Revista Brasileira de Buiatria

EVALUATION OF THE IN VITRO INFECTION OF *TRYPANOSOMA THEILERI* AND *TRYPANOSOMA THEILERI-LIKE* IN A MONOLAYER OF EMBRYONIC CELLS OF *RHIPICEPHALUS MICROPLUS* (CANESTRINI) (ACARI: IXODIDAE)

AVALIAÇÃO DA INFECÇÃO IN VITRO DE *TRYPANOSOMA THEILERI* E *TRYPANOSOMA THEILERI-LIKE* EM MONOCAMADA DE CÉLULAS EMBRIONÁRIAS DE *RHIPICEPHALUS MICROPLUS* (CANESTRINI) (ACARI:IXODIDAE)

Ingridi Braz de Oliveira Manhães[1], Renata Tonhosolo[2], Leidiane Lima-Duarte[3,4], Jaciara de Oliveira Jorge Costa[4], Ryan Emiliano da Silva[2], Pablo Henrique Nunes[5], Fernanda Aparecida Nieri-Bastos[4], Thamiris Nascimento dos Santos[1], Darci Moraes Barros-Battesti[4,6] and Arlei Marcili[1]

1 Programa de Medicina Veterinária e Bem-estar animal e Saúde Única, Universidade Santo Amaro (UNISA), São Paulo, SP, Brasil.

2 Faculdade de Medicina, Universidade Santo Amaro (UNISA), São Paulo, SP, Brasil.

3 Centro de Desenvolvimento Experimental para Medicina e Biologia, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brasil.

4 Laboratório de Doenças Parasitárias, Departamento de Medicina Veterinária Preventiva e Saúde Animal (VPS), Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade de São Paulo (USP), São Paulo, SP, Brasil.

5 Instituto Latino-Americano de Ciências da Vida e da Natureza (ILACVN), Universidade Federal da Integração Latino-Americana (UNILA), Foz do Iguaçu, PR, Brasil.

6 Laboratório de Imunoparasitologia, Departamento de Patologia, Reprodução e Saúde Única (DPRSU), Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Jaboticabal, SP, Brasil.

Autor para correspondência: **amarcili@prof.unisa.br**

Revista Brasileira de Buiatria
Volume 1, Número 2, p. 1-11, 2026

Publicado em 02 de agosto de 2026

ISSN 2763-955X

DOI: 10.70061/2763-955X.2026.05

Associação Brasileira de Buiatria

ABSTRACT

The genus *Trypanosoma* infects a wide range of domestic and wild animals, with *Trypanosoma theileri* being a highly prevalent parasite in cattle worldwide. Members of the subgenus *Megatrypanum* are typically host-specific, infecting ungulates and ruminants. Experimental studies show that *T. theileri* from cattle does not infect sheep, indicating a lack of cross-species transmission. In buffaloes (*Bubalus bubalis*), distinct *T. theileri*-like lineages occur, with no evidence of cross-infection between cattle and buffalo parasites. Tabanids and hippoboscids are known as natural vectors of this disease. *Rhipicephalus microplus* is a monoxenous tick species that has cattle as its main host. We evaluated the growth and differentiation of *T. theileri* and *T. theileri*-like in a monolayer of embryonic cells (RBME-6) from *R. microplus*. The amastigote forms were evaluated by fluorescence microscopy and confocal microscopy with DAPI and Phalloidin staining. The cultures of *T. theileri* and *T. theileri*-like trypanosomes in the tick cell line RBME-6 were successful. *Trypanosoma theileri* and *T. theileri*-like trypanosomes invaded and remained active in the cell culture. The results of this study may be used as a tool to evaluate the adaptability of the invertebrate host and to provide information on the parasite-host relationship. Our results may facilitate the use of *R. microplus* embryonic cells as an *in vitro* model for the cultivation of trypanosomatids, as this represents a fast, low-cost method.

Keywords: Bovine trypanosomatid, experimental infection, monoxenous tick, tick cell line.

RESUMO

O gênero *Trypanosoma* infecta uma ampla variedade de animais domésticos e silvestres, sendo *Trypanosoma theileri* um parasita altamente prevalente em bovinos em todo o mundo. Membros do subgênero *Megatrypanum* são tipicamente específicos de hospedeiro, infectando ungulados e ruminantes. Estudos experimentais demonstram que *T. theileri* proveniente de bovinos não infecta ovinos, o que indica a ausência de transmissão entre as espécies. Em búfalos (*Bubalus bubalis*), ocorrem linhagens distintas semelhantes a *T. theileri*, sem evidências de infecção cruzada entre parasitas de bovinos e de búfalos. Tabanídeos e hipoboscídeos são conhecidos como vetores naturais desta doença. *Rhipicephalus microplus* é uma espécie de carrapato monoxeno, com o bovino como principal hospedeiro. Avaliamos o crescimento e diferenciação dos tripanossomas *T. theileri* e *T. theileri*-like em monocamada de células embrionárias (linhagem RBME-6) de *R. microplus*. As formas amastigotas foram avaliadas por microscopia de fluorescência e por microscopia confocal de transmissão com coloração DAPI e Faloidina. As culturas de tripanossomas *T. theileri*-like e *T. theileri* na linhagem celular de carrapatos RBME-6 foram bem-sucedidas. *Trypanosoma theileri* e *T. theileri*-like invadiram e permaneceram ativos na cultura celular. O resultado deste estudo poderá ser utilizado como ferramenta para avaliar a adaptabilidade ao hospedeiro invertebrado, bem como para apresentar informações sobre a relação parasita-hospedeiro. Nossos resultados propiciarão principalmente o uso de células embrionárias de *R. microplus* como modelo in vitro para o cultivo de tripanossomatídeos, por representarem um método rápido e de baixo custo.

