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Leptospira spp. in wild small mammals from two forest areas under different anthropogenic pressures in the Western Brazilian Amazon[1]

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ABSTRACT.- Albuquerque NF, Di Azevedo MIN, D'Andrea PS, De Moura ALB, Teixeira BR, Lilenbaum W, Medeiros LS. ***Leptospira* spp. in wild small mammals from two forest areas under different anthropogenic pressures in the Western Brazilian Amazon**. *Pesquisa Veterinária Brasileira 46:e07861, 2026.* Departamento de Saúde Coletiva Veterinária e Saúde Pública, Universidade Federal Fluminense, Rua Doutor Vital Brazil Filho 64, Vital Brasil, Niterói, RJ 24230-340, Brazil. E-mail: luciana_medeiros@id.uff.br

This study evaluated the frequency of *Leptospira* spp. infection in wild small mammals from two areas in Western Brazilian Amazonia: the Chandless State Park (PEC), a protected area with low human population density, and the Santa Cecília Forest Fragment (FFSC), an area under anthropogenic pressure. A total of 65 animals representing 17 species were captured, and blood, kidney, and urine samples were obtained for serological, molecular, and bacteriological analyses. Serum samples (n = 51) were tested by the microscopic agglutination test (MAT), kidney and urine samples by *lipL32* polymerase chain reaction (PCR), and urine samples by bacterial culture. Species richness was higher in the PEC (13 species) than in the FFSC (six species). Overall, 49.0% (25/51) of serum samples showed seroreactivity to at least one tested serogroup, including 42.1% (8/19) in the PEC and 53.1% (17/32) in the FFSC. The most frequently detected serogroups were Panama, Icterohaemorrhagiae, and Sejroe, with co-agglutination observed in some samples. All bacterial cultures were negative. PCR positivity was similar between areas: 18.5% (5/27) in the PEC and 17.6% (6/34) in the FFSC. The marsupial *Didelphis marsupialis* was the only PCR-positive species in the PEC, while the rodent *Proechimys gardneri* was the only PCR-positive species in the FFSC. The results suggest that *Leptospira* spp. are naturally maintained among wild small mammals in Amazonia. No significant differences in infection rates were detected between the studied areas, although differences in the circulation of *Leptospira* spp. serogroups were observed.

INDEX TERMS: *Leptospira* spp., marsupials, rodents, PCR, serogroups.

RESUMO.- [***Leptospira* spp. em pequenos mamíferos silvestres de duas áreas florestais na Amazônia Ocidental Brasileira sob diferentes pressões antrópicas.**] Este estudo avaliou a frequência de infecção por *Leptospira* spp. em pequenos mamíferos silvestres de duas áreas da Amazônia Ocidental Brasileira: o Parque Estadual Chandless (PEC), uma unidade de conservação com baixa densidade populacional humana, e o Fragmento Florestal Santa Cecília

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(FFSC), uma área sob influência antrópica. Um total de 65 animais representando 17 espécies foi capturado. Amostras de sangue, rim e urina foram coletadas para análises sorológicas, moleculares e bacteriológicas. As amostras de soro (n = 51) foram analisadas pela técnica de soroaglutinação microscópica (MAT), enquanto as amostras de rim e urina foram submetidas à reação em cadeia da polimerase (PCR) para o gene *lipL32*, e as amostras de urina a cultura bacteriana. A riqueza de espécies foi maior no PEC (13 espécies) em comparação ao FFSC (seis espécies). No total, 49,0% (25/51) das amostras séricas apresentaram sororreatividade para pelo menos um dos sorogrupos testados, sendo 42,1% (8/19) no PEC e 53,1% (17/32) no FFSC. Os sorogrupos predominantes foram Panama, Icterohaemorrhagiae e Sejroe, com ocorrência de coaglutinação em algumas amostras. Todas as culturas bacterianas apresentaram resultado negativo. A positividade na PCR foi semelhante entre as áreas estudadas: 18,5% (5/27) no PEC e 17,6% (6/34) no FFSC. O marsupial *Didelphis marsupialis* foi a única espécie PCR-positiva no PEC, enquanto o roedor *Proechimys gardneri* foi a única espécie PCR-positiva no FFSC. Os resultados sugerem que *Leptospira* spp. circula naturalmente entre pequenos mamíferos silvestres da Amazônia. Não foram observadas diferenças significativas nas taxas de infecção entre as áreas avaliadas, embora tenham sido verificadas diferenças na circulação dos sorogrupos de *Leptospira* spp.

