Arquivos do Instituto Biológico
ISSN 1808-1657
https://doi.org/10.1590/1808-1657000112022

Investigation of the parasitoid *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in *Amblyomma sculptum* (Acari: Ixodidae) ticks in the municipality of Salto, São Paulo, Brazil

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ABSTRACT

The presence of capybaras (*Hydrochoerus hydrochaeris*) in urban and periurban areas has caused increased numbers of cases of Brazilian spotted fever. With the aim of investigating the presence of the parasitoid *Ixodiphagus hookeri* in *Amblyomma sculptum* ticks in the municipality of Salto, state of São Paulo, samples were collected monthly from 14 sites. Thirty samples were placed in containers for observation of the emergence of microhymenopterans and 88 samples were subjected to molecular testing to identify the presence of *I. hookeri* DNA. Neither dissections nor observation of emergence indicated any presence of *I. hookeri* larvae in ticks. Samples subjected to polymerase chain reaction (PCR) amplification of the mCOX I region of *I. hookeri* did not reveal its presence, although fragments corresponding to mRNA 16S of *Amblyomma sculptum* ticks were amplified in all samples tested.

Keywords: microhymenopterans; biological control; polymerase chain reaction.

Increases in the population of capybaras (*Hydrochoerus hydrochaeris*) in public parks and in residential areas and forest fragments around lakes and reservoirs have been reported in most municipalities in the state of São Paulo, Brazil. According to FERRAZ et al. (2007), this has been due to prohibition of hunting through the fauna protection law, declining numbers of potential natural predators and the high reproductive rate of this species. There has also been a reduction in the capacity for environmental support associated with increased cultivation of sugarcane.

The presence of capybaras in urban areas with bodies of water has caused population increases among the ticks *Amblyomma sculptum* and *Amblyomma dubitatum* at unbearable levels of environmental infestations (BRITES-NETO et al., 2014). This situation has become a matter of concern at government level due to the risk of acquiring Brazilian spotted fever. Over recent years, there has been an increase in the number of human cases of this disease. According to POLO et al. (2018), this increase can be correlated with the presence and expansion of the range of capybaras, which are the primary host for *A. sculptum* ticks, given that these ticks are amplifiers of the causative agent of Brazilian spotted fever, *Rickettsia rickettsii*. According to SCINACHI (2015), when this disease remains untreated, it can reach a lethality rate of 80%. The epidemiology of this rickettsiosis is determined by the geographical and environmental distribution of the vector (SZABÓ et al., 2007). In addition to the possibility of transmission of *Rickettsia*, high infestation by *A. sculptum* and *A. dubitatum* in their different stages can give rise to direct damage to humans and animals, such as allergies and irritation, and in certain cases can cause severe skin reactions.

Among the control methods, use of natural enemies for implementing biological control has been reported as a tool for use within an integrated tick control system, with the aims of reducing losses and reducing the use of chemical

Received: Jun 30, 2022. Accepted: Nov 04, 2022
Section Editor: Silvia Galleti
Peer Review History: Double-blind Peer Review.

products, thereby minimizing the residual effects of these products on the environment (SILVA, 2019). Biological control with parasitoids and predators is already practiced in agricultural production with the aim of attaining sustainable agriculture (PARRA, 2014).

The genus *Ixodiphagus* is composed of insects with great importance in biological control. The first researcher to report that this genus parasitizes ticks was Howard (1907), through the description of *Ixodiphagus texanus*. The following year, Howard (1908) described a second species in the state of Texas, which he called *Hunterellus hookeri*. Both are now included in the genus *Ixodiphagus* of the family Encyrtidae, and *Ixodiphagus hookeri* is the most widespread species (DAVIS, 1986; MWANGI et al., 1994).

The parasitoids of the species *I. hookeri* (Fig. 1) are microhymenopterans with an approximate size of 2 mm. The parasitoid larvae develop inside the engorged nymphs of the ticks, but oviposition can be performed in the larvae of the ticks (KRAWCZYK et al., 2020).

