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Evaluation of infection by *Borrelia* sp. in domestic and wild mammals and ticks from the Catimbau National Park, Pernambuco[1]

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ABSTRACT.- Farias I.F., Moura L.M.D., de Sá J.C.B., Souza D.S., Torres-Santos P.T., Oliveira J.B., Muñoz-Leal S. & Horta M.C. 2023. **Evaluation of infection by *Borrelia* sp. in domestic and wild mammals and ticks from the Catimbau National Park, Pernambuco.** *Pesquisa Veterinária Brasileira 43:e07203, 2023*. Universidade Federal do Vale do São Francisco, Av. José de Sá Maniçoba s/n, Centro, Petrolina, PE 56304-917, Brazil. E-mail: mauricio.horta@univasf.edu.br

Spirochetes in the genus *Borrelia* (Spirochaetes) are the etiological agents of two zoonotic diseases that have rodents as reservoirs: the Lyme disease, transmitted by ixodid ticks (Ixodidae), and tick-borne relapsing fever, which is transmitted mostly by argasid ticks (Argasidae). The information on the epidemiology of pathogens that affect animals is scarce, especially in places that have been barely studied, such as the Caatinga biome. Thus, in the present study, we aimed to detect DNA of *Borrelia* in the blood of wild and domestic mammals, as well as in ticks collected in the Catimbau National Park, State of Pernambuco, Northeast Brazil. Blood was collected from 77 captured wild animals belonging to Didelphidae (45), Echimyidae (21), Cricetidae (7), Caviidae (2), and Dasypodidae (2) families. Additionally, blood samples from 120 sheep, 119 goats, 70 dogs, and 47 cattle were obtained. A total of 286 ticks were collected in the environment and identified as *Ornithodoros* cf. *tabajara* (188) and *Ornithodoros rietcorreai* (98); in addition, larvae of *Ornithodoros* spp. were collected on *Thrichomys laurentius* (4 specimens) and *Monodelphis domestica* (1); larvae of *O. rietcorreai* on *Didelphis albiventris* (2) and *T. laurentius* (1); *Amblyomma parvum* on *T. laurentius* (4); *Rhipicephalus sanguineus* (*sensu lato*) on dogs (121) and *Rhipicephalus microplus* on cattle (47). All blood samples, 120 free-living ticks, 46 *O. rietcorreai* and 74 *O.* cf. *tabajara*, and 12 *R. sanguineus* s.l. were submitted to DNA extraction to perform a polymerase chain reaction (nested PCR), aiming at amplifying a fragment of the *flaB* gene present in all species of *Borrelia*. Only one free-living specimen of *O.* cf. *tabajara* (1.3%) was positive. Negative detections in mammals may be related to the absence of infection or the low concentration of the spirochetes in the hosts. The low prevalence of *Borrelia* found in ticks can be explained by the low infection rates that these spirochetes depict in their vectors. New studies focusing on the detection of antibodies, as well as on the sequencing and isolating *Borrelia* spp. should be encouraged for a better understanding of the epidemiology of these bacterial agents in the region.

INDEX TERMS: *Ornithodoros* spp., borrelioses, Catimbau National Park.

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RESUMO.- [**Avaliação de infecção por *Borrelia* sp. em mamíferos domésticos, selvagens e carrapatos do Parque Nacional do Catimbau, Pernambuco.**] No gênero *Borrelia* (Spirochaetes) encontram-se dois grupos de agentes responsáveis por duas doenças zoonóticas, que têm roedores como reservatórios e acometem humanos: o grupo de Lyme, transmitido por carrapatos ixodídeos, e a febre recorrente, que pode ser transmitida por carrapatos argasídeos ou piolhos. Poucas são as informações sobre epidemiologia e agentes patogênicos que acometem animais, podendo representar uma riquíssima fonte de informações, principalmente em locais nunca estudados, pois podem ser bioindicadores de qualidade do ambiente e saúde. Desta forma, no presente estudo foi realizada a pesquisa de *Borrelia* spp. em amostras de sangue de mamíferos silvestres e domésticos, assim como de carrapatos, do Parque Nacional do Catimbau, Pernambuco. Foi realizada a captura e colheita sanguínea de 77 animais pertencentes às famílias Didelphidae (45), Echimyidae (21), Cricetidae (7), Caviidae (2) e Dasypodidae (2). Adicionalmente, amostras de sangue foram obtidas de 120 ovinos, 119 caprinos, 70 cães e 47 bovinos. Foram coletados 286 carrapatos no ambiente, identificados como *Ornithodoros* cf. *tabajara* (188) e *Ornithodoros rietcorreai* (98); além de carrapatos coletados nos animais: *Ornithodoros* spp. em *Thrichomys laurentius* (4 espécimes) e *Monodelphis domestica* (1); *O. rietcorreai* em *Didelphis albiventris* (2) e *T. laurentius* (1); *Amblyomma parvum* em *T. laurentius* (4); *Rhipicephalus sanguineus* (*sensu lato*) em cães (121) e *R. microplus* em bovinos (47). Todas as amostras sanguíneas, 120 carrapatos de vida livre, sendo 46 *O. rietcorreai* e 74 *O.* cf. *tabajara*, e 12 *R. sanguineus* s. l. foram submetidos à extração de DNA para realização da reação em cadeia da polimerase (nested-PCR), visando amplificação de um fragmento do gene *flaB* presente em todas as espécies de *Borrelia*. Não houve amplificação nas amostras sanguíneas dos animais, no entanto em um espécime de *O.* cf. *tabajara* de vida livre (1,3%) foi detectado o DNA de *Borrelia*. A não detecção nos mamíferos pode estar relacionada com a ausência de infecção ou à baixa concentração do agente nos hospedeiros. A baixa prevalência encontrada nos carrapatos pode ser justificada devido à baixa infecção nos vetores. Novos estudos focando na determinação de anticorpos, assim como no sequenciamento e isolamento de *Borrelia* devem ser estimulados para uma melhor compreensão da epidemiologia do agente na região.

TERMO DE INDEXAÇÃO: *Ornithodoros* spp., borrelioses, Parque Nacional do Catimbau.

INTRODUCTION

The Catimbau National Park (CNP) is a conservation unit in the Caatinga biome, located in the Pernambuco State, Northeast region of Brazil. It was created in 2002 and aims to preserve the ecosystems within the geological complex of Serra of Catimbau (ICMBio 2019). Although wildlife is abundant, the presence of man in and around the park leads eventually to domestic animals sharing the environment with wild animals, a fact that facilitates the transmission of vector-borne agents between animals and humans (Carvalho et al. 2009).

Spirochetes of the genus *Borrelia* are motile cells that infect argasid ticks (i.e. genus *Argas, Ornithodoros*), ixodid ticks (i.e. genus *Amblyomma*, *Bothriocroton*, *Hyalomma*, *Ixodes* and *Rhipicephalus*), and the human lice (*Pediculus humanus humanus*) (Faccini-Martínez et al. 2022). These spirochetes are the etiological agents of tick-borne relapsing fever (TBRF) and Lyme disease (Faccini-Martínez et al. 2021, Trevisan et al. 2021). Sylvatic transmission cycles of the spirochetes involve vertebrate hosts and their ticks (Sánchez et al. 2020), with small mammals acting as main reservoirs (Lopez et al. 2021, Trevisan et al. 2021).

TBRF is a zoonosis caused by several species of *Borrelia* (Lopez et al. 2021, Faccini-Martínez et al. 2022), endemic to southern British Columbia, the highlands of Mexico, the western United States, the Mediterranean, Central Asia, Central, and South America, and Africa (Dworkin et al. 2002). The symptoms of the disease are nonspecific, among these, malaise, myalgia, headache, chills, anorexia, nausea, vomiting, muscle pain, and neurological symptoms; if not treated, the disease can lead to death, and may be confused with other febrile diseases such as malaria, dengue, and typhoid fever (Cutler 2015, Faccini-Martínez et al. 2021, 2022).

Lyme disease is also zoonosis, caused by *Borrelia burgdoferi* (*sensu lato*), and occurs currently in the Northern Hemisphere only. In the acute stage, the disease provokes flu-like symptoms, low-grade fever, chills, myalgia, arthralgia, headache, and adenomegaly (Stanek & Strle 2018). In a secondary stage, the spirochetes can cause joint, cardiac, and neurological complications, days to months after the initial infection (Stanek & Strle 2018). In Brazil, an allegedly similar affection is named as the Baggio-Yoshinari Syndrome (Yoshinari et al. 2010). However, the circulation of *Borrelia* in animals, and the disease in humans has yet-to-be confirmed in the country.

Wild animals can be excellent bioindicators of environmental health, and may represent a very rich source of information for circulating microorganisms, especially in places never studied before (Soares et al. 2000). In Brazil there is little information about the epidemiology of pathogenic agents that affect wild animals, and tick-borne spirochetes as agents of disease are still neglected (Yoshinari et al. 2010, Faccini-Martínez et al. 2022).

Several studies attempted to understand the epidemiology of borreliosis, and the identity of vector ticks fauna of ticks and *Borrelia* associated with them, in the Northeast region (Dantas-Torres et al. 2019, Labruna et al. 2014, Oliveira et al. 2018, Muñoz-Leal et al. 2018a, 2018b, 2019a, 2019b, 2021a, 2021b). However, the knowledge about the occurrence of tick-borne spirochetes in the State of Pernambuco is vague and needs attention. Therefore, the present study aimed at the molecular detection of *Borrelia* in small wild mammals, domestic animals, and ticks from the CNP, to evaluate these hosts as reservoirs or sentinels of infection.

MATERIALS AND METHODS

Study area. The CNP (8°24'00'' and 8°36'35'' S; 37°09'30'' and 37°14'40'' W) has an area of near 62,300 hectares, covers part of the municipalities of Buíque, Ibimirim, and Tupanatinga, and is located in a transition area between the Sertão and Agreste regions within the Caatinga biome, in the State of Pernambuco (Freire et al. 2020, ICMBio 2019). The period of highest rainfall is between March and June and the lowest, between September and January, with an annual average temperature of 26°C, and average rainfall of 450 to 950mm (Sadmet/Inmet 2021).

Sample collection. Small wild mammals were captured using live traps (Sherman® – HB Sherman Traps, Tallahassee/FL, USA and Tomahawk® Tomahawk Live Traps, Tomahawk/WI, USA), baited

with a mixture of sardines, cornmeal, banana, pineapple, cod liver oil, and soybean oil (Graipel et al. 2006). Traps were set for five consecutive nights along randomly selected transects, with a distance of approximately 10m and alternating trap types along the transects. The capture was carried out in seven areas, one in the Sertão region: Ibimirim (1); and seven in the Agreste region: Açude Velho (2), Camelo (3), Alcobaça (4), Toca do Vale (5), ICMBio (6) and Vila dos Moradores (7) (Fig.1). The traps were set up in the afternoon, shift and inspected the following morning, in two visits to CNP: January and September 2021. The total capture effort was 6,790 traps. The taxonomic identification of wild animals was based on external morphology according to Bonvicino et al. (2008). The sampling of domestic animals was carried out in three visits to the park, in January, June, and September 2021, involving dogs, goats, sheep, and cattle, present in and around the park (Fig.1). Blood collection in wild mammals was performed by puncturing the caudal vein or intracardiacly, in dogs through the cephalic vein and in domestic ungulates through the jugular vein. Samples were stored in tubes containing EDTA. After blood collection, an inspection was carried out on the animals to search for ectoparasites, which were placed in tubes containing 70% ethanol. Blood samples were centrifuged to obtain plasma and whole blood and stored at -20°C until DNA extraction. Free-living argasids were collected in naturally infested places, such as rocks with animal burrows containing fresh feces and animal tracks. CO2 expelled by the researchers also attracted the ticks, which were stored in 70% ethanol. Taxonomic identification was carried out in the Laboratory of Parasitic Diseases of the "Universidade Federal do Vale do São Francisco" (Univasf), according to Barros-Battesti et al. (2006) for the genus *Rhipicephalus* and argasid larvae, Martins et al. (2010) for nymphs of *Amblyomma* spp., Labruna et al. (2016) and Muñoz-Leal et al. (2021b) for argasids nymphs.