Palavras-chave: Carrapato monoxeno, células embrionárias de carrapato, infecção experimental, tripanossomatídeo bovino.

INTRODUÇÃO

The genus *Trypanosoma* has been reported to infect domestic and wild animals of several orders[1-3]. In the subgenus *Megatrypanum,* the type species is *Trypanosoma theileri*[4], which routinely infects domestic cattle herds[5,6], and is one of the most prevalent bovine parasite species in the world[2,3,7-11].

The trypanosomes present in the subgenus *Megatrypanum* are species-specific, infecting ungulate and ruminant mammalian hosts[5,12-15]. Experimental infections of *T. theileri* obtained from cattle have been performed in sheep, but showed no cross-species infection[5,16,17]. In buffaloes (*Bubalus bubalis*), infection with *T. theileri*-like trypanosomes, which belong to a lineage exclusive to these parasites, has been described, while cross-infection between *T. theileri* infecting cattle and *T. theileri*-like trypanosomes infecting bubalines is not known[5,9,10]. The natural vectors of *T. theileri* species are flying tabanids and hippoboscids, which transmit the parasite by releasing metacyclic trypomastigotes in their feces, which penetrate a new host through the lesion of the vector bite or skin abrasions, with the possibility of infection through host ingestion of the feces or the vector itself[7,18-20]. The transmission of *T. theileri* by ticks is not well established. However, ticks of the Ixodidae family are the second-largest group of arthropods involved in disease transmission,[1,5,20-27]. Experimental and natural transmission routes demonstrated potential vector competence of Ixodidae tick families[26]. *T. theileri* was detected in haemolymph from *Rhipicephalus microplus*[27].

R. microplus is a monoxenous tick species[28], native to Asia[29-33], that expanded to tropical and subtropical regions. After introduction, the species remains widely distributed in the country[32-35], through its association with cattle and bubaline breeding[36,37] and its distribution now spans most continents[38]. Domestic cattle represent the main host of *R. microplus*[39], as well as descriptions of frequent parasitism in cervids and bubalines, indicating the latter as a suitable host for *R. microplus*[40,41]. *R. microplus* causes notable losses to cattle and sheep herds[42,43], not only due to anemia in animals caused by blood spoliation[44], but also from the transmission of pathogens[45-51].

The present study aimed to establish and observe the in vitro development of *T. theileri* and *T. theileri*-like trypanosomes in cells of *R. microplus*, one of the main tick species that infest bovine and bubaline populations in Brazil. It is also noteworthy that the results obtained in this work will enable the use of the *R. microplus* cell line as an *in vitro* model for the cultivation of pathogens.

MATERIALS AND METHODS

Culture and maintenance of *Trypanosoma theileri* and *Trypanosoma theileri*-like trypanosome isolates

Isolate of *T. theileri* (CBT 114) from a bovine (*Bos taurus*) from the state of Rondônia, Brazil, in 2012/2013[52], and isolate of *T. theileri*-like trypanosomes (CBT 253) from a water buffalo (*B. bubalis* Linnaeus, 1758) from the state of Pará, Brazil, in 2015 were used in this study.

For parasite isolation, blood samples were inoculated into Vacutainer® tubes containing a biphasic medium consisting of 15% sheep red blood cells as the solid phase (blood agar base), overlain by liquid LIT medium supplemented with 20% FBS[53]. After growth and culture establishment, the parasites were cryopreserved in liquid nitrogen. These isolates were deposited in the "Coleção Brasileira de Tripanossomatídeos (CBT)" located in the Department of Preventive Veterinary Medicine and Animal Health of the Faculty of Veterinary Medicine and Zootechnics of the University of São Paulo - SP, Brazil.

For maintenance and growth of isolates, the

solid phase was used in BAB (blood agar) with 15% defibrinated sheep blood, and the liquid phase was LIT (liver infusion tryptose) medium supplemented with 10% fetal bovine serum (FBS), and incubated at 28°C[54].

