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Records of ectoparasites on humans and wildlife in southeastern Brazil

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ABSTRACT

We report ectoparasites found on humans and wildlife from different locations in southeastern Brazil. Ectoparasites were stored in 70% alcohol and visually identified under a stereo microscope with the help of identification keys. Genomic DNA of ticks was extracted and used in the Polymerase Chain Reaction (PCR) to detect the presence of tick-borne pathogens. Sixteen species of ectoparasites (six ticks, one louse, four fleas, four flies, and one bedbug) were identified on ten host species, including humans, other mammals, and birds. We report the first record of the parasitism of *Amblyomma longirostre* on *Didelphis aurita*, *Microlynchia pussila* on *Turdus leucomelas*, *Ornithoica vicina* and *Ornithocoris pallidus* on *Psittacara leucophthalmus* and *O. pallidus* on *Homo sapiens*. In addition, we also report the first record of *O. vicina* in the state of Minas Gerais and the infection by *Rickettsia* sp. in *Amblyomma varium*.

1. Introduction

Brazil is a continental country, with six biomes, a long marine coast, and a plethora of hydrographic basins holding a huge diversity of animal and plant species. Among these biomes, the Atlantic Forest and the Cerrado, are considered biodiversity hotspots due to their high species richness and destruction levels. Part of the threat to the country's biodiversity comes from the urbanization process and agricultural and livestock practices (MMA 2017, Myers et al., 2000). These environmental changes have been responsible for the invasion of the habitats of many wild animals, and more frequent encounters with humans (Gardner 2008).

Despite several studies on the Brazilian fauna, basic information such as the richness of parasitic species is still incipient if compared to that of domestic animals (Grisi et al., 2014, Mays et al., 2014, Valli et al., 2018, Hurtado and Giraldo-Ríos 2019, Rashid et al., 2019, Pérez de León et al., 2020). However, neglecting this may be quite mistaken, as wild animals can act as important pathogenic hosts that impact both rearing animals and humans (Bezerra-Santos et al., 2021a).

Ectoparasites are responsible for transmitting pathogens, toxic injuries, allergic reactions causing illness and even death (Potti et al., 1999, Schoeler and Wikel 2001, Szabó et al., 2002, Wagner et al., 2008, Pitala et al., 2009, Webster et al., 2014). Several pathogens that use ectoparasites as vectors are zoonotic, with *Rickettsia rickettsii* being the most harmful bacteria to humans in Brazil, causing high fever, severe hemorrhagic manifestations, and high lethality (Labruna 2009, Barbieri et al., 2014). In this study we present new data of ectoparasites infesting wildlife and humans, including new records of the host-parasite relationship, from Southeastern Region of Brazil and the molecular identification of pathogens.

2. Materials and Methods

The Ectoparasites collected from wild animals were donated to the Parasitology and Parasitic Diseases Laboratory of the Department of Veterinary Medicine/ Universidade Federal de Viçosa. These ectoparasites were preserved in 70% ethanol and identified using a stereomicroscope and taxonomic keys for ticks (Barros-Battesti et al., 2006, Martins et al., 2010, 2016), fleas (Bicho and Ribeiro 1998, Linardi et al., 2014, Linardi and Guimarães 2000), lice (Tuff 1977), flies (Graciolli and Carvalho 2001, 2003), and bedbugs (Usinger 1966, di Iorio et al., 2010).

Genomic DNA was extracted from ticks according to Ramos et al. (2015) and ectoparasites were screened by a Polymerase Chain Reaction (PCR) protocol for *Rickettsia* spp., A*naplasmataceae*, *Borrelia* spp., and *Babesia* spp.

using primers and amplification conditions described in Table 1. DNA of *Rickettsia vini* (Preventive Veterinary Medicine and Animal Health Department of the University of São Paulo), *Borrelia garini* (Epidemiology and Public Health Department of the Federal Rural University of Rio de Janeiro), *Ehrlichia canis* and *Babesia canis* (Veterinary Medicine Department of the Federal University of Viçosa) were used as positive control, and nuclease free water as negative control. Amplification was carried out using Taq Pol Master Mix Green 2X) according to the manufacturer's instructions and protocols previously described for each primer (Table 1).

