.; RIBEIRO, M. F. B. Epidemiologic aspects of an outbreak of *Trypanosoma vivax* in a dairy cattle herd in Minas Gerais state, Brazil. *Veterinary Parasitology*, v. 69, n. 3-4, p. 320-326. 2010.

DAGNACHEW, S.; BEZIE, M. Review on *Trypanosoma vivax*. *African Journal of Basic & Applied Sciences* v. 7, n. 1, p. 41-64, 2015.

FIDELIS JUNIOR, O. L.: SAMPAIO, P. H.; GONÇALVES, L. R.; ANDRÉ, M. R.; MACHADO, R. Z.; WIJFFELS, G.; CADIOLI, F.A. Comparison of conventional and molecular techniques for *Trypanosoma vivax* diagnosis in experimentally infected cattle. *Revista Brasileira de Parasitologia Veterinária*,v. 28, n.2, p. 203-209, 2019.

GUEDES JUNIOR, D.S; ARAÚJO, F.R.; SILVA, F.J.M.; RANGEL, C. P.; NETO, J. D. B.; FONSECA, A. Frequency of antibodies to *Babesia bigemina, B. bovis, Anaplasma marginale, Trypanosoma vivax* and *Borrelia burdgorfer*in cattle from the northeastern region of the state of Pará, Brazil. *Revista Brasileira de Parasitologia,* v.08, n.17, p.105-109, 2008.

JONES. T.W.; DÁVILA, A. M. *Trypanosoma vivax*-out of Africa. *Trends in Parasitology* v. 17, n. 2, p.99-101, 2001.

LINHARES, G.F.C.; DIAS FILHO, F.C.; FERNANDES, P.R.; DUARTE S.C. Tripanossomíase em bovinos no município de formoso do Araguaia, Tocantins (relato de caso). *Ciência Animal Brasileira,* v.7, n.4, p.455-460, 2006.

OSÓRIO, A.L.; MADRUGA, C. R.; DESQUESNES, M., SOARES, C. O.; RIBEIRO, L. R. L.; COSTA, S. C. G. *Trypanosoma* (Duttonella) *vivax:* its biology, epidemiology, pathogenesis, and introduction in the New World-a review. *Mem*órias do *Instituto Oswaldo Cruz*. v. 103, n. 1, p. 1-13, 2008.

PAIVA, F.; LEMOS, R.A.A.; NAKAZATO, L.; MORI, A.E.; BRUM, K.B.; BERNARDO, K.C. *Trypanosoma vivax* em bovinos no pantanal do estado do Mato Grosso do Sul, Brasil: I – acompanhamento clínico, laboratorial e anatomopatológico de rebanhos infectados. *Revista Brasileira de Parasitologia Veterinária*, v.9, n.2, p.135-141. 2000.

PIMENTEL, D. S.; RAMOS, C. A. N.; RAMOS, R. A. N.; Araújo, F. R,; Borbac, M. L.; Faustino, M. A; G.; Alves. L. C. First report and molecular characterization of *Trypanosoma vivax* in cattle from state of Pernambuco, Brazil. *Veterinary Parasitology*, n.185, p.286-289, 2012.

REIS, M.O.; SOUZA, F. R.; ALBUQUERQUE, A. S., MONTEIRO, F.; OLIVEIRA, L. F. D.S,; RAYMUNDO, D. L.; WOUTERS, F.; WOUTERS, A. T. B.; PECONICK, A. P.; VARASCHIN, M. S. Epizootic Infection by *Trypanosoma vivax* in cattle from the state of Minas Gerais, Brazil. *Korean Journal of Parasitology*. v. 57, n.2, p. 191-195, 2019.

SHAW, J.J.; LAINSON, R. *Trypanosoma vivax* in Brazil. *Annals of Tropical Medicine Parasitology*, v.66, n.1, p.25-33, 1972

SILVA, A. S.; COSTA, M. M.; POLENZ, M.; POLENZ, C. H.; TEIXEIRA, M. M. G. LOPES, S. T. A.; MONTEIRO, S.G. Primeiro registro de *Trypanosoma vivax* em bovinos no Estado do Rio Grande do Sul, Brasil. *Ciência Rural*, v. 39, n. 8, p. 2550-2554, 2009.

SOUSA, F.G.; Souza, R.C.; Andrade, L. S. New Detection of the occurrence of Trypanosomiasis in a bovine herd in a rural property in the state of Bahia/BA- case report. *Revista Brasileira de Ciências Veterinárias*, v. 28 n. 3 p. 138-141, 2021.

UZOIGWE, N. R. Self-cure in Zebu calves experimentally infected with *Trypanosoma vivax*. *Veterinary Parasitology*, v.22, p.141-146, 1986.

World Organisation for Animal Health - *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* Chapter 2.4.18. OIE, Paris, 2008.