Origin and maintenance of tick embryonic cells

The RBME-6 cell line derived from the *R. microplus* embryo was provided and maintained as described previously[55]. For trypanosome infection, RBME-6 cells were maintained in L-15 medium (Leibovitz) supplemented with 20% FBS and incubated at 28 °C.

Infection of tick embryonic cell monolayer with *Trypanosoma* species and fluorescence microscopy

A standardized concentration of 2 x 10^5 *T. theileri* and *T. theileri*-like trypanosome epimastigotes from axenic medium (LIT) was used for infection of *R. microplus* embryonic cell monolayers. After infection, the tick cells were evaluated every 48 hours to count epimastigote forms in a Neubauer-type chamber under a phase-contrast microscope with a 40x objective. In addition, cells were scraped every 48 hours to prepare slides for evaluation of trypomastigote forms. Infection in the monolayer in tick cells was followed for 10 days or as long as the cell monolayer remained preserved.

In parallel, epimastigotes from the isolates were also inoculated in L15 medium supplemented with 20% fetal bovine serum to evaluate growth in axenic medium. For fluorescence microscopy, embryonic cell monolayers of R. microplus infected with *T. theileri* and *T. theileri-like* trypanosomes at different time points were washed with L15 medium to remove extracellular parasites. The monolayer was then scraped off, transferred to conical tubes, and centrifuged at 1500 rpm for fluorescence and confocal microscopy. For fluorescence microscopy, the pellet was transferred to microtubes, washed with PBS (140 mM NaCl; 10 mM Na_2HPO_4; 1.76 mM KH_2PO_4; 2.68 mM KCl, pH 7.2), and fixed in 4% paraformaldehyde. After this process, slides were prepared for staining with DAPI (4',6-diamidino-2-phenylindole) - deoxyribonucleic acid staining - and Phalloidin (Actin-F staining) (Molecular Probes, USA), and the images were obtained using a Nikon Eclipse TS100 microscope.

Confocal microscopy

Samples were washed twice in PBS (phosphate-buffered saline, pH 7.4) and then fixed in 4% paraformaldehyde for 2 hours. Subsequently, the material was washed again in PBS and permeabilized with 0.1% Triton X-100 for 20 minutes. For F-actin labeling, cells were incubated with Alexa Fluor 488 Phalloidin solution (Molecular Probes, USA) (5 µL of Alexa Fluor 488 Phalloidin stock solution + 200 µL of PBS + 2 µL of BSA) for 30 minutes at room temperature in the dark.

The material was then washed twice in PBS for 5 minutes each and incubated with 3 µM DAPI solution for 20 minutes. After this step, the material was washed again in PBS and mounted on a glass slide with ProLong® Gold mounting medium (Molecular Probes, USA). The material was analyzed and photographed in a Laser Scanning Confocal Microscope, LEICA TCS SP5 II, and the software LAS-AF.

RESULTS

The parasites inoculated in a monolayer of embryonic cells of *R. microplus* initially multiplied intensively in epimastigote forms on the supernatant and adhered to the cells. After 48 hours of infection,

the number of trypomastigotes was higher (58%) than the number of epimastigotes observed in the supernatant, and the number of tick embryonic cells gradually decreased. The evaluation of trypomastigote forms in the cell supernatant showed 37.4% at 48 hours post-infection. After 96 hours of infection, the embryonic cell monolayer showed extensive lysis, with only a few cells remaining.

The presence of amastigotes evaluated by fluorescence microscopy with DAPI/Phalloidin staining and by transmission electron microscopy showed, in the markings after 24 hours of infection, the presence of a few amastigotes in the cells, the presence of more elongated nuclei, suggesting the presence of trypomastigotes inside the cell, and the formation of a parasitophorous vacuole.

After 48 hours of infection, the number of amastigotes within tick cells was higher (81%) than at 24 hours after infection. At 72 hours, the number of amastigotes per cell decreased (38%), as did the number of cells present in the monolayer. The same results were obtained with both fluorescence and confocal microscopy (Figure 1). No differences were observed in the infection by *T. theileri* or *T. theileri*-like trypanosomes in the embryonic cell monolayer.