Pathogens	Gene	Amplicon size (bp)	DNA amplification	Primer sequence	References
Rickettsia spp.	gtlA	300-380	35 cycles of denaturation (20s at 95°C), annealing (30s at 48°C), and extension (2 min at 60°C)	5'GGGGGCCTGCTCACGG CGG3' and 5'ATTGCAAAAAGTACAG TGAACA3	(Regnery et al., 1991)
Anaplasmataceae	16S	345	40 cycles of denaturation (20s at 94°C), annealing (30s at 55°C), and extension (5 min at 72°C)	5'GGTACCYACAGAAGAA GTCC3' and 5'TAGCACTCATCGTTT ACA GC3'	(Martin et al., 2005)
Borrelia spp.	Fla	482	35 cycles of denaturation (20s at 94°C), annealing (30s at 55°C), and extension (5 min at 72°C)	5'AGAGCAACTTACAGAC GAAATTAAT3' and 5'CAAGTCTATTTTGGAAA GCACCTAA3'	(Skotarczak et al., 2002)
Babesia spp.	18S	411-452	35 cycles of denaturation (20s at 95°C), annealing (20s at 60°C), and extension (7 min at 72°C)	5'GTCTTGTAATTGGAATG ATGG3' and 5'TAGTTTATGGTTAGGAC TACG3'	(Casati et al., 2006)

Table 1 *– Primers used for molecular analysis.*

A total of 16 species of ectoparasites, including ticks, lice, fleas, flies and bedbugs, were collected on 12 host species, including humans, mammals and wild birds, in southeastern Brazil (Table 2).

Host	Ectoparasites	Municipalities/ States
	Ticks	
"Javaporco"	*Amblyomma sculptum* (4M, 1F)	Bueno Brandão/ MG
Bradypus variegatus	*Amblyomma varium* (1M[a], 1 F)	Linhares/ ES
Didelphis aurita	*Amblyomma longirostre*[b] (1M) and *Ixodes loricatus* (2F)	Viçosa/ MG
Nasua nasua	*Amblyomma ovale* (4M, 4F)	Viçosa/ MG
Cariama cristata	*Amblyomma sculptum* (2N)	Viçosa/ MG
Homo sapiens	*Amblyomma* sp. (1L)	Ipatinga/ MG, Viçosa/ MG, Ponte Nova/ MG, Bueno Brandão/ MG
	Amblyomma sculptum (3M, 5F, 16N)	
	Amblyomma ovale (1F)	Carmo do Rio Claro/ MG
	Rhipicephalus microplus (1M)	Viçosa/ MG
	Lice	
"Javaporco"	*Haematopinus suis* (2)	Bueno Brandão/ MG
	Fleas	
Didelphis aurita	*Ctenocephalides felis* (1) and *Xenopsylla cheopis* (1)	Viçosa/ MG
"Javaporco"	*Tunga penetrans* (S*)	Bueno Brandão/ MG
Nasua nasua	*Ctenocephalides felis* (12)	Viçosa/ MG
	Flies	
Artibeus lituratus	*Paratrichobius longicrus* (S**)	Viçosa/ MG
Columba livia	*Pseudolynchia canariensis* (1M)	Viçosa/ MG
Turdus leucomelas	*Microlynchia pussila*[b] (1F)	Viçosa/ MG
Psittacara leucophthalmus	*Ornithoica vicina*[b,c] (2F)	Coimbra/MG
	Bedbugs	
Psittacara leucophthalmus' nest	*Ornithocoris pallidus*[b]	Viçosa/ MG
Homo sapiens	*Ornithocoris pallidus* (S***)[b]	Viçosa/ MG

Table 2 *– Hosts and their ectoparasites were collected in southeastern Brazil.*

3. Discussion

3.1. Tick records

Our study presents for the first time the parasitism of *Amblyomma longirostre* on *Didelphis aurita*, which is popularly known as black-eared opossum and found across the eastern Neotropical region, from Brazil to southeastern Paraguay and northeastern Argentina, and is the most widely distributed species of *Didelphis* in Minas Gerais (Gardner 2008, Cáceres et al., 2009, Bezerra-Santos et al., 2021b). This genus is frequently infested by ectoparasites such as the ticks *Amblyomma dubitatum* (Horta et al., 2007), *Amblyomma incisum* (Lamattina et al., 2018a), *Amblyomma aureolatum* (Luz et al., 2018a), *Amblyomma brasiliense* (Szabó et al., 2013, Lamattina et al., 2018a, 2018b), A*mblyomma coelebs* (Szabó et al., 2013, Lamattina et al., 2018a, 2018b), *Amblyomma ovale* (Szabó et al., 2013, Lamattina et al., 2018b, 2018c), *Amblyomma fuscum* (Martins et al., 2009, Szabó et al., 2013), *Amblyomma sculptum* (Saraiva et al., 2012, Szabó et al., 2013, Bezerra-Santos et al., 2020), *Haemaphysalis juxtakochi* (Lamattina et al., 2018b), I*xodes amarali* (Oliveira et al., 2014), *Ixodes auritulus* (Saraiva et al., 2012, Szabó et al., 2013), *Ixodes luciae* (Saraiva et al., 2012, Szabó et al., 2013), and *Ixodes loricatus* (Bezerra-Santos et al., 2020, Horta et al., 2007, Luz et al., 2018a, Oliveira et al., 2014, Saraiva et al., 2012, Szabó et al., 2013). The occurrence of *A. longirostre* on this synanthropic opossum species (Jansen 2002) may be worrying because this tick can harbor *Rickettsia* spp. (Labruna et al., 2004, McIntosh et al., 2015, Ogrzewalska et al., 2008, Żeringóta et al., 2017), as well as some other tick species that parasitize this opossum species (Barbieri et al., 2014).