TERMOS DE INDEXAÇÃO: *Leptospira* spp., marsupiais, roedores, sorogrupos, PCR.

INTRODUCTION

Leptospirosis is a zoonotic disease caused by spirochete bacteria of the genus *Leptospira*. It is widely distributed worldwide, particularly in tropical regions, where a wide diversity of animal species has been identified as reservoirs. Recent molecular analyses have revealed that the Amazon biome accounts for a substantial proportion of circulating *Leptospira* strains in Brazil, with wild animals representing a significant share of positive hosts (Di Azevedo & Lilenbaum 2025). Investigating the enzootic cycle of *Leptospira* in wild animals provides important insights into the epidemiological dynamics of the disease, which affects wild, domestic, and human populations.

Infections caused by *Leptospira* spp. have been detected in a broad range of small-bodied mammals, particularly rodents and marsupials, across different Brazilian ecosystems (Santos et al. 2017, Mesquita et al. 2018, Abreu et al. 2019, Vieira et al. 2019). These groups are recognized as important renal reservoirs in highly impacted landscapes of the western Amazon basin, where deforestation has resulted in a mosaic of forest fragments (Medeiros et al. 2020). However, data on the occurrence of *Leptospira* in protected areas with low levels of anthropogenic disturbance are still limited. Therefore, data on the presence of *Leptospira* in relatively preserved ecosystems are essential for understanding the diversity of reservoirs of leptospirosis in the Amazon region and the potential influence of anthropogenic impacts on the prevalence of this disease.

Within this context, the present study investigated the occurrence of *Leptospira* spp. in wild small mammals from two forest areas in the western Brazilian Amazon: the Chandless State Park ("Parque Estadual Chandless" – PEC), a large protected area with low levels of anthropogenic disturbance, and the Santa Cecília Forest Fragment ("Fragmento Florestal Santa Cecília" – FFSC), a disturbed area located in a periurban area of the municipality of Rio Branco, Acre State.

MATERIALS AND METHODS

Ethical approval. All necessary biosecurity procedures, including level 3 personal protective equipment (PPE), were adopted for handling specimens and processing and analyzing samples. The project was assessed and approved by the "Instituto Oswaldo Cruz" Committee for the Ethical Use of Animals (CEUA-Fiocruz: protocol L-036/2018), and the collection of zoological samples was authorized by "Sistema de Autorização e Informações de Biodiversidade" of the "Instituto Chico Mendes de Conservação da Biodiversidade" (SISBIO-ICMBIO) through permanent license 13373.

The selection of the study areas. The two areas selected for the present study – PEC and FFSC – represent distinct levels of anthropogenic impact in the Brazilian state of Acre (Fig. 1). The Chandless State Park (9° 55' 07.7" S, 70° 09' 34.7" W) is a fully protected conservation unit with approximately 695,303 ha of forest, located primarily in the municipality of Manoel Urbano, with some overlap into the neighboring municipalities of Sena Madureira and Santa Rosa do Purus on the Brazilian frontier with Peru. The PEC is a well-preserved area with a vegetation mosaic dominated by open rainforest, including extensive stands of bamboo and open forest with palms (Sema-AC 2010). The local mammalian fauna is well conserved and includes many species that are highly sensitive to anthropogenic impacts (Borges et al. 2015). The park is inhabited by only 11 families, with a population density of less than 0.5 inhabitants per square kilometer.