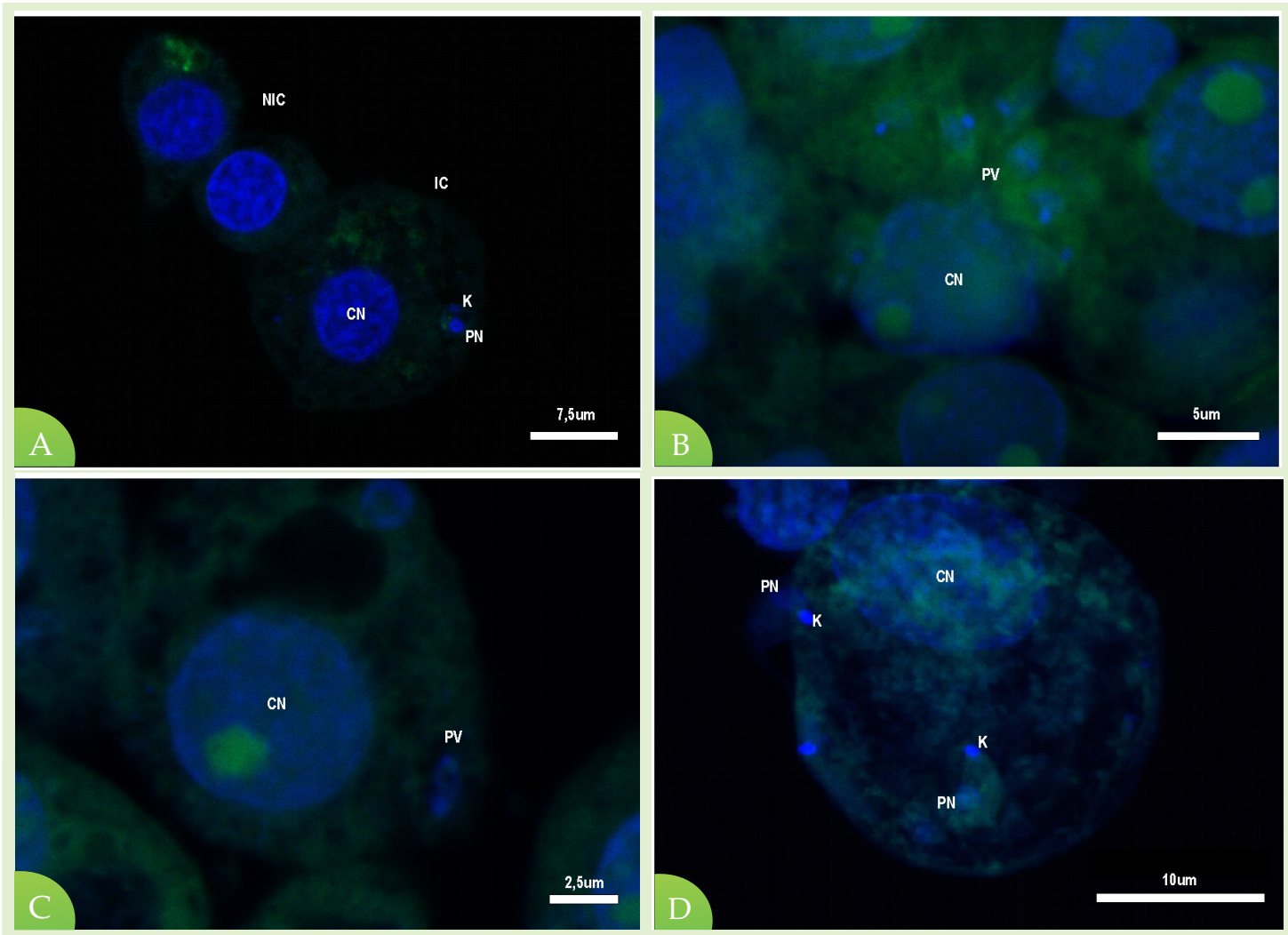

Figure 1. Phalloidin and DAPI merged staining in a monolayer of embryonic cells of *Rhipicephalus microplus* (RBME-6) infected with *Trypanosoma theileri* (CBT 114), comparing different infection times. Time of infection: (A) 24 hours, (B-C) 48 hours, and (D) 72 hours. Legend: Parasite nucleus (PN), Kinetoplast (K), Cell nucleus (CN), Parasitophorus vacuole (PV), Non-infected cell (NIC), and Infected cell (IF).

DISCUSSION

The cultivation of parasites in cell culture can represent an alternative to *in vivo* experiments. In *Trypanosoma cruzi*, a substitute used *in vitro* to induce the change of the parasite from the infective to the replicative forms, is to expose them to an acid pH, mimicking the process that occurs when the infective forms penetrate the host cell, through the parasitophorous vacuole, which acidifies the environment, stimulating the migration of the parasite to the cytoplasm and thus the beginning of the transformation process[56,57]. In *T. theileri*, the intracellular pathways of development are not shown, and this is the first study to demonstrate intracellular infection. New studies should be proposed to elucidate intracellular infection and the proteins and receptors involved.

In vitro cultivation of *T. theileri* and *T. theileri*-like in *R. microplus* cells was successful in the present study. A similar result was previously demonstrated in the successful in vitro culture of the genus *Trypanosoma* in naturally infected Brazilian ticks of the species *R. microplus*[58].

Additionally, the *R. microplus* was evaluated as an alternative vector of *Trypanosoma vivax* by experimental transmission, but this was not well established[59].