We highlight the record of *Rickettsia* sp. among the zoonotic pathogens that use ticks as vectors and the detection of *Rickettsia* sp. in a male of *Amblyomma varium* collected in a Brown-throated Sloth (*Bradypus variegatus*). This tick species is popularly known as Sloth's Giant Tick, whose geographical range matches that of the sloth which is widely distributed throughout the Atlantic Forest (Marques et al., 2002). This parasitism was already recorded in the states of São Paulo (Gonzalez et al., 2017, Marques et al., 2002, Martins 2017a, 2017b, Sanches et al., 2014), Rio de Janeiro (Spolidorio et al., 2012), Pernambuco (Marques et al., 2002), and Paraíba (Dantas-Torres et al., 2010).

Amblyomma ovale was another tick species found in our records. This species is of medical and veterinary importance due to its potential to act as a vector of *Rickettsia*, more specifically the species *Rickettsia parkeri* (Lamattina et al., 2018c). Parasitism by this tick on coatis, *Nasua nasua*, has already been reported in Argentina (Lamattina et al., 2018b, 2018c), Minas Gerais and Mato Grosso do Sul states, and elsewhere in Brazil (Sousa et al., 2017, Estevam et al., 2020, Garcia et al., 2013, Labruna et al., 2005).

Our study also significantly contributed to the knowledge of relationships of ticks and humans in Brazil (Nogueira et al., 2022). In our country studies reporting this parasitism are scarce when compared to other countries in which these data are easily collected because people seek medical assistance for guidance and removal of attached ticks (Blanda et al., 2017, Briciu et al., 2016, Karasartova et al., 2018, Orkun et al., 2014). Although our findings were concentrated in the state of Minas Gerais, they are quite relevant since the only records of this parasitism in the state are on the following species: *Ornithodoros mimon* (Labruna et al., 2014), *Ornithodoros talaje* (Carvalho 1942), *Amblyomma auricularium* (Szabó et al., 2020), *A. brasiliense* (Aragão 1936, Luz et al., 2018b), *Amblyomma oblongoguttatum* (Aragão 1936), *A. ovale* (Aragão 1936), *Amblyomma parvum* (Szabó et al., 2020), *A. sculptum* (Lemos et al., 1997, Martins et al., 2016, Szabó et al., 2018), *Dermacentor nitens* (Szabó et al., 2020) and *Rhipicephalus microplus* (Szabó et al., 2020). In addition, parasitism by *A. ovale* and *R. microplus* was registered only once in Minas Gerais. Furthermore, the most common species found in humans in our results was *A. sculptum*, which is an extremely important species in the One Health concept, as it is a vector of pathogens such as *Rickettsia rickettsii* (Esteves et al., 2019), *Theileria equi* (Scoles and Ueti 2013), and *Babesia caballi* (Sousa et al., 2018), which may cause injuries to the endothelial tissue and in the circulatory, digestive, nervous, urinary and respiratory systems, affecting development and leading to the death of the host (Fiol et al., 2010, Onyiche et al., 2019, Vieira et al., 2002).

In addition, we also found *A. sculptum* in *Cariama cristata* and in “Javaporco”. This parasitism has been also previously reported (Luz et al., 2016, Nogueira et al., 2020) and is an important finding for public health, as *C. cristata* is a species that has been in contact with humans, mainly in pasture areas (Silva et al., 2016, Alexandrino et al., 2019). The “Javaporco” is an invasive species worldwide distributed, which had widely spread in Brazil in the last three decades (Ramos et al., 2014, Pedrosa et al., 2015). It is frequently found in rural areas close to houses, showing aggressive behavior towards people and animals (Pimentel et al., 2005), and certainly presents the potential for dispersal of ectoparasites in the environment.

