International Journal for Parasitology: Parasites and Wildlife 29 (2026) 101187

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Molecular detection of vector-borne and hemotropic pathogens in raccoons (*Procyon lotor*) from a tropical ecotourism area in Costa Rica

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ARTICLE INFO

Keywords:
Vector-borne diseases
Infectious disease
Mycoplasma haemocanis
Canine protoparvovirus 1
Wildlife health

ABSTRACT

Interactions between wildlife, domestic animals, and humans in ecotourism settings could facilitate the circulation of pathogens with zoonotic potential. Raccoons (*Procyon lotor*), due to their synanthropic behavior and adaptability, may serve as hosts for several infectious agents at these interfaces. This study aimed to investigate the presence of vector-borne and hemotropic pathogens in free-ranging raccoons inhabiting Manuel Antonio National Park (MANP) and its surrounding communities, a major tourist destination in Costa Rica. Between 2021 and 2022, nineteen raccoons were captured using Tomahawk traps, anesthetized, clinically examined, and sampled for hematological and molecular analyses. DNA extracted from blood was screened using real-time and conventional PCR assays targeting *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Trypanosoma* spp., *Mycoplasma* spp., and canine protoparvovirus 1 (CPPV-1). Of the individuals tested, 6/19 (31.6 %) were positive for *Anaplasmataceae*, 4/14 (28.6 %) for *Mycoplasma* spp., and 6/14 (42.9 %) for CPPV-1. One raccoon was confirmed to carry *Mycoplasma haemocanis* (99 % nucleotide identity with GenBank accession MN294708), representing the first molecular identification of this species in raccoons worldwide. Coinfections were detected in five animals. No raccoons tested positive for *Rickettsia* spp. or *Trypanosoma* spp. Hematological profiles were largely within reference ranges; however, mild leukogram variations and occasional hyperglobulinemia were observed, with no consistent infection-associated pattern. These findings provide molecular evidence of pathogen presence in raccoons from a high-tourism area and highlight their potential epidemiological relevance at the wildlife-human interface. The results underscore the need for sustained One Health surveillance to better assess pathogen transmission risks in tropical ecotourism settings.

1. Introduction

The close interaction between wildlife, domestic animals, and humans is a key driver of pathogen emergence and spillover events, particularly in tropical regions with high biodiversity and increasing anthropogenic pressure (Keesing et al., 2010; Ellwanger and Chies, 2021). Raccoons (*Procyon lotor*), due to their synanthropic behavior, dietary plasticity, and tolerance of human disturbance, can act as hosts for a wide range of infectious agents, including zoonotic pathogens (Stope, 2019).

In Costa Rica, most investigations on infectious agents in raccoons have focused on urban and peri-urban settings within the Greater Metropolitan Area (Baldi et al., 2016, 2019; Meneses et al., 2016; Quesada et al., 2024). However, data from natural or protected environments remain scarce, despite their relevance for understanding wildlife health and disease ecology. Manuel Antonio National Park

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https://doi.org/10.1016/j.ijppaw.2026.101187
Received 25 November 2025; Received in revised form 2 January 2026; Accepted 3 January 2026
Available online 3 January 2026

(MANP), one of the most visited protected areas in Central America with over 400,000 tourists annually, represents a unique scenario in which raccoons frequently interact with humans and free-roaming domestic animals, often accessing anthropogenic food sources such as waste or pet food (Farrera-Hernández, 2017; Duscher et al., 2021).

Such interactions increase opportunities for pathogen exchange at the wildlife–human–domestic animal interface, making raccoons an important model species for One Health surveillance. The present study aimed to detect vector-borne and hemotropic pathogens of zoonotic relevance, including *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Trypanosoma*, *Mycoplasma*, and canine protoparvovirus 1 (CPPV-1), in free-ranging raccoons inhabiting MANP and surrounding communities.

2. Materials and methods

2.1. Ethical approval

All procedures complied with Costa Rican legislation on wildlife research and animal welfare. The study was authorized by the National System of Conservation Areas (SINAC) and the Ministry of Environment and Energy (MINAE) under research permit No. M-PC-SINAC-PNI-ACOPAC-021-2019 and approved by the National Commission for Biodiversity Management (CONAGEBIO) under permit R-CM-UNA-008-2021-OT-CONAGEBIO. Fieldwork was conducted in accordance with the ethical guidelines of the Universidad Nacional, Costa Rica.