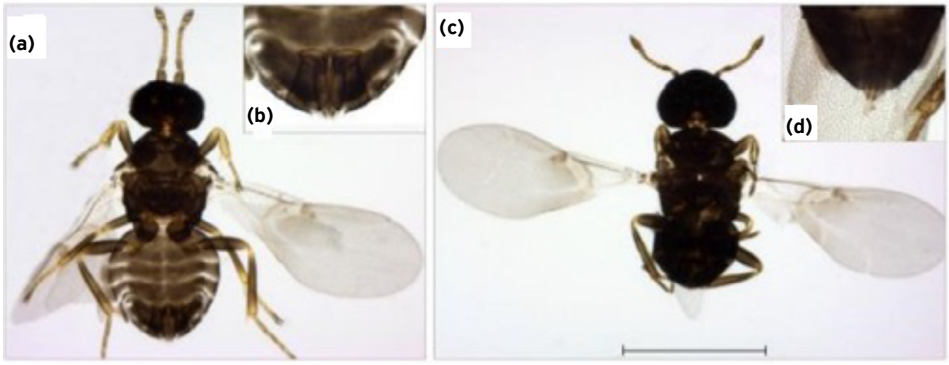

Figure 1. (a) Adult female of *Ixodiphagus hookeri*; (b) Magnified female genitalia; (c) Adult male of *Ixodiphagus hookeri*; (d) Magnified male genitalia.
Source: Retrieved from Bohacsova et al. (2016, p. 5).

These parasitoids reproduce sexually and the females release their eggs inside the ticks by perforating the cuticle using the ovipositor. After hatching, the larvae feed on the internal organs of the engorged tick. The adult wasps emerge between 30 and 57 days after oviposition (WOOD, 1911). The adult parasitoids then mate and the cycle is repeated (HU; HYLAND, 1997).

Tick collections were performed in Salto, state of São Paulo, monthly from March 2019 to February 2021, in areas with a history of presence of capybaras and/or horses. These sites were mostly on the banks of watercourses (Tietê River, Jundiaí River, Buru River and Ajudante Stream). The collection sites were at the company Toyobo do Brasil; São João square; Rocha Moutonnée park and Chácara Maninho, district of São Pedro and São Paulo; Federal Institute of Education, Science and Technology of the State of São Paulo, Salto campus; Lago park, in the district of Nair Maria; Lagos do Icaraí condominium; Zuleika Jabour condominium; the former "fishery of the Portuguese man", in the Jardim Buru district; Santa Rosa condominium; and the natural park of the hydroelectric island. Ticks were also collected in the vicinity of the homes of citizens who had died due to spotted fever (São João square, Icaraí condominium and Santa Rosa condominium).

After collection, the tick samples were taken to the Animal Parasitology Laboratory of the Biological Institute. They were then separated according to their stage of development: larvae, nymphs and adult males and females. The males and females were identified under a stereoscopic microscope, using the key proposed by GUIMARÃES et al. (2001). The larvae and nymphs were identified as *Amblyomma* spp.

Twenty ticks according to the stage of development were placed in test tubes. These were identified according to the collection site and placed in a chamber for subsequent dissection. Thirty engorged nymphs of *Amblyomma* genus were collected in plastic containers that were closed with fine-gauze tissue to allow oxygen to enter. They remained in the containers for a period of 60 days for observation regarding the emergence of parasitoids.

Among the ticks collected between October and December 2020 at these collection points in the municipality of Salto, ninety individuals were subjected to molecular testing: 74 larvae and nymphs of *Amblyomma* sp. and 16 adults of *A. sculptum*. The specimens were cut along their bilateral symmetry plane using a scalpel and were subsequently subjected to DNA extraction using the Quick-DNA Miniprep Plus Kit (Zymo Research), following the manufacturer's instructions. The products obtained were subjected to the polymerase chain reaction (PCR) technique for amplification of different gene segments corresponding to the mitochondrial gene 16S of *Amblyomma* spp., with the objective of confirming the extraction, along with a segment of the mitochondrial gene COX I of the hymenopteran *I. hookeri*. The primers used were 16S + 1 (5' – TCGGTITAAACTCAGATCATGT – 3') and 16S – 1 (5' – CTGCTCAATGAIIIIIITTAAtTGCTGTGG – 3') for

amplification of the 16S gene of *Amblyomma* spp. and Iphag 583f (5' – TTGCTGTTCCAACAGGAGTAAA – 3') and Iphag820r (5' – CAAAAAATTGCAAAAACTGC – 3') for amplification of the COX I gene of *I. hookeri* (BOHACSOVA et al., 2016). The positive control for the reaction was performed under the same conditions described above, by adding a DNA segment of 180 base pairs in which the 5'- and 3'- terminal sequences corresponded to the sequences of the primers Iphag583f and Iphag820r.