The Santa Cecília Forest Fragment (9° 59' 25.4" S, 67° 42' 38.3" W) is a reserve of 830 ha located in the municipality of Rio Branco. The vegetation at this site is dominated by open rainforest with palms. This forest fragment is located within an anthropogenic matrix of cattle ranches and roads surrounding its perimeter. The surrounding area has a relatively high population density and is in a region popular for sporting and recreational activities.

Capture of the animals and collection of biological samples. The animals were captured using Sherman® and Tomahawk® live traps distributed along 15-meter transects across two vegetation strata (ground and forest understory). The traps were baited with a mixture of oats, banana, bacon, and peanut butter. The traps were checked daily for the presence of animals. Pitfall traps were also used at the two sites. The total capture effort comprised 600 trap-nights with live traps and 120 trap-nights with pitfall traps at PEC, and 375 and 100 trap-nights, respectively, at FFSC. The difference in trapping effort reflects the larger size and more remote location of PEC, requiring a more extensive sampling regime. In FFSC, access was easier, and the fragment is smaller, allowing a more concentrated effort. This difference may have influenced capture rates and should be considered when comparing species richness between areas.

Once captured, the small mammals were transported to the field laboratory, where they were anesthetized with 10% ketamine hydrochloride and 2% xylazine hydrochloride before sample collection. Blood samples were collected by cardiac puncture. Euthanasia was performed according to CEUA/Fiocruz recommendations, followed by necropsy for the collection of kidney and urine samples for the molecular and bacteriological diagnosis of *Leptospira*. The kidneys were sectioned and placed in 1.5-mL microtubes (Eppendorf®, São Paulo, SP, Brazil) containing 1 mL of absolute alcohol. Urine was collected directly from the bladder using a 1-mL syringe, accessed through an incision in the abdominal wall. These biological samples

were preserved at temperatures below -20 °C until processing in the laboratory.

Bacterial culture. Three to five drops of urine were inoculated into 5 mL of EMJH liquid medium (Difco, BD, Franklin Lakes/NJ, USA), 5 mL of Fletcher semisolid medium (Difco, BD, Franklin Lakes/NJ, USA), and 5 mL of EMJH liquid medium supplemented with a cocktail of antibiotics, denominated STAFF (Chakraborty et al. 2011). The culture media were sent for analysis from the Veterinary Microbiology Laboratory of the "Universidade Federal do Acre" (UFAC), in Rio Branco, to the Veterinary Bacteriological Laboratory of the "Universidade Federal Fluminense" (UFF), in Niterói, where bacterial growth was monitored over a 24-week period.

Serology. Anti-*Leptospira* antibodies were detected using the microscopic agglutination test (MAT) with 10 live antigens. This set of antigens included serovars Australis (Australis serogroup), Autumnalis (Autumnalis serogroup), Bataviae (Bataviae serogroup), Canicola (Canicola serogroup), Pomona (Pomona serogroup), Panama (Panama serogroup), Grippotyphosa (Grippotyphosa serogroup), Hardjo (Hardjoprajitno; Sejroe serogroup), and the Copenhageni and Icterohaemorrhagiae (RGA) serovars of the Icterohaemorrhagiae serogroup.

Blood samples were centrifuged at 1,500 × g for 10 minutes to obtain serum. A dilution of 1:50 was used to identify the seroreactive animals. The reactive samples were then examined using serial dilutions from 1:50 to 1:1600, with the highest positive dilution being considered the titer of the serum. The titration cutoff was 100 (Fornazari et al. 2018), and the sample was considered reactive when it showed at least 50% agglutination at 100x magnification under a dark-field microscope.

Molecular analysis. The DNA was extracted from the urine samples using the Wizard SV Genomic DNA Purification System kit (Promega, Madison, USA). The DNA of the kidney samples was extracted using the DNA easy Blood & Tissues kit (Qiagen, Iowa, USA). A partial sequence of the *lipL32* gene (241 bp), which is present in pathogenic leptospires, was amplified by polymerase chain reaction (PCR) using the primers LipL32-45F: 5′-AAG CAT TAC CGC TTG TGG TG-3′ and LipL32- 286R: 5′-GAA CTC CCA TTT CAG CGA TT-3′ (Stoddard et al. 2009). For each test, ultrapure water was used as a negative control in all reactions. In contrast, 10 fg of DNA extracted from *Leptospira interrogans* serovar Copenhageni (Fiocruz L1-130) was used as a positive control. The PCR products were analyzed by electrophoresis on a 1.5–2% agarose gel and visualized under ultraviolet light after being stained with GelRed®.

In addition, the concentration of DNA amplified from the PCR-positive samples was not sufficient for sequencing; therefore, the *Leptospira* species infecting the animals could not be identified.

Statistical analyses. The prevalence of seroreactive and PCR-positive samples was calculated for each area, based on the proportion of the captured animals that tested positive for each type of analysis. The frequency of each serogroup was also calculated for the results of

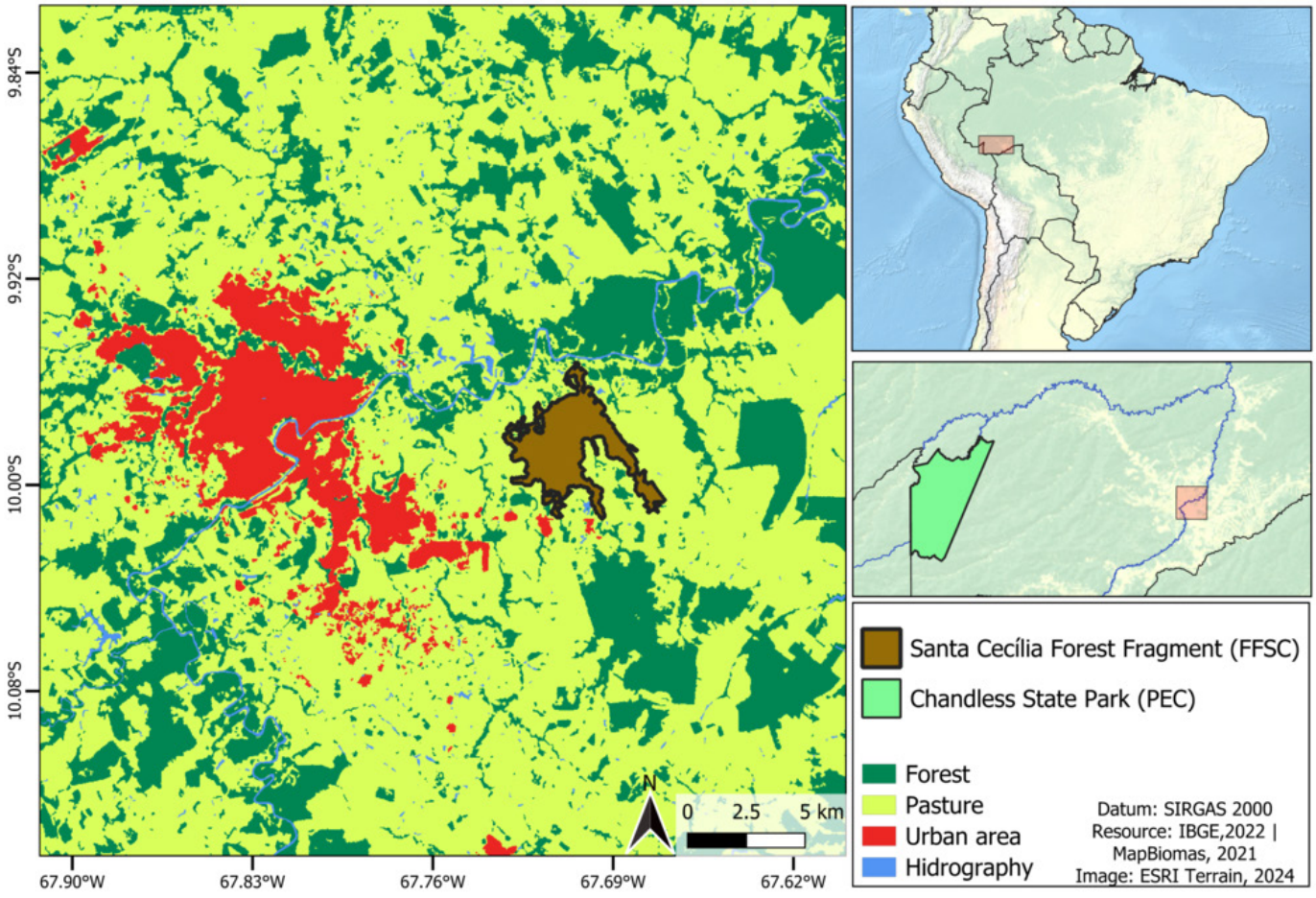


Fig. 1. Location of the "Parque Estadual Chandless" (PEC) and the "Fragmento Florestal Santa Cecília" (FFSC) in Acre state, Western Brazilian Amazon.

the MAT. The variation in the prevalence of positive samples between areas was evaluated using Fisher's exact test due to the small sample sizes, with a significance level of $p < 0.05$. This analysis was performed using the statistical software jamovi (The Jamovi Project 2025) and R version 4.5 (R Core Team 2025).

RESULTS

A total of 65 animals were captured during the present study, including 29 from PEC and 36 from FFSC. The FFSC had the lowest species richness of the two areas, with only six species being identified, of which four were rodents (Rodentia) — *Proechimys brevicauda*, *Proechimys gardneri*, *Mesomys hispidus* (Echimyidae: Eumysopinae), and *Oecomys* gr. *bicolor* (Cricetidae: Sigmodontinae) — while the two others — *Philander canus* and *Monodelphis glirina* — were marsupials (Didelphimorphia: Didelphidae). By contrast, a total of 13 species were recorded in the PEC, including seven rodents and six marsupials. The rodents included five sigmodontines — *Euryoryzomys nitidus*, *Hylaeamys perenensis*, *Neacomys musseri*, *Neacomys amoenus*, and *Oecomys* gr. *bicolor* — and two echimyids, *Proechimys longicaudatus*, and *Rhipidomys gardneri*. The marsupials were all didelphids — *Didelphis marsupialis*, *Marmosa* (*Micoureus*) *constantiae*, *Marmosa* (*Micoureus*) *rutteri*, *Marmosops noctivagus*, *Marmosops ocellatus*, and *P. canus.*

Each captured specimen was assigned an individual identification code (Table 1 and 2). Most animals were tested for *Leptospira* by both PCR and serology, except for a few individuals for which blood (n = 14), urine (n = 12), or kidney (n = 6) samples could not be collected due to field constraints.

PCR positivity was similar between areas: 18.5% (5/27; 95% CI: 6.3–38.1%) in the PEC and 17.6% (6/34; 95% CI: 6.8–34.5%) in the FFSC (Fisher's exact test, $p = 1.000$). The marsupial *D. marsupialis* was the only PCR-positive species in the PEC, while the rodent *P. gardneri* was the only PCR-positive species in the FFSC.

Serological analysis was performed on 51 serum samples. Seroprevalence was 42.1% (8/19; 95% CI: 20.3–66.5%) in

Table 1. Molecular (polymerase chain reaction – PCR) and serological (microscopic agglutination test – MAT) detection of *Leptospira* spp. in wild small mammals from "Parque Estadual Chandless" (PEC), Acre, Brazil