2.2. Study area and sampling design

An observational study was conducted between 2021 and 2022 in MANP and its surrounding areas (9.391990° N, 84.145442° W), located on the Pacific coast of Costa Rica. The park is one of the most visited protected areas in Central America, receiving over 400,000 tourists annually. Its unique combination of protected habitats and high human visitation creates an interface where wildlife, domestic animals, and humans frequently interact, increasing opportunities for pathogen exchange.

Previous studies estimated a local raccoon (*Procyon lotor*) population of approximately 12 individuals in the park's intensive use zone (Farrera-Hernández, 2017). For this study, areas adjacent to the park were also included, and a total population of 24 individuals was assumed. The sample size was calculated using the WinEpi program, with an expected minimum prevalence of 10 %, a 95 % confidence level, and 80 % power. Under these conditions, a sample of 19 individuals was required to detect at least two infected animals within this population.

2.3. Capture and sample collection

Raccoons were live trapped using Tomahawk traps (Tomahawk, WI, USA) baited with fried bacon and placed in locations of frequent raccoon activity identified by park rangers. Captures were performed between 06:00 and 17:00. Animals were chemically immobilized with tiletamine–zolazepam (Zoletil®, Virbac) following standard wildlife protocols (Pitt et al., 2006). Each raccoon was physically examined, sexed, and aged (juveniles or adults). Blood was collected from the jugular, cephalic, or saphenous vein into EDTA, heparinized, and plain (without anticoagulant) tubes. After recovery from anesthesia, animals were released at the capture site. Whole blood samples were transported on ice and stored at −20 °C until further processing.

2.4. Hematology and biochemistry

Basic hematological and serum biochemical analyses were performed using standard veterinary analyzers (Abaxis VetScan HM5 and VS2, Zoetis, Inc., NJ, USA). Parameters were interpreted based on available reference ranges for *Procyonidae* (Ramsay, 2015).

2.5. DNA extraction and molecular assays

DNA was extracted from 100 µL of EDTA blood using the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. Samples were screened by real-time and conventional polymerase chain reaction (PCR) assays for the detection of *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Trypanosoma*, *Mycoplasma*, and canine protoparvovirus 1 (CPPV-1). Real-time PCRs (qPCRs) targeted the 16S rRNA gene for Anaplasmataceae (Li et al., 2001), *gltA* for *Rickettsia* spp. (Stenos et al., 2005), and VP2 for CPPV-1 (Balboni et al., 2018). Each qPCR run included a no-template control (molecular-grade water). Reactions were run in single replicate due to limited sample. Samples with Ct values < 35 were considered positive and were selected for downstream confirmatory PCR assays (conventional/nested/semi-nested) when sample availability permitted: *gltA* (Labruna et al., 2004) for *Rickettsia*, 16S rRNA for *Anaplasma* spp. (Zobba et al., 2014), *groEL* for *Anaplasma platys* and *A. phagocytophilum* (Alberti et al., 2005), and 16S rRNA for *Ehrlichia* spp. (Kocan et al., 2000). *Trypanosoma* spp. was detected using SSU-rRNA primers (Uliana et al., 1994), and *Mycoplasma* spp. with 16S rRNA primers (Kuppeveld et al., 1994; Kong et al., 2001). PCR products were visualized on agarose gels, purified with the QIAquick® PCR Purification Kit (Qiagen), and sequenced by Macrogen (Seoul, Republic of Korea). Sequences were aligned using ClustalW in BioEdit (Hall, 1999) and compared to GenBank entries via BLASTn for species identification. Due to limited sample, not all individuals were tested for every assay; therefore, denominators vary by pathogen and are indicated in each table. The 16S rRNA sequence obtained for *Mycoplasma haemocanis* in this study was deposited in GenBank under accession number PX794531.

2.6. Statistical and interpretative approach

Pathogen detection frequencies were calculated as percentages of positive individuals among total samples tested for each pathogen. Prevalence estimates were reported as proportions with 95 % confidence intervals (95 % CI) calculated using the Wilson score method. Coinfections were recorded when an animal tested positive for more than one pathogen. No statistical correlations between hematological variables and infection status were performed due to the small sample size; instead, descriptive statistics were performed.

3. Results

A total of 19 raccoons (15 adults and 4 juveniles; 5 females and 14 males) were captured between 2021 and 2022. All appeared clinically healthy at the time of capture and recovered uneventfully after anesthesia.

Of the 19 individuals analyzed, 6/19 (31.6 %; 95 % CI: 15.4–54.0) tested positive for Anaplasmataceae by qPCR, 6/14 (42.9 %; 95 % CI: 21.4 %–67.4 %) were positive for CPPV-1, and 4/14 (28.6 %; 95 % CI: 11.7–54.6) for *Mycoplasma* spp. One raccoon carried *Mycