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Detection of *Rickettsia felis* in ectoparasites collected from domestic animals

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Abstract

Ticks and fleas are arthropods widely distributed around the world involved in the transmission of various vector-borne diseases (VBDs), including Brazilian Spotted Fever (BSF), Baggio-Yoshinari Syndrome and the plague, with outstanding consequences for the public health. The aim of this study was to investigate the presence of *Rickettsia* spp., *Borrelia* spp. and *Yersinia pestis* in arthropods collected from dogs, cats and horses living in the state of Pernambuco, Northeastern Brazil. From January 2017 to April 2019, ectoparasites were collected, morphologically identified and molecularly analysed through PCR and sequencing. In total 401 specimens were collected from 86 animals, being 68% (n = 273) and 32% (n = 128) from rural and urban areas, respectively. The most commonly detected species were the ticks *Dermacentor nitens*, *Amblyomma sculptum*, *Rhipicephalus sanguineus* sensu lato (s.l.), *Rhipicephalus microplus*, and *Amblyomma ovale*, and the fleas *Ctenocephalides felis* and *Ctenocephalides canis*. DNA of *Rickettsia felis* was detected in *D. nitens* collected from horses, and *C. felis*, and *R. sanguineus* s.l. collected from dogs. All samples scored negative for *Borrelia* spp. and *Y. pestis* DNA. This study provides valuable data on ectoparasite fauna from domestic animals and identifies the circulation of a zoonotic pathogen (i.e., *R. felis*) in the population of the arthropods assessed. Therefore, preventive measures should be adopted in order to reduce the risk of occurrence of neglected VBD caused by this pathogen in animal and human hosts.

Keywords *Rickettsia felis* · Vector-borne pathogens · Fleas · Ticks

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Introduction

The majority of emerging and re-emerging diseases affecting humans originate from zoonotic pathogens (Zanela 2016) transmitted by vectors (e.g., mosquitoes, ticks and fleas) (Ewald 1983). In fact, the involvement of blood-sucking vectors in ancient epidemics, as that caused by plague, has been speculated for a long time (Simond 1898) and currently the development of molecular techniques has opened a new chapter on the study of these diseases, revealing unprecedented information on the interaction between host, vector and parasite at the molecular level.

In Brazil the role of ticks as vectors of pathogens such as *Rickettsia* spp. (Moraes-Filho 2017; Aguirre et al. 2018) and the role of fleas as vectors of *Yersinia pestis* (Linardi and Guimarães 2000; Linardi 2017) is well documented. Accordingly, zoonoses such as the Brazilian Spotted Fever (BSF) and plague have acquired a great importance over time. BSF is a disease of public health concern caused by *Rickettsia rickettsii* and transmitted mainly by *Amblyomma* ticks (Szabó et al. 2013; Moraes-Filho 2017). Clinically, this parasitic condition is characterized by fever, joint pain and general vasculitis (Del Fiol et al. 2010). In recent years, several cases have been reported, especially from the Southeast and South regions of the country (Oliveira et al. 2016). At the same time, there is speculation that the prevalace of the infection in the Northeastern region is underestimated, the first fatal case having been documented only in 2016 (Oliveira et al. 2018). It is important to highlight the current importance of *Rickettsia felis*, which has been considered an emerging rickettsial pathogen and whose distribution overlaps the occurence area of *Ctenocephalides felis* fleas (Brown and Macaluso 2016). The spreading of this pathogen represents a threat to the human population due to the lack of host specificity for the cat flea (Pérez-Osorio et al. 2008).

On the other hand, the plague caused by *Y. pestis* is responsible for a severe, acute and progressing febrile illness, with significant mortality rates and clinically characterized by three clinical conditions (bubonic, pneumonic and septicemic diseases) (Brasil 2008). It is important to note that in some Brazilian regions this disease is still a threat (CDC 2019) due to the existence of two natural foci—Foco do Nordeste and Foco da Serra dos Órgãos—located in areas with specific ecological and geographical conditions (Brasil 2017). Although the last human case in Brasil occurred in 2005 (Tavares et al. 2012), the rich fauna of rodents and fleas allow the circulation of *Y. pestis* in these foci.