Molecular analyses. DNA was extracted from the blood of animals and adult ticks using a commercial kit (Promega, Madison/WI, USA), following the manufacturer recommendations. Collected nymphs were individually subjected to DNA extraction by boiling at 100°C for 20 minutes, as described by Horta et al. (2007), and stored at -20°C. Molecular analyses were performed in the above referred laboratory. All extracted samples were submitted to a nested PCR using FLaLL (5'- ACA TAT TCA GAT GCA GAC AGA GGT-3') and FLaRL (5'- GCA ATC ATA GCC ATT GCA GAT TGT-3') primers in the first round, and FLaLS (5'- AAC AGC TGA AGA GCT TGG AAT G -3') and FLaRS (5'- CTT TGA TCA CTT ATC ATT CTA ATA GC -3') in the second round aiming to amplify a fragment of 354 base pair of the flagellin gene (*flaB*), present in all *Borrelia* species, following Stromdahl et al. (2003). PCR products were submitted to electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and visualized with an ultraviolet transilluminator.

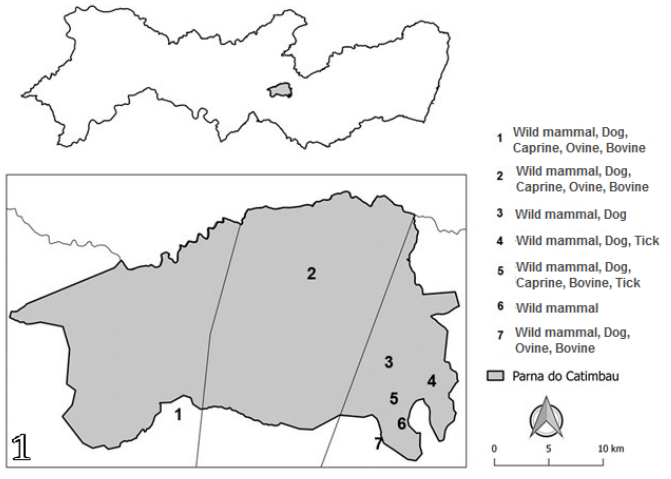

Fig.1. Capture sites for small wild mammals, domestic animals, and free-living ticks in the Catimbau National Park, Pernambuco. Points: Ibimirim (1), Açude Velho (2), Camelo (3), Alcobaça (4), Toca do Vale (5), ICMBio (6), Vila dos Moradores (7).

Ethical aspect. The study was approved by the Ethics and Deontology in Studies and Research Committee of the Univasf, under protocol, 0008/270320. Authorization for research in the CNP Conservation Unit and capture of wild animals was granted by the Biodiversity Authorization and Information System (SISBIO), under protocol 74584-1.

RESULTS

Sample collection

A total of 77 small wild mammals were captured: 45 marsupials, 30 rodents, and two cingulates (Table 1). Of these, four specimens (two *Didelphis albiventris* and two *Euphractus sexcinctus*) were actively caught. Blood samples were collected from 76 animals. Of the captured animals, 7.8% (6/77) were infested by *Ornithodoros rietcorreai*, an undetermined *Ornithodoros* sp., or *Amblyomma parvum* (Table 2).

Of the 70 sampled dogs, 25.7% were females (18/70) and 74.3% males (52/70); 32.9% (23/70) of them were parasitized by *Rhipicephalus sanguineus* (*sensu lato*), for which we collected 121 specimens (8L, 14N, 55F, 44M). Of the 47 bovines, 42.6% (20/47) were females and 57.4% (27/47) were males, with 17% of them (8/47) parasitized by *Rhipicephlaus microplus*, for which we collected 6 nymphs, 35 females, and six males. Of the 119 goats, 83.2% (99/119) were females and 16.8% (20/119), and of the 120 sheep, 68.3% (82/120) were females and 31.7% (38/120) males. Neither goats or sheep were parasitized by ticks (Table 2). All the collection points had parasitized animals with the exception of ICMBio, Camelo, and Ibimirim.

Regarding free-living ticks, a total of 286 specimens were collected in two areas within the municipality of Buíque, Toca do Vale and Alcobaça. These specimens were identified as *O. rietcorreai* (n=98) and *Ornithodoros* cf. *tabajara* (n=188) (Table 3).

Table 1. Species of small wild mammals captured in the Catimbau National Park, Pernambuco, Brazil

Order	Family	Species	Female	Male	Total
Didelphimorphia	Didelphidae	*Didelphis albiventris*	4	2	6
		Monodelphis domestica	13	26	39
Cingulate	Dasypodidae	*Euphrates sexcinctus*	0	2	2
Rodentia	Caviidae	*Galea spixii*	0	2	2
	Echimyidae	*thrichomys laurentius*	11	10	21
	cricetidae	*Cerradomys subflavus*	3	3	6
		Rhipidomys mastacalis	1	0	1
TOTAL			32	45	77

Polymerase chain reaction

Blood samples from 76 wild animals, 120 sheep, 119 goats, 47 cattle, and 70 dogs were tested. Of the 286 collected ticks, only 132 specimens were tested, 46 *O. rietcorreai*, 74 *O.* cf. *tabajara*, and 12 *R. sanguineus* (*sensu lato*) (Table 4). None of the blood samples amplified borrelial DNA. However, one specimen of *O.* cf. *tabajara* (1.3%) collected at Toca do Vale was positive.

DISCUSSION

The CNP is located in the Caatinga biome, and represents a barely explored region. Although the Park is rich in fauna and flora, there are no reports of diseases carried by ticks such as borreliosis in the region. Noteworthy, before the Park became a Conservation Unit, the place was populated, and nowadays residents still live inside and survive through family farming and animal husbandry (Freire et al. 2020). The park stands out for being a pole of tourism, and the human presence within and around favors the maintenance of domestic animals coexisting with wild animals, a fact that facilitates the sharing of ectoparasites and the transmission of diseases between animals and humans (Carvalho et al. 2009, Freire et al. 2020).

Two specimens of *D. albiventris* were captured within the Park, near the Vila dos Moradores. Marsupials are considered synanthropic animals and live near human dwellings searching for food or shelter. Importantly, they are hosts of zoonotic pathogens of animal and human health relevance such as *Leishmania infantum, Trypanosoma cruzi*, *Rickettsia rickettsii,* and *Toxoplasma gondii* (Bezerra-Santos et al. 2021).

In the study by Geise et al. (2010), carried out in the park, 43 rodents (*Cerradomys langguthi, Necromys lasiurus, Oligoryzomys stramineus, Wiedomys pyrrhorhinos, Rhipidomys* sp., *Galea spixii, and Thrichomys laurentius*) and 29 marsupials (*Gracilinanus agilis* and *Monodelphis domestica*) were captured and identified. After more than 10 years, our study brings novelty after capturing 30 rodents (*G. spixii, T. laurentius, Cerradomys subflavus,* and *Rhipidomys mastacalis*) and 43 marsupials (*M. domestica and D. albiventris*), showing difference in the diversity of species compared with the previous study.

Table 2. Tick species of domestic and wild mammals in the Catimbau National Park, Pernambuco, Brazil

Collection point	*Didelphis albiventris*	*Monodelphis domestica*	Thrichomys laurentius	Dogs	Cattle
Açude Velho	-	-	*Ornithodoros* sp. 2L (1/21)	*Rhipicephalus sanguineus* 6N, 8F, 7M (8/70)	-
Alcobaça	-	-	*Ornithodoros* sp. 2L (2/21) *Ornithodoros rietcorreai* 1N (1/21) *Amblyomma parvum* 4N (1/21)	-	-
Toca do Vale	-	*Ornithodoros* sp. 1L (1/39)	-	*Rhipicephalus sanguineus* 16F, 19M (2/70)	*Rhipicephalus microplus* 3N, 20F, 4M (3/47)
Vila dos Moradores	*Ornithodoros rietcorreai* 2N (1/6)	-	-	*Rhipicephalus sanguineus* 8L, 8N, 31F, 18M (13/70)	*Rhipicephalus microplus* 4N, 14F, 2M (5/47)

L = larva, N = nymph, F = female, M = male.