ID	Order/species	Sample type (PCR)	*lipL32*-PCR	Serology
	Rodentia			
21895	*Hylaeamys perenensis*	Kidney	Negative	*
21912	*Hylaeamys perenensis*	Kidney	Negative	Hardjo(400)
21933	*Hylaeamys perenensis*	Kidney	Negative	Aus/Can/Pan(100);Aut/Bat (400)
21934	*Hylaeamys perenensis*	Kidney	Negative	*
21940	*Hylaeamys perenensis*	Kidney	Negative	*
21952	*Hylaeamys perenensis*	Kidney	Negative	*
21955	*Hylaeamys perenensis*	Kidney	Negative	Hardjo/Pan(100)
21910	*Oecomys* gr. *bicolor*	Kidney	Negative	—
21946	*Oecomys* gr. *bicolor*	—	—	—
21953	*Oecomys* gr. *bicolor*	Kidney	Negative	Aus/Cop (100); Aut/Bat/Pan (200); Gri/Hardjo (400)
21954	*Oecomys* gr. *bicolor*	Kidney	Negative	Aus/Bat/Cop/Pan (100); Gri/Hardjo (200)
21941	*Euryoryzomys nitidus*	Kidney	Negative	*
21932	*Rhipidomys gardneri*	Kidney	Negative	—
21944	*Neacomys musseri*	Kidney	Negative	—
21945	*Neacomys amoenus*	Kidney	Negative	—
21947	*Proechimys longicaudatus*	—	—	—
	Didelphimorphia			
21897	*Didelphis marsupialis*	Urine	Negative	*
21911	*Didelphis marsupialis*	Urine	Positive	*
21915	*Didelphis marsupialis*	Urine	Positive	*
21937	*Didelphis marsupialis*	Urine	Positive	*
21943	*Didelphis marsupialis*	Urine	Positive	Not reactive
21957	*Didelphis marsupialis*	Urine	Positive	*
21896	*Marmosops noctivagus*	Kidney	Negative	—
21913	*Marmosa* (*Micoureus*) *rutteri*	Kidney	Negative	Hardjo (200)
21935	*Marmosa* (*Micoureus*) *constantiae*	Kidney	Negative	—
21936	*Marmosa* (*Micoureus*) *constantiae*	Kidney	Negative	Aut/Bat/Pan (100)
21914	*Marmosops ocellatus*	Kidney	Negative	—
21942	*Philander canus*	Kidney	Negative	Hardjo(100)
21956	*Marmosops ocellatus*	Kidney	Negative	—

* Reactive at 1:50 dilution (titer < 100, below the established cutoff of ≥ 100); (—) Not collected (sample unavailable for the indicated analysis); Aus = Australis, Aut = Autumnalis, Bat = Bataviae, Can = Canicola, Cop = Copenhageni, Gri = Grippotyphosa, Pan = Panama, Pom = Pomona, RGA = Icterohaemorrhagiae, Hardjo = Sejroe serogroup; All captured individuals are shown to document species richness.

the PEC and 53.1% (17/32; 95% CI: 34.7–70.9%) in the FFSC. The difference was not statistically significant (Fisher's exact test, $p = 0.196$). The predominant serogroups were Panama, Icterohaemorrhagiae, and Sejroe. The relative frequency of each serogroup recorded in the MAT is shown for the two study areas in Table 3.

DISCUSSION

This study seems to be the first to detect antibodies against the Bataviae, Canicola, Pomona, Icterohaemorrhagiae, Sejroe, and Grippotyphosa serogroups in rodents and marsupials from this region. Also, it represents the first serological investigation in a strictly protected conservation unit in the Western Brazilian Amazon.

Most seroreactive animals showed low titers (100), indicating past exposure. Although infrequent, higher titers (400 and 800) suggest recent infection, especially the titer of 800 (Autumnalis) recorded in a *Proechimys gardneri* from FFSC. The predominance of low titers is consistent with findings in capybaras from the same region (Albuquerque

Table 2. Molecular (polymerase chain reaction – PCR) and serological (microscopic agglutination test – MAT) detection of *Leptospira* spp. in wild small mammals from "Fragmento Florestal Santa Cecília" (FFSC), Acre, Brazil