Another important disease is caused by spirochetes within the *Borrelia burgdorferi* complex, which are primarily transmitted by ticks of the genus *Ixodes*. In Brazil, this disease is known as the Baggio-Yoshinari Syndrome (BYS) and the main clinical sign observed in patients is the Erythema migrans, often associated with arthritis (Yoshinari et al. 2010; Pritt et al. 2016). Until now, only few cases have been notified (Yoshinari et al. 2007; Carranza-Tamayo et al. 2012; Rosa Neto et al. 2014), and although the clinical suspect exists since 1987 (Talhari et al. 1987) the isolation of the *Borrelia* species was only achieved in 2010 (Talhari et al. 2010; Santos et al. 2011).

Recently, global warming has facilitated and increased contact between humans and vectors in part due to the spreading of these arthropods or their growing abundance in endemic areas (Estrada-Peña et al. 2012). As a matter of fact, the risk of infection by vector-borne pathogens has increased worldwide (Ogden and Lindsay 2016; Semenza and Suk 2018; Sonenshine 2018; Petersen et al. 2019) and (re) emerging infections such as BSF, BYS and plague are still a real threat. Therefore, the aim of this study was to investigate the presence of *Rickettsia* spp., *Borrelia* spp. and *Y. pestis* in ectoparasites

collected from dogs, cats and horses living in the state of Pernambuco, Northeastern Brazil.

Materials and methods

Study area and ethical aspects

This study was conducted in various municipalities (Fig. 1) of the state of Pernambuco, Northeastern Brazil. The region is situated at a mean altitude of 842 m above sea level, with a semi-arid climate and an annual temperature mean of 22 °C (ranging from 17 to 30 °C), rainfall mean of 147 mm (ranging from 25 to 295 mm) and a relative air humidity of about 90%. It is an ecological area defined as high altitude swamp which is characterized by an oasis of humid vegetation surrounding the Caatinga. Therefore, it presents favorable natural conditions for the establishment and development of vector populations (Rodrigues et al. 2008; Santos et al. 2014). In addition, it is inserted in a natural foci (i.e., Foco do Nordeste) of risk for the occurrence of plague.

The Ethics Committee for Animal Experimentation of the 'Universidade Federal Rural de Pernambuco' approved all procedures herein performed (approval number: 94/2018).

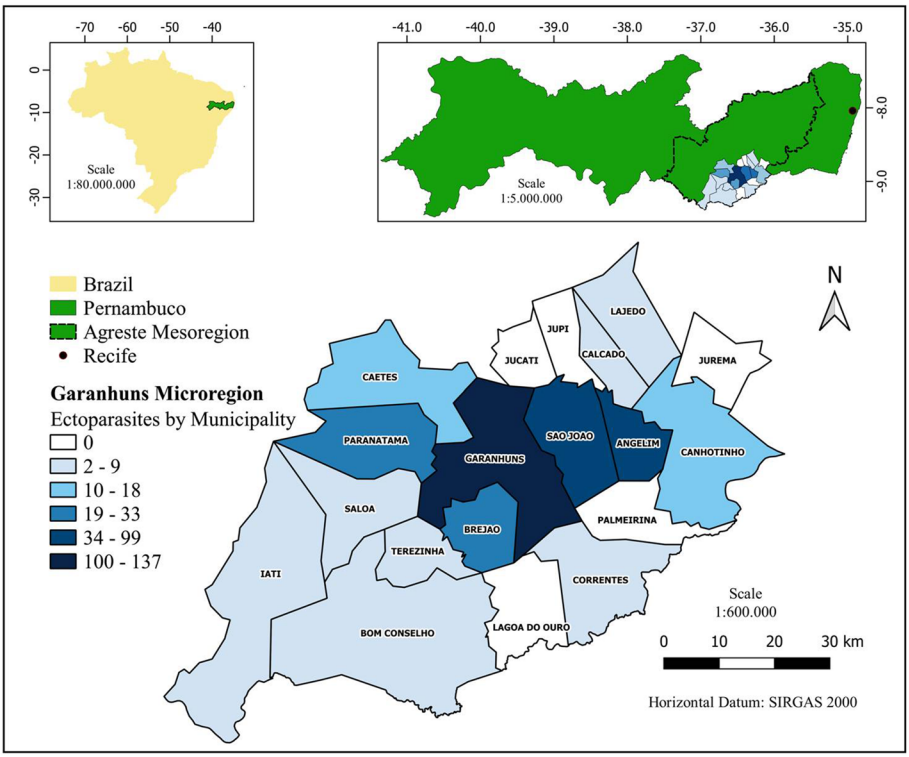

Fig. 1 Study area—Microregion of Garanhuns, state of Pernambuco, Northeastern Brazil

Sampling and morphological identification

From January 2017 to April 2019 ectoparasites were collected from dogs, cats and horses living in urban and rural zones. Samples obtained in urban areas were from domiciled animals living inside the urban perimeters of each municipality. Conversely, those obtained in rural areas were from farms of bovine milk production, which is one of the most important economical activities of the region. Animals were selected by convenience irrespective of their sex, age or breed.