Among arthropod vectors of pathogens, the tick *R. microplus* is of great importance due to its wide adaptability to modified environments and hosts61, in addition to, as already demonstrated, its capacity to infest *R. microplus* naturally in bubalines that cohabit with cattle[61]. A demonstration of the occurrence of *R. microplus* parasitism in bubalines was conducted in Asia, where the tick parasitized water buffaloes (*B. bubalis*). In India and Pakistan, it was observed that the most abundant species among these animals was R. microplus, with 80% and 53%, respectively[62,63]. In Brazil, the species *R. microplus* remains one of the main causes of economic losses in livestock[35,60,64], because this tick carries pathogens of various diseases, hide depreciation, and blood spoliation[68-69].

Moreover, flagellate forms of *T. theileri* have been described previously in the hemolymph of larvae of ticks belonging to the Ixodidae family[68,69], and in the species *Rhipicephalus sanguineus* flagellate structures, suggestive of organisms belonging to the Trypanosomatidae family, were found[70]. Besides these, the occurrence of flagellates of *Crithidia hyalomma* in eggs and hemolymph of ticks *Hyalomma aegyptium*[71] and *Boophilus calcaratus*[72] was reported. There are also suggestions, experimentally, that Ixodid ticks could possibly transmit trypanosomes of the Stercoraria section (*T. cruzi*) by regurgitation after ingestion of many parasites[73], or by accidental ingestion of the invertebrate host containing the parasite forms[74]. To confirm the role of *R. microplus* as a vector agent of those hemiparasitic protozoa, it is necessary to establish the tick's ability to support the growth and multiplication of protozoa of the genus *Trypanosoma*.

The result of the study on the maintenance of these protozoa in ticks may become a tool to evaluate the adaptability to the invertebrate host.

CONCLUSION

T. theileri and *T. theileri*-like trypanosomes invade and differentiate in the tick cell line (RBME-6) of the tick *R. microplus*. This is the first study to demonstrate the differentiation of *T. theileri* in amastigotic forms in a tick cell monolayer. *T. theileri* and *T. theileri*-like have invaded and remained active in cultures. The result of the study on the maintenance of these protozoa in ticks may become a tool to evaluate the adaptability to the invertebrate host. Above all, the results obtained in this work may lead to the use of *R. microplus* embryonic cells as an in vitro model for the cultivation of trypanosomatids and other pathogens of human and veterinary relevance.

ACKNOWLEDGMENTS

This research was financially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Universidade Santo Amaro (UNISA), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – financial code 001.

REFERENCES

1. BRAUN, U. et al. *Trypanosoma theileri* in the cerebrospinal fluid and brain of a heifer with suppurative meningoencephalitis. *Veterinary Record*, v.150, n.1, p.18–19, 2002.

2. HOARE, C.A. The classification of mammalian trypanosomes. In: Ergebnisse der Mikrobiologie Immunitätsforschung und Experimentellen Therapie. Berlin: Springer, 1966. p.43-57.

3. MORALES, G.A. et al. The capybara (*Hydrochoerus hydrochaeris*) as a reservoir host for *Trypanosoma Evansi*. *Journal of Wildlife Diseases*, v.12, n.4, p.572–574, 1976.

4. SCHLAFER, D.H. *Trypanosoma theileri*: a literature review and report of incidence in New York cattle. *The Cornell Veterinarian*, v.69, n.4, p.411–425, 1979.

5. RODRIGUES, A.C. et al. Brazilian Isolates of *Trypanosoma* (*Megatrypanum*) *theileri:* diagnosis and differentiation of isolates from cattle and water buffalo based on biological characteristics and randomly amplified DNA sequences. *Veterinary Parasitology*, v.116, n.3, p.185–207, 2003.

6. JAIMES-DUEÑEZ, J. et al. Spatial-temporal and phylogeographic characterization of *Trypanosoma* spp. in cattle (*Bos taurus*) and buffaloes (*Bubalus bubalis*) reveals transmission dynamics of these parasites in Colombia. *Veterinary Parasitology*, v.249, p.30–42, 2018.

7. BÖSE, R. et al. Characterization of *Megatrypanum trypanosomes* from European cervidae. *Parasitology*, v.107, n.1, p.55–61, 1993.

8. RODRIGUES, A.C. et al. Phylogeny of *Trypanosoma* (*Megatrypanum*) *Theileri* and related trypanosomes reveals lineages of isolates associated with artiodactyl hosts diverging on SSU and ITS ribosomal sequences. *Parasitology*, v.132, n.2, p.215–224, 2006.

9. RODRIGUES, A.C. et al. Characterization of spliced leader genes of *Trypanosoma* (*Megatrypanum*) *Theileri*: phylogeographical analysis of Brazilian isolates from cattle supports spatial clustering of genotypes and parity with ribosomal markers. *Parasitology*, v.137, n.1, p.111–122, 2010.

10. RODRIGUES, A.C. et al. Cysteine proteases of *Trypanosoma* (*Megatrypanum*) *theileri:* cathepsin L-like gene sequences as targets for phylogenetic analysis, genotyping diagnosis. *Parasitology International*, v.59, n.3, p.318–325, 2010.