3.2. Lice and flea records

The lice *Haematopinus suis* and the flea *Tunga penetrans* on feral pigs were recorded before in Minas Gerais State. This louse species is often found on pigs (Gipson et al., 1999, Foata et al., 2006, Acosta et al., 2019), while this flea can have multiple hosts, including companion animals and humans (Linardi et al., 2014). This parasitism also may be worrying given the potential of wildboars to act as hosts for ectoparasites and the studies of pathogens that they can transmit are scarce. In addition, the parasitism of the opossum by fleas is important, as mentioned previously, and has already been reported for other flea species such as *Ctenocephalides felis felis* (Horta et al., 2007, Bezerra-Santos et al., 2020), *Polygenis rimatus* (Urdapilleta et al., 2019), *P. occidentalis* (Pinto et al., 2009), *P. atopus* (Horta et al., 2007),

Adiratiosylla intermedia (Pinto et al., 2009), *A. antiquorum* (Pinto et al., 2009), *Leptosylla segnis* (Oliveira et al., 2007) and *Xenopsylla cheopis* (Oliveira et al., 2007, Bezerra-Santos et al., 2020).

3.3. Fly records

Among the four species of flies found in our study, *Paratrichobius longicrus* belongs to the Streblidae family and *Pseudolynchia canariensis*, *Microlynchia pussila* and *Ornithoica vicina* belong to the Hippoboscidae family. The flies of both families are parasites, with Streblidae species being already known as parasites of bats (Dick and Miller 2010), and Hippoboscidae of other mammals and birds (Bequaert 1957).

We found *P. longicrus* on the bat *Artibeus lituratus* and *P. canariensis* on a specimen of Rock Dove (*Columba livia*) both of them being well-known host-parasite relationships (Arcoverde et al., 2007, Bertola et al., 2005, Amaral et al., 2013, Graciolli and Rui 2001, Rui and Graciolli 2005, Silva and Baldo 2007, Valim and Gazêta 2007). We also found the Pale-breasted Thrush (*Turdus leucomelas*) parasitized by *M. pussila*, a species of louse fly that is recorded mainly in Columbidae (pigeons and doves) species in Brazil (Bequaert 1957, Silva and Pichorim 2013, Valim et al., 2004). Although Luz et al. (1915) recorded *M. pussila* in Minas Gerais, this is the first record of this fly on *T. leucomelas*.

Our study also presents the first record of *O. vicina* in the state of Minas Gerais and the first parasitism of this fly on P*sittacara leucophthalmus*. This parakeet is quite common in the study area, being so well adapted to human environments that it is considered a pest or nuisance species in some regions of southeastern Brazil due to damage to crops, and synanthropic habits in proximity to humans and domestic animals, and damages to house roofs and electric and other wires (Carvalho et al., 2019). Despite this, the few existing studies of ectoparasites for this species cover only feather mites (Pedroso and Hernandes 2016, Hernandes and Pedroso 2017). In addition, *O. vicina* is a fly ranging across several countries in the Americas, with records in Brazil in the states of São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (Graciolli and Carvalho 2003). About 86 genera of birds have been recorded as hosts of this fly (Maa 1969) In Brazil, encompassing passerines (Passeriformes) and non-Passerines such as owls (Strigiformes) (Bequaert 1954, Graciolli and Carvalho 2003, Vaz and Teixeira 2016).

3.4. Bedbug records

We have found a male of *Ornithocoris pallidus* in a P*sittacara leucophthalmus*' nest in Viçosa and in another locality of the city. This type of bedbug was frequently parasitizing a woman while she slept and was later identified a nest close to her residence. This bedbug belongs to the Cimicidae family, which is composed of exclusively hematophagous species (Forattini 1990), often found in bird nests and crevices present in the environment (Usinger 1966). Three species of this family stand out in public health due to their association with humans: *Cimex lectularius*, which frequently occurs in cities in temperate climate zones, *Cimex hemipterus*, which is widely distributed in the Neotropics and *Leptocimex boueti* which is restricted to Africa (Usinger 1966, Forattini 1990). The species *O. pallidus*, until then, has been recorded in the swallows *Pygochelidon cyanoleuca* and *Progne subis*, and in the chicken (*Gallus gallus*) (Usinger 1966, Carpintero et al., 2011). The associations of these species found in this study presented have been made for the first time and are worrying as their host birds are close to humans and bed bugs can accumulate in large numbers in the houses, impairing human's life quality through dermatitis generated by their parasitism (Forattini 1990).

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