Table 3. Free-living tick species collected in the Catimbau National Park, Pernambuco, Brazil

Species	Location	Larva	Nymph	Female	Male	Total
Ornithodoros rietcorreai	Toca do Vale	0	56	14	10	80
Ornithodoros rietcorreai	Alcobaça	0	15	0	3	18
Ornithodoros cf. tabajara	Toca do Vale	0	118	7	1	126
Ornithodoros cf. tabajara	Alcobaça	0	38	19	5	62
TOTAL		0	227	40	19	286

Table 4. Species of ticks subjected to the polymerase chain reaction

Species	Location	Nymph	Female	Male	Total
Ornithodoros rietcorreai	Toca do Vale	(0/20)	(0/10)	(0/5)	35
Ornithodoros rietcorreai	Alcobaça	(0/10)	(0/0)	(0/1)	11
Ornithodoros cf. tabajara	Toca do Vale	(1/40)	(0/2)	(0/0)	42
Ornithodoros cf. tabajara	Alcobaça	(0/20)	(0/10)	(0/2)	32
Rhipicephalus sanguineus	Miscellaneous	(0/2)	(0/2)	(0/8)	12
TOTAL		92	24	16	132

Several factors may have contributed to these differences, such as capture locations, capture method, study period, food supply, anthropization, and hunting, which is commonly practiced by some of the residents.

The Caatinga biome is exclusive to Brazil and presents a wide diversity of fauna and flora. However, knowledge of the tick fauna in this biome is scarce and there is little information about parasitism by these ectoparasites, especially in small wild mammals. The study of these arthropods in wild environments stands out for providing ecological information, distribution, and host-parasite relationship, as well as the transmission of pathogenic agents (Horta et al. 2011).

Nymphs of the genus *Ornithodoros* tend to feed quickly and stay long on the host, being more easily found in the burrows of their hosts, as the larvae take up to hours to complete feeding (Muñoz-Leal et al. 2018a). Although *O. rietcorreai* nymphs were found in one rodent and one marsupial, it is highly possible that the animals attracted the ticks once in the trap, and we collected them while feeding on the animals. Conversely, *M. domestica* and *T. laurentius* would be likely natural hosts of larvae.

Ornithodoros rietcorreai, described by Labruna et al. (2016) in the Caatinga biome in the states of Piauí and Paraíba, has *Kerodon rupestris* (mocó) as a possible host. In the present study, we reported the presence of *O. rietcorreai* nymphs infesting *D. albiventris* and *T. laurentius*, which may be considered possible hosts, corroborating the study by Oliveira et al. (2020), who found *O. rietcorreai* larvae in *T. laurentius*. Although *K. rupestris* was not found in the present study, residents report its presence throughout the park.

Amblyomma parvum has domestic animals, medium to large wild animals, and humans as hosts, in addition to being a species implicated as a vector of putative pathogens of importance in public health (Lado et al. 2016). Its immature stages can infest rodents (Horta et al. 2011), which was corroborated in the present study because nymphs of this tick species were collected in *T. laurentius*.

According to Szabó et al. (2001), the fact of living with cattle may favor accidental parasitism of sheep and goats by *R. microplus*. The fact that in this study goats and sheep were not parasitized by ticks, could be justified because these animals did not live with cattle, which is the primary host and spreader of *R. microplus*.

Most of the dogs living inside the Park have free access to wild areas, keep in touch with other animals, and expose themselves to ectoparasites and pathogens of wild animals. However, the prospected dogs were parasitized only by *R. sanguineus* (*sensu lato*). The coexistence of domestic animals in the environment of wild animals, as well as the contact with forest areas, can favor mixed infestations by different species of ticks, which may increase the transmission of pathogenic agents from wild populations to domestic animals and humans (Guimarães et al. 2017).