Out of the total of 1,674 individuals collected, 448 were identified as larvae and 622 as nymphs of *Amblyomma* spp., and 278 males and 326 females were identified as *A. sculptum*. The number of larvae and nymphs exceeded the number of adults collected. Most of the larvae and nymphs were caught between May and October, and most of the adult ticks were found between December and April. According to OLIVEIRA et al. (2000), the population dynamics of *A. sculptum* in the southeastern region of Brazil are characterized by predominance of larvae in the months of April to July, nymphs from July to October and adults from October to March.

The largest quantity of *Amblyomma* spp. ticks was caught in the Rocha Moutonnée park, followed by Santa Rosa condominium, São João square, Lago park, hydroelectric island park, the fishery of the Portuguese man and the company Toyobo do Brasil. These places are characterized by a vast area of forest with native fauna of high diversity, and are close to the Tietê River, except for São João square, which is next to the Jundiaí River. All these sites have histories of abundant circulation of capybaras, a factor that favors the presence of ticks.

The places with intermediate quantities of *Amblyomma* spp. collected, i.e., Zuleika Jabour, Icaraí and the district of São Pedro and São Paulo, are close to urban areas and are residential neighborhoods, where weeding is performed frequently. In the Zuleika Jabour and Icaraí condominiums, there have been reports of application of insecticides with the objective of reducing the numbers of ticks.

The areas with the lowest quantities of individuals of *A. sculptum* collected were the Nair Maria district, Chácara Maninho and Jardim Buru, which are dedicated to horse breeding, where the owners of the animals control ectoparasites frequently through the use of acaricides. The use of acaricides in these places can explain the smaller quantity of ticks here, compare with the other areas. This result was compatible with the findings of LABRUNA et al. (2002) and SOUZA (1990), who reported that farms involved in horse breeding contributed to controlling the spread of ticks in the environment, through spraying their animals with acaricide. Thus, it was more likely that smaller numbers of ticks would be caught in these places, compared with other collection points.

Neither the dissections and nor the observations of emergence showed any presence of *I. hookeri* larvae in the tick samples collected.

The PCR tests showed that fragments corresponding to mRNA 16S of *A. sculptum* were amplified in all samples. However, the mCOX I region of *I. hookeri* was not amplified in any of the samples (Fig. 2). This result demonstrated that there was no presence of *I. hookeri* in the samples evaluated, considering that there was amplification of mitochondrial mRNA 16S in any of the samples of *A. sculptum* tested, thus confirming the results from the dissections, which did not show any presence of parasitoid larvae.

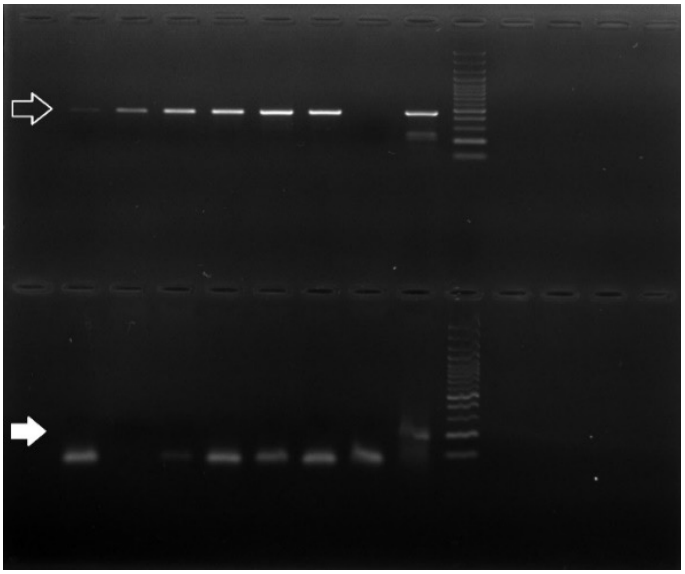

Figure 2. PCR amplification of the fragments corresponding to the mitochondrial RNA 16S of *Amblyomma sculptum* (hollow arrow) and mCOX I of *Ixodiphagus hookeri* (filled arrow). In both panels, the penultimate and the last sample correspond to the negative and positive controls, respectively.

BOHACSOVA et al. (2016) investigated the presence of the parasitoid *I. hookeri* in samples from a population of *Ixodes ricinus* collected in Slovakia. They found this parasitoid in 14% of the nymphs examined, regardless of whether they were engorged. This occurrence was probably linked to the environmental conditions of these authors' collection site, which perpetuated the population dynamics of the ticks and parasitoids. Those conditions contrast with those of present study, which was conducted in a neotropical region that is composed of urban areas and farms that have been highly modified from the original natural habitat through human activity.

AUTHORS' CONTRIBUTIONS

Conceptualization: Mendes, M.C. **Data curation:** Soares, L.A.; Fiorini, L.C.; Duarte, F.C.; Mendes, M.C. **Formal analysis:** Duarte, F.C.; Mendes, M.C. **Investigation:** Soares, L.A.; Fiorini, L.C.; Duarte, F.C.; Sampaio, P.H.S. **Methodology:** Soares, L.A.; Mendes, M.C; Romano, D.M.M; Almeida, I.B. **Project administration:** Mendes, M.C. **Supervision:** Mendes, M.C. **Validation:** Mendes, M.C. **Writing – original draft:** Soares, L.A.; Fiorini, L.C.; Duarte, F.C.; Mendes, M.C. **Writing – review & editing:** Soares, L.A.; Fiorini, L.C.; Duarte, F.C.; Mendes, M.C.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are available in the Acervo Digital at Animal Parasitology Laboratory of the Biological Institute in São Paulo city.

FUNDING

Not applicable

CONFLICTS OF INTEREST

The authors declare to have no competing interests regarding results with potentially profitable applications of the manuscript.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

Not applicable.

REFERENCES

BOHACSOVA, M.; MEDIANNIKOV, O.; KAZIMIROVA, M.; RAOULT, D.; SEKEYOVA, *Z. Arsenophonus nasoniae* and *Rickettsiae* infection of *Ixodes ricinus* due to parasitic wasp *Ixodiphagus hookeri*. *PLoS ONE*, San Francisco, v.11, n.2, e0149950, 2016. https://doi.org/10.1371/journal.pone.0149950

BRITES-NETO, J.; .BRASIL, J.; TAKEDA, G.A.C.G.; GUILLEN, A.C.; LABRUNA, M.B.; PINTER, A. Diferenciação morfométrica entre larvas de *Amblyomma sculptum* Berlese, 1888 e *Amblyomma dubitatum* Neumann, 1899. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v.70, n.5, p.1521-1528, 2014. https://doi.org/10.1590/1678-4162-9774

DAVIS, A.J. Bibliography of the Ixodiphagini (Hymenoptera, Chalcidoidea, Encyrtidae), parasites of ticks (Acari, Ixodidae), with notes on their biology. *Tijdschrift voor Entomologie*, Leiden, v.129, p.181-190, 1986.

FERRAZ, K.M.P.M. de B.; FERRAZ, S.F. de B.; MOREIRA, J.R.; COUTO, H.T.Z.; VERDADE, L.M. Capybara (*Hydrochoerus hydrochaeris*) distribution in agroecosystems: a cross-scale habitat analysis. *Journal of Biogeography*, Oxford, v.34, n.2, p.223-230, 2007. https://doi.org/10.1111/j.1365-2699.2006.01568.x

GUIMARÃES, J.H.; BATTESTI, D.M.B.; TUCCI, E.C. *Ectoparasitos de importância veterinária*. São Paulo: Plêiade/Fapesp, 2001.