Each animal was physically examined for a period of 5 min. The presence of arthropods was assessed through the examination of the following body regions: head, ears, breast-neck, thorax, abdomen, fore and back limbs, inter-digital areas (dogs and cats), axilla, tail and inguinal area. Ectoparasites were removed with the aid of tweezers, washed in saline solution (0.9% NaCl) and placed in plastic vials containing 70% ethanol until laboratory analysis. Specimens were quantified, separated according to life stage/sex, and then identified morphologically by using dichotomous keys (Linardi and Guimarães 2000; Aragão and Fonseca 1961; Guimarães et al. 2001; Barker and Murrell 2004). All animals sampled did not use ectoparasiticide compounds over the previous 4 months before sampling.

Pools (n = 131) containing 1–3 individuals were prepared and kept at − 20 °C until molecular analysis. The segregation of pools was based on the species and life stage of ectoparasites. In addition, each pool included samples merely from a single animal.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from pools using a protocol previously described (Ramos et al. 2015). Each pool was tested for DNA detection of *Rickettsia* spp., *Borrelia* spp. and *Y. pestis* using the primers reported in Table 1. All reactions included positive and negative controls. Amplified products were revealed through electrophoresis using 1.5% agarose gel, stained with GelRed (Biotium) and viewed under an UV transilluminatior. Amplicons were purified using ExoSAP-IT (Thermo Fisher Scientific), according to manufacturer's instructions, and sequenced in both directions by the Sanger method (Sanger et al. 1977) using an automatic sequencer ABI 3130 Genetic Analyser (Applied Biosystems). The DNA sequences identity was defined through comparison with others from the GenBank using the BLASTn search tool (Altschul et al. 1990).

Data analysis

A descriptive analysis was performed to obtain absolute and relative frequencies. In addition, the differences of species collected in rural and urban areas were analysed by using the χ^2 test ($\alpha = 0.05$). All analyses were carried out using the statistical software BioEstat v.5.3 (Ayres et al. 2007).

Table 1 Primers used for amplifying selected tick-borne pathogens

Pathogens	Target gene	Primer/sequence (5′–3′)	Product size (bp)	References
Rickettsia spp.	*gltA*	CS-78 (GCAAGTATCGGTGAGGATGTAAT) CS-323 (GCTTCCTTAAAATTCAATAAATCAGGAT)	401	Labruna et al. (2004)
Borrelia spp.	*flagE*	flgE 262 (TCCTCCGGGATTCATACAAG) flgE 262 (TGGGTGCAAATGTAGGTGAA)	262	Rezende et al. (2016)
Yersinia pestis	*Pla*	Yp1 (ATCTTACTTTCCGTGAGAA) Yp2 (CTTGGATGTTGAGCTTCCTA)	478	Hinnebusch and Schwan (1993)

Results

In total 401 ectoparasites (male = 96; female = 259; and nymphs = 46) were collected from 86 animals (cats = 8; dogs = 22; horses = 56) during the whole study period (Table 2), 68% (n = 273) from rural areas and 32% (n = 128) from urban areas. In particular, fleas predominated in urban areas whereas ticks were more common in rural zones ($\chi^2 = 56.94$, $p < 0.0001$).

Two flea species (*Ctenocephalides canis* and *C. felis*) and five tick species (*Amblyomma ovale*, *A. sculptum*, *Dermacentor nitens*, *Rhipicephalus microplus* and *R. sanguineus* s.l.) were identified. Table 2 summarizes the results of molecular examination according to the arthropod species. Out of all positive samples only (*D. nitens* from a horse) was collected from an animal from a rural area. All scored negative for *Borrelia* spp. and *Y. pestis* DNA.

The sequences derived from the amplicons obtained in PCR for *Rickettsia* spp. showed identity > 99% with *R. felis* sequences available in the GenBank. The DNA sequences obtained in the present study were deposited in the GenBank under the access numbers shown in Table 2.