11. CHÁVEZ-LARREA, M.A. et al. Molecular identification of *Trypanosoma theileri* (Laveran, 1902) in cattle from two slaughterhouses in Ecuador and its relation with other haemotropic agents. *Frontiers in Veterinary Science*, v.10, 1153069, 2023.

12. HOARE, C.A. The trypanosomes of mammals. A zoological monograph. *Science*, v.179, p.60, 1972.

13. KINGSTON, N.; MORTON, J.K. *Trypanosoma cervi* sp. n. from elk (*Cervus canadensis*) in wyoming. *The Journal of Parasitology*, v.61, n.1, p.17–23, 1975.

14. SUGANUMA, K. et al. Molecular characterization of a new *Trypanosoma* (*Megatrypanum*) *theileri* isolate supports the two main phylogenetic lineages of this species in Japanese cattle. *Parasitology Research*, v.118, n.6, p.1927–1935, 2019.

15. GARCIA, H.A. et al. Pan-American *Trypanosoma* (*Megatrypanum*) *trinaperronei n. sp.* in the white-tailed deer *Odocoileus virginianus* (Zimmermann) and its deer ked *Lipoptena mazamae* (Rondani), 1878: morphological, developmental and phylogeographical characterisation. *Parasites & Vectors*, v.13, n.1, p.308, 2020.

16. WELLS, E.A. Biology of the Kinetoplastida. London: Academic Press, 1976. p.257-275.

17. GARCIA, H.A. et al. Multilocus phylogeographical analysis of *Trypanosoma* (*Megatrypanum*) genotypes from sympatric cattle and water buffalo populations supports evolutionary host constraint and close phylogenetic relationships with genotypes found in other ruminants. *International Journal for Parasitology*, v.41, n.13–14, p.1385–1396, 2011.

18. BÖHM, M. et al. Wild Deer as a source of infection for livestock and humans in the UK. *The Veterinary Journal*, v.174, n.2, p.260–276, 2007.

19. CALZOLARI, M. et al. Isolation of a trypanosome related to *Trypanosoma theileri* (Kinetoplastea: Trypanosomatidae) from *Phlebotomus perfiliewi* (Diptera: Psychodidae). *BioMed Research International*, v.2018, p.1–8, 2018.

20. BALASHOV Y.S. Bloodsucking ticks (Ixodoidea): vectors of diseases of man and animals. *Miscellaneous Publications of the Entomological Society of America*, n.8, p.159-376, 1972.

21. KRINSKY, W.L.; BURGDORFER, W. Trypanosomes in amblyomma americanum from Oklahoma. *The Journal of Parasitology*, v.62, n.5, p.824–825, 1976.

22. SHASTRI, U.V.; DESHPANDE, P.D. *Hyalomma anatolicum anatolicum* (Koch, 1844) as a possible vector for transmission of *Trypanosoma theileri*, Laveran, 1902 in cattle. *Veterinary Parasitology*, v.9, n.2, p.151–155, 1981.

23. CUPP, E.W. Biology of ticks. *Veterinary Clinics of North America: Small Animal Practice*, v.21, n.1, p.1–26, 1991.

24. LATIF, A.A. et al. High infection rates of the tick *Hyalomma anatolicum anatolicum* with *Trypanosoma theileri*. *The Onderstepoort Journal of Veterinary Research*, v.71, n.4, p.251–256, 2004.

25. BRITES-NETO, J. et al. Tick-borne infections in human and animal population worldwide. *Veterinary World*, v.8, n.3, p.301–315, 2015.

26. KERNIF, T. et al. Ticks as vectors of Trypanosomatidae with medical or veterinary interest: insights and implications from a comprehensive systematic review and meta-analysis. *Helyon*, v.10, n.24, e40895, 2024.

27. MOREL, N. et al. A *Trypanosoma* species detected in *Rhipicephalus (Boophilus) microplus* ticks from Argentina. *Ticks and Tick-Borne Diseases*, v.12, n.1 p.101573, 2021.

28. AHMAD, Z. et al. Collection and prevalence of ticks in cattle and buffaloes from free-range management systems of Islamabad. *The Journal of Basic and Applied Zoology*, v.80, n.1, p.12, 2019.

29. SCHUNTNER, C.A. et al. Toxicity of piperonyl butoxide to *Boophilus microplus*. *Nature*, v.249, n.5455, p.386–386, 1974.

30. HOOGSTRAAL, H. Argasid and Nuttalliellid Ticks as Parasites and Vectors. *In*: *Advances in Parasitology*. Amsterdam: Elsevier, 1985. p.135–238.

31. OSTERKAMP, J. et al. Host-odor recognition in two tick species is coded in a blend of vertebrate volatiles. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, v.185, n.1, p.59–67, 1999.

32. BARRÉ, N.; UILENBERG, G. Spread of parasites transported with their hosts: case study of two species of cattle tick. *Revue Scientifique et Technique (International Office of Epizootics)*, v.29, n.1, p.149–160, 2010.