ID	Order/species	Sample type (PCR)	*lipL32*- PCR	Serology
	Rodentia			
22081	*Proechimys gardneri*	Kidney	Negative	Aus/Can(100); Aut/Bat/Cop/Pan/Pom (200); Gri/RGA (400)
22083	*Proechimys gardneri*	Kidney	Negative	Pan (200)
22085	*Proechimys* sp.	Kidney	Negative	—
22086	*Proechimys* sp.	Kidney	Negative	*
22088	*Proechimys gardneri*	Kidney	Negative	Gri/Pan/Pom (100)
22089	*Proechimys brevicauda*	Kidney	Negative	Gri (100); Pan (200)
22090	*Proechimys gardneri*	Kidney	Positive	Hardjo/RGA (100); Gri/Pan (200)
22099	*Proechimys gardneri*	Kidney	Positive	*
22100	*Proechimys gardneri*	Kidney	Positive	*
22101	*Proechimys gardneri*	Kidney	Positive	Pan (100)
22103	*Proechimys gardneri*	Kidney	Negative	—
22105	*Proechimys brevicauda*	Kidney	Negative	Cop (100);Gri (200);Pan (400)
22133	*Proechimys gardneri*	Kidney	Positive	*
22134	*Proechimys gardneri*	Kidney	Negative	*
22135	*Proechimys gardneri*	Kidney	Negative	*
22137	*Proechimys gardneri*	Kidney	Negative	Aus/Bat/Cop/Pom/RGA (100)/Gri/Pan(200)
22138	*Proechimys brevicauda*	Kidney	Negative	Bat/Gri (100);Pan (200)
22139	*Proechimys gardneri*	Kidney	Negative	Pan (100)
22155	*Proechimys brevicauda*	Kidney	Negative	*
22156	*Proechimys gardneri*	Kidney	Negative	Not reactive
22158	*Proechimys gardneri*	Kidney	Negative	Cop (100)
22162	*Proechimys gardneri*	Kidney	Positive	Can/Cop/Pan (100); Gri/Hardjo (200);Aut (800)
22164	*Proechimys gardneri*	Urine	Negative	*
22643	*Proechimys gardneri*	Urine	Negative	Not reactive
22644	*Proechimys gardneri*	Urine	Negative	Not reactive
22645	*Proechimys* sp.	Urine	Negative	*
22646	*Proechimys gardneri*	Urine	Negative	Not reactive
22159	*Mesomys hispidus*	Kidney	Negative	Bat (100);Aus/Aut/Cop/Hardjo (200)
22161	*Mesomys hispidus*	Kidney	Negative	*
22641	*Mesomys hispidus*	Urine	Negative	Aut/Bat/Hardjo/Pan (100)
22082	*Mesomys hispidus*	Kidney	Negative	Gri/Hardjo/RGA (100);Pan (400)
22166	*Oecomys* gr. *bicolor*	—	—	—
22647	*Oecomys* gr. *bicolor*	—	—	Aut/Bat/Gri/Pan(100);Hardjo(200)
22642	*Oecomys* gr. *bicolor*	Kidney	Negative	—
	Didelphimorphia			
22141	*Philander canus*	Kidney	Negative	Not reactive
22104	*Monodelphis glirina*	Kidney	Negative	Cop/Pom (100);Pan (200)
ID	Order/species	Sample type (PCR)	*lipL32*- PCR	Serology

*Reactive at 1:50 dilution (titer < 100, below the established cutoff of ≥ 100); (—) Not collected (sample unavailable for the indicated analysis); Aus = Australis, Aut = Autumnalis, Bat = Bataviae, Can = Canicola, Cop = Copenhageni, Gri = Grippotyphosa, Pan = Panama, Pom = Pomona, RGA = Icterohaemorrhagiae, Hardjo = Sejroe serogroup; All captured individuals are shown to document species richness.

et al. 2017), which also showed predominantly low titers for the Shermani, Pomona, and Icterohaemorrhagiae serogroups.

In the present study, the predominant serogroups differed between areas: Panama and Sejroe in PEC vs. Panama and Icterohaemorrhagiae in FFSC. The presence of the Sejroe serogroup in a protected area is noteworthy and suggests that this serogroup (or its serovars) may circulate among wild reservoirs in preserved environments. However, within the limits of this two-area comparison, we did not detect a significant difference in infection rates between areas, although serogroup composition varied. Although numerically different (42.1% in PEC vs. 53.1% in FFSC), this difference was not statistically significant (Fisher's exact test, $p = 0.196$), likely due to the relatively small sample sizes, which limited statistical power.