We report the presence of free-living *O. rietcorreai* and *O.* cf. *tabajara* in the CNP, contributing to knowledge about the tick fauna in the Brazilian Northeast. Several species of *Ornithodoros* are implicated as vectors of *Borrelia* that cause relapsing fever in humans (Faccini-Martínez et al. 2022). Although the disease has never been reported in Brazil, several argasid species such as *O. brasiliensis, O. rietcorreai, Ornithodoros mimon, O. fonsecai, O. rostratus, Ornithodoros hasei,* and *Ornithodoros tabajara* do parasitize humans and cause allergic reactions (Muñoz-Leal et al. 2021a, 2021b). Indeed, one of the researchers of the present study was bitten by ticks of the genus *Ornithodoros* (results not shown).

Borrelia sp. DNA was amplified in one free-living nymph of *O.* cf. *tajabara* using primers directed to a fragment of the *flaB* gene. Several studies carried out in Brazil have demonstrated infection by borreliae of the relapsing fever group in ticks of the genus *Ornithodoros*. For instance, in a study carried out in Maranhão, *Borrelia venezuelensis* was isolated from a female of *Ornithodoros rudis* (Muñoz-Leal et al. 2018a). Probable new species of *Borrelia* from the relapsing fever group were detected in *O. mimon*, *O. hasei*, *O. rietcorreai,* and *O. tabajara* (Muñoz-Leal et al. 2021a, 2021b). An ongoing study with ticks from Parna do Catimbau, demonstrated the presence of the bacteria in the park, corroborating with the present exposure (Oliveira 2022).

According to Mantovani et al. (2012), molecular tests may have reduced sensitivity in detecting certain agents when the animals are not in the acute phase of the disease. Socoloski et al. (2018) suggested that a reduced spirochetemia, attributed to the conditions of chronicity of the disease in vertebrate hosts, may result in the non-detection of *Borrelia* spp. One of these two facts might be reason why we did not DNA of *Borrelia* in blood samples.

Work carried out in Chile focused on the molecular identification of *Borrelia* genotypes in ticks removed from small mammals, demonstrating that these animals may be able to act as efficient hosts of the agent in that country and South America (Thomas Sánchez et al. 2020). Additionally, the study carried out by Weck et al. (2022) in eight areas of forest fragments in São Paulo, Mato Grosso, and Mato Grosso do Sul, reported a new genospecies "*Candidatus* Borrelia paulista" in two rodents *Oligoryzomys mattogrossae,* thus suggesting that rodents might maintain spirochetes in enzootic cycles.

In tick-borne relapsing and Lyme disease, an accurate diagnosis during the afebrile period of both diseases is challenging because bacteria are below the detection limit. In order to confirming the circulation of the agent in an unknown region it would be important to implement serological surveys, as this approach will provide evidence on exposure or active infection by a given spirochetes. While serological assays searching for *Borrelia anserina* and *Borrelia burgdorferi* antibodies have been implemented in Brazil, the former is a tick-borne relapsing fever that affects bird and not humans, and the latter is distributed only in the Northern Hemisphere. In the meantime, serological tests to assess the circulation of other *Borrelia* spp. that have been detected or isolated in the country are still lacking.

CONCLUSION

In this study, we report the presence of DNA of a *Borrelia* sp. in the Catimbau National Park (CNP), Pernambuco. We also report two species of ticks for this conservation unit, *Ornithodoros rietcorreai,* and *Ornithodoros* cf. *tabajara*, contributing to the knowledge of the tick fauna of the Northeast region. For what seems to be the first time, we also report parasitism by nymphs of *O. rietcorreai* on *Didelphis albiventris* and *Thrichomys laurentius.* Although the prevalence of *Borrelia* was low in ticks, and we did not detect the spirochetes in domestic and wild vertebrates, the presence of people in the park justifies the

need to perform further surveillance to monitor the exposure to *Borrelia* spp., and the eventual use of serological techniques to detect antibodies in animals and humans.

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