Discussion

This study confirms the presence of *R. felis* in ectoparasites collected from dogs and horses in the study area. All species of ticks and fleas reported in this study have already been described as infesting cats, dogs and horses in tropical regions (Ehlers et al. 2019). The climatic conditions observed in these areas favour the establishment of these arthropods in vertebrate hosts (Kumsa et al. 2019). Most of the ectoparasites collected were ticks from horses, including *A. sculptum*, which is the vector of *R. rickettsii*, the etiological agent of BSF (Moraes-Filho 2017). *Amblyomma ovale* was collected from a dog living in an urban area. This tick species is frequently reported in wild carnivores, occasionally sharing the same environment with domestic dogs (Labruna et al. 2000). It has already been demonstrated that the proximity among animals, ectoparasites and humans may be considered a risk due to the possibility of sharing pathogens with each other (Esch and Petersen 2013).

The presence of *R. felis* in *C. felis* and *R. sanguineus* s.l. collected from dogs, and in *D. nitens* collected from horses is important due to the possibility of transmission to vertebrate hosts, including human beings (Pacheco et al. 2011; Angerami et al. 2012). The detection of *R. felis* DNA in these invertebrates does not confirm the vector role of these arthropods, but it indicates the circulation of this pathogen in the area of study. It is known

Table 2 Ectoparasites (numbers) collected from domestic animals and positivity for pathogen tested

Host	Ectoparasite	No.	Stage and sex (adult)			Area		Positive pools/ tested pools	*Rickettsia felis*	GenBank accession numbers
			Nymphs	Male	Female	Rural	Urban			
Cat	*Ctenocephalides felis*	10	0	2	8	3	7	0/9	0	–
Dog	*C. canis*	5	0	5	0	0	5	0/3	0	–
	C. felis	42	0	9	33	8	34	4/18	4	MN726355, MN726356, MN726357, MN726358
	Amblyomma ovale	1	0	0	1	0	1	0/1	0	–
	Rhipicephalus sanguineus	48	5	16	27	31	17	1/31	1	MN726359
Horse	*A. sculptum*	52	3	15	34	52	0	0/7	0	–
	Dermacentor nitens	234	38	47	149	170	64	1/57	1	MN726354
	R. (Boophilus) microplus	9	0	2	7	9	0	0/5	0	–
	Total	401	46	96	259	273	128	6/131	6	

that *C. felis* is recognized as the most relevant vector of *R. felis* due to its ability to infect progeny by transovarian transmission (Azad et al. 1992). The absence of *R. rickettsi* was an interesting finding. Although a fatal case of BSF has already been reported in Northeastern Brazil (Oliveira et al. 2018), this kind of infection in vertebrate hosts and arthropods in this area has been poorly investigated and data are scarse. Also, the presence of *R. felis* does not exclude the possibility of detection of other rickettsial organisms, rather it may suggest the predominance of this pathogen in invertebrates in the study area.

From an epidemiological perspective, the detection of this emerging vector-borne pathogen in urban areas is interesting and follows a similar trend reported in other regions of the world (Raoult et al. 2001). The disease in humans is called flea-born spotted fever and the symptoms of infection range from non-specific flu-like illness to severe multisystemic disease with generalized vasculitis (Teoh et al. 2016). In Brazil, these clinical signs are also observed in diseases caused by other rickettsial organisms (murine typhus and Q fever) and dengue, which makes diagnosis difficult (Oliveira et al. 2002). This suggests that infections caused by *R. felis* are underestimated, therefore their real impact on public health remains unknow.

Unfortunately, in this study animals were not investigated for detection or exposure to these pathogens. To have information about the real condition of animals it would be important to corroborate our findings. The non detection of *Y. pestis* and *Borrelia* spp. DNA does not confirm the absence of both pathogens in this area, but indicates that these invertebrates most likely have not established contact with vetebrate hosts involved in the epidemiological cycle of these organisms.

In conclusion, the data herein reported indicates the circulation of a zoonotic pathogen (i.e., *R. felis*) in the population of arthropods assessed. Therefore, preventive measures should be adopted in order to reduce the risk of occurrence of neglected vector-borne disease caused by this pathogen in animal and human hosts.

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Author contributions JCPdO and RANR conceived and designed this study. Material preparation and data collection was performed by JCPdO. Data analysis was conducted by AG, GAdC and LCA. Molecular analysis was performed by JCPdO, GHR and CAdNR. JCPdO and RANR wrote the original draft and all co-authors commented and contributed intellectually on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author declares that they have no competing interests.

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