As the conditions found in the Amazon biome are highly favorable for the persistence of *Leptospira* spp. in the environment, the high levels of seroreactivity recorded in the present study were expected. Recent data show that the Amazon biome accounts for a substantial proportion of circulating *Leptospira* strains in Brazil, with wild animals representing a significant share of positive hosts (Di Azevedo & Lilenbaum 2025). Overall seroreactivity was high in both study areas, with frequencies similar to those previously reported in capybaras from western Amazonia (Albuquerque et al. 2017). In contrast, previous studies in small mammals from the Atlantic Forest and other Amazonian regions reported lower seroprevalence values (Lins & Lopes 1984, Vieira et al. 2019, Medeiros et al. 2020), reinforcing *Leptospira* circulation in Amazonian ecosystems. Although most antibody titers were low, a few individuals presented titers of 400 and 800, which may suggest recent exposure. In addition, seroprevalence in PEC was higher than reported for other protected areas in Brazil (Santos et al. 2017, Abreu et al. 2019).

In contrast, a recent study in Atlantic Forest conservation units found a markedly higher PCR prevalence (56%) in wild small mammals, with identification of *Leptospira interrogans* belonging to the Icterohaemorrhagiae serogroup by sequencing (Di Azevedo et al. 2025). In contrast with previous studies in the Peruvian Amazon (59%), western Brazilian Amazon (44.7%), and Brazilian Atlantic Forest (30%) (Bunnell et al. 2000, Vieira et al. 2019, Medeiros et al. 2020), the prevalence of PCR-positive individuals was low in both areas surveyed in the present study (approximately 18%). The marsupial *Didelphis marsupialis* was the only PCR-positive species in PEC, while *Proechimys gardneri* was the only one in the FFSC (Patton et al. 2000, Santos-Filho et al. 2015, Borges-Matos et al. 2016). However, the absence of quantitative data prevents us from confirming these species as true reservoirs.

As expected, species richness was higher in PEC (13 species) than in FFSC (six species). Despite this difference, no significant differences in infection rates were observed between the two areas ($p > 0.05$). These findings suggest that *Leptospira* circulates among wild small mammals in both studied environments, although differences in serogroup composition were observed between sites.

Several limitations should be acknowledged. The absence of sequencing prevented species-level identification of the leptospires detected by PCR. The relatively small sample size (n = 65) limited statistical power to detect subtle differences between areas, as reflected in the wide confidence intervals for prevalence estimates. Additionally, MAT detects antibodies, indicating exposure rather than active infection; therefore, we cannot distinguish between past and current infections.

CONCLUSION

The study detected the circulation of *Leptospira* spp. among wild small mammals in two forest areas of the western Brazilian Amazon. No significant differences in infection rates were observed between the studied areas, although differences in serogroup distribution were detected. These findings indicate natural circulation of *Leptospira* in both areas, but further studies with larger sample sizes and molecular characterization of circulating strains are necessary to confirm the role of these species as reservoirs and to better understand the epidemiology of *Leptospira* in Amazonian environments.

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Table 3. Serogroup-specific reactivity among microscopic agglutination test (MAT)-positive wild small mammals from "Parque Estadual Chandless" (PEC) and "Fragmento Florestal Santa Cecília" (FFSC), Acre, Brazil

Serogroup	Reactivity to each serogroup among MAT-positive animals (%)	
	PEC (n = 8)	FFSC (n = 17)
Australis	25.0%	17.6%
Autumnalis	50.0%	35.3%
Bataviae	50.0%	35.3%
Canicola	12.5%	11.8%
Icterohaemorrhagiae (Copenhageni)	25.0%	41.2%
Grippotyphosa	25.0%	58.8%
Sejroe	75.0%	41.2%
Panama	62.5%	88.2%
Pomona	0.0%	23.5%
Icterohaemorrhagiae (RGA)	0.0%	23.5%

Values represent the percentage of reactive animals that tested positive for each serogroup. Because individual animals often reacted to multiple serogroups (co-agglutination), the percentages are not mutually exclusive and sum to more than 100%.

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