33. LÉGER, E. et al. Changing distributions of ticks: causes and consequences. *Experimental and Applied Acarology*, v.59, n.1–2, p.219–244, 2013.

34. ESTRADA-PEÑA, A. et al. The known distribution and ecological preferences of the tick subgenus *Boophilus* (Acari: Ixodidae) in Africa and Latin America. *Experimental and Applied Acarology*, v.38, n.2–3, p.219–235, 2006.

35. KLAFKE, G.M. et al. Brazil's battle against *Rhipicephalus (Boophilus) microplus* ticks: current strategies and future directions. *Revista Brasileira de Parasitologia Veterinária*, v.33, n.2, e001423, 2024.

36. FAMADAS, K.M.; FACCINI, J.L.H. Variação morfológica de *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae) no Brasil. *Arquivos da Universidade Federal Rural do Rio de Janeiro*. v.12, n.1-2, p.73-81, 1989.

37. MINERVINO, A.H.H. et al. *Bubalus bubalis*: a short story. *Frontiers in Veterinary Science*, v.7, 570413, 2020.

38. MADDER, M. et al. New foci of *Rhipicephalus microplus* in West Africa. *Experimental and Applied Acarology*, v.56, n.4, p.385–390, 2012.

39. SILVA FILHO, M.L. et al. Efeito do extrato aquoso e etanólico do angico preto sobre larvas de *Rhipicephalus (Boophilus) microplus*. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, v.65, n.3, p.637–644, 2013.

40. ROCHA, U.F. et al. Infestação natural de búfalos *Bubalus bubalis* L., 1758 dos estados de São Paulo e Minas Gerais, Brasil, por *Boophilus microplus* (Canestrini, 1887) e por *Anocentro nitens* (Neumann, 1897) (Acari: Ixodidae). *Arquivos do Instituto Biológico*, v.36, p.197-199, 1969.

41. BECHARA, G. H. et al. Skin test and tick immune status in susceptible and resistant cattle in Brazil. *Annals of the New York Academy of Sciences*, v.916, n.1, p.570–575, 2000.

42. GARCIA, M.V. et al. Contributions of the hair sheep breed Santa Ines as a maintenance host for *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Brazil. *Parasites & Vectors*, v.7, n.1, p.515, 2014.

43. CALVANO, M.P.C.A. et al. Economic efficiency of *Rhipicephalus microplus* control and effect on beef cattle performance in the Brazilian Cerrado. *Experimental and Applied Acarology*, v.79, n.3–4, p.459–471, 2019.

44. SENBILL, H. et al. Life cycle of the southern cattle tick, *Rhipicephalus (Boophilus) microplus* Canestrini 1888 (Acari: Ixodidae) under laboratory conditions. *Systematic and Applied Acarology*, v.23, n.6, p.1169, 2018.

45. GUERRERO, F.D. et al. *Rhipicephalus (Boophilus) microplus* aquaporin as an effective vaccine antigen to protect against cattle tick infestations. *Parasites & Vectors*, v.7, n.1, p.475, 2014.

46. DE LA FUENTE, J. et al. Perception of ticks and tick-borne diseases worldwide. *Pathogens*, v.12, n.10, p.1258, 2023.

47. FERREIRA, G.C.M. et al. Prevalence of bovine *Babesia spp.*, *Anaplasma marginale*, and *their* co-infections in Latin America: systematic review-meta-analysis. *Ticks and Tick-borne Diseases*, v.13, n.4, p.101967, 2022.

48. GUGLIELMONE, A.A. Epidemiology of babesiosis and anaplasmosis in South and Central America. *Veterinary Parasitology*, v.57, n.1–3, p.109–119, 1995.

49. BOCK, R. et al. Babesiosis of cattle. *Parasitology*, v.129, n.S1, p.S247–S269, 2004.

50. KOCAN, K.M. et al. The natural history of *Anaplasma marginale*. *Veterinary Parasitology*, v.167, n.2–4, p.95–107, 2010.

51. JAIMES-DUEÑEZ, J. et al. Parasitological and molecular surveys reveal high rates of infection with vector-borne pathogens and clinical anemia signs associated with infection in cattle from two important livestock areas in Colombia. *Ticks and Tick-borne Diseases*, v.8, n.2, p.290–299, 2017.

52. PACHECO, T.D.A. et al. Genetic diversity and molecular survey of *Trypanosoma (Megatrypanum) theileri* in cattle in Brazil's western Amazon region. *Revista Brasileira de Parasitologia Veterinária*, v.27, n.4, p.579–583, 2018.

53. MARCILI, A. et al. Isolation and phylogenetic relationships of bat trypanosomes from different biomes in Mato Grosso, Brazil. *Journal of Parasitology*, v.99, n.6, p.1071–1076, 2013.