HOWARD, L.O. A chalcidid parasite of a tick. *Entomological News*, Philadelphia, v.18, n.9, p.375-378, 1907.

HU, R.; HYLAND, K.E. Prevalence and seasonal activity of the wasp parasitoid, *Ixodiphagus hookeri* (Hymenoptera: Encyrtidae) in its tick host. *Systematic and Applied Acarology*, London, v.2, p.95-100, 1997. https://doi.org/10.11158/saa.2.1.12

KRAWCZYK, A.I.; BAKKER, J.W.; KOENRAADT, C.J.M.; FONVILLE, M.; TAKUMI, K.; SPRONG, H.; DEMIR, S. Tripartite interactions among *Ixodiphagus hookeri*, *Ixodes icinus* and deer: differential interference with transmission cycles of tick-borne pathogens. *Pathogens*, Basel, v.9, n.5, 339, 2020. https://doi.org/10.3390/pathogens9050339

LABRUNA, M.B.; KASAI, N.; FERREIRA, F.; FACCINI, J.L.H.; GENNARI, S.M. Seasonal dynamics of ticks (Acari: Ixodidae) on horses in the state of São Paulo, Brazil. *Veterinary Parasitology*, v.105, n.1, p.65-77, 2002. https://doi.org/10.1016/S0304-4017(01)00649-5

MWANGI, E.N.; KAAYA, G.P.; ESSUMAN, S.; KIMONDO, M.G. Parasitism of *Amblyomma variegatum* by a hymenopteran parasitoid in the laboratory, and some aspects of its basic biology. *Biological Control*, v.4, n.2, p.101-104, 1994. https://doi.org/10.1006/bcon.1994.1017

OLIVEIRA, P.R.; BORGES, L.M.F.; LOPES, C.M.L.; LEITE, R.C. Population dynamics of the free-living stages of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) on pastures of Pedro Leopoldo, Minas Gerais State, Brazil. *Veterinary Parasitology,* v.92, n.4, p.295, 2000. https://doi.org/10.1016/S0304-4017(00)00322-8

PARRA, J.R.P. Biological Control in Brazil: an overview. *Scientia Agricola*, Piracicaba, v.71, n.5, p.420-429, 2014. https://doi.org/10.1590/0103-9016-2014-0167

POLO, G.; LABRUNA, M.B.; FERREIRA, F. Basic reproduction number for the Brazilian Spotted Fever. *Journal of Theoretical Biology*, Amsterdam, v.458, p.119-124, 2018. https://doi.org/10.1016/j.jtbi.2018.09.011

SCINACHI, C.A. *Avaliação soroepidemiológica de animais sentinelas para febre maculosa brasileira e correlação com fragmentação vegetal na periferia sul da região metropolitana da cidade de São Paulo*. Dissertation (Master in Epidemiology) – Universidade de São Paulo, São Paulo, 2015. https://doi.org/10.11606/D.6.2015.tde-01062015-111615

SILVA, S.B.A. *Controle integrado de Amblyomma sculptum em zonas rurais*. Dissertation. (Master in Health, Food and Environmental Safety in Agribusiness) – Agência Paulista de Tecnologia dos Agronegócios, Secretaria de Agricultura e Abastecimento do Estado de São Paulo, São Paulo, 2019. https://doi.org/10.31368/PGSSAAA.2019D.SS07

SOUZA, A.P. *Variação populacional dos principais ixodídeos parasitas de bovinos e equinos em diferentes condições de manejo, nos municípios de Paracambi e Itaguaí no estado do Rio de Janeiro*. Thesis (PhD in Veterinary Sciences) – Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, 1990. Available from: https://tede.ufrrj.br/jspui/handle/jspui/3905. Access on: 24 Nov. 2020.

SZABÓ, M.P.; OLEGARIO, M.M.; SANTOS, A.L. Tick fauna from two locations in the Brazilian savannah. *Experimental and Applied Acarology*, v.43, 73, 2007. https://doi.org/10.1007/s10493-007-9096-8

WOOD, H.P. Notes on the life history of the tick parasite *Hunterellus hookeri* Howard. J*ournal of Economic Entomology*, v.4, p.425-431, 1911.