54. MARCILI, A. et al. Phylogenetic relationships of leishmania species based on trypanosomatid barcode (SSU rDNA) and gGAPDH genes: taxonomic revision of *Leishmania (L.) infantum chagasi* in South America. *Infection, Genetics and Evolution*, v.25, p.44–51, 2014.

55. LIMA-DUARTE, L. et al. Establishment and characterization of a cell line (RBME-6) of *Rhipicephalus (Boophilus) microplus* from Brazil. *Ticks and Tick-borne Diseases*, v.12, n.5, 101770, 2021.

56. ANDRADE, L.O.; ANDREWS, N.W. The *Trypanosoma cruzi*–host-cell interplay: location, invasion, retention. *Nature Reviews Microbiology*, v.3, n.10, p.819–823, 2005.

57. YOSHIDA, N. Molecular basis of mammalian cell invasion by *Trypanosoma cruzi*. *Anais da Academia Brasileira de Ciências*, v.78, n.1, p.87-111, 2006.

58. MAROTTA, C.R. et al. *Trypanosoma rhipicephalis sp*. Nov. (Protozoa: Kinetoplastida) isolated from *Rhipicephalus microplus* (Acari: Ixodidae) ticks in Rio de Janeiro, Brazil. *Parasitology Open*, v.4, p.e2, 2018.

59. LÓPEZ, G. et al. Transmisión experimental de *Trypanosoma vivax* por la garrapata *Boophilus microplus*. *Revista do Instituto Colombiano Agropecuário*, n.14, p.93-96, 1979.

60. CAMILLO, G. et al. Eficiência *in vitro* de acaricidas sobre carrapatos de bovinos no estado do Rio Grande do Sul, Brasil. *Ciência Rural*, v.39, n.2, p.490–495, 2008.

61. BENITEZ, D. et al. *Rhipicephalus (Boophilus) microplus* ticks can complete their life cycle on the water buffalo (*Bubalus bubalis*). *Journal of Buffalo Science*, v.1, n.2, p.193-197, 2012.

62. MIRANPURI, G.S. Ticks parasitising the indian buffalo (*Bubalus bubalis*) and their possible role in disease transmission. *Veterinary Parasitology*, v.27, n.3–4, p.357–362, 1988.

63. MANAN, A. et al. Prevalence and identification of ixodid tick genera in frontier region Peshawar. *Journal of Agricultural and Biological Science*, v.2, n.1, p.21-25, 2007.

64. SATO, T.P. et al. *Rhipicephalus* (*Boophilus*) *Microplus*: rickettsiae infection in Brazil. *International Journal of Acarology*, v.46, n.2, p.88–93, 2020.

65. BROWN, W.C. et al. Immune control of *Babesia bovis* infection. *Veterinary Parasitology*, v.138, n.1–2, p.75–87, 2006.

66. JONSSON, N.N. The productivity effects of cattle tick (*Boophilus microplus*) infestation on cattle, with particular reference to *Bos indicus* cattle and their crosses. *Veterinary Parasitology*, v.137, n.1–2, p.1–10, 2006.

67. JONSSON, N.N. et al. Productivity and health effects of anaplasmosis and babesiosis on *Bos indicus* cattle and their crosses, and the effects of differing intensity of tick control in Australia. *Veterinary Parasitology*, v.155, n.1–2, p.1–9, 2008.

68. BURGDORFER, W. et al. Detection of *Trypanosoma theileri* in Ethiopian cattle ticks. *Acta Tropica*, v.30, n.4, p.340–346, 1973.

69. RIBEIRO, M.F.B. et al. Ocorrência de *Trypanosoma theileri* (Laveran, 1902) em *Boophilus microplus* no estado de Minas Gerais. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia*, v.40, n.1, p.65-68, 1988.

70. SHERLOCK, I. A. Notes on the transmission of visceral leishmaniasis in Brazil. *Revista Brasileira de Malariologia e Doenças Tropicais*, v.16, p.19–26, 1964.

71. O'FARRELL, W.R. Preliminary note on a new flagellate *Crithidia hyalommae* sp. nov. found in the tick *Hyalomma aegyptium* (Linnaeus, 1758). *Journal of*

Tropical Medicine Hygiene, v.16, n.16, p.245-246, 1913.

72. MURATOV, E.A.; KHEISIN E.M. The development of *Piroplasma bigeminum* in the tick *Boophilus calcaratus*. *Zoologicheskii Zhurnal*, v.38, p.970-985, 1959.

73. DIAS, J.C.P. et al. Mecanismos alternativos de transmissão do *Trypanosoma cruzi* no Brasil e sugestões para sua prevenção. *Revista da Sociedade Brasileira de Medicina Tropical*, v.44, n.3, p.375–379, 2011.

74. KRIGE, A.-S. et al. 'Hang on a tick' - Are ticks really the vectors for Australian trypanosomes? *Trends in Parasitology*, v.35, n.8, p.596–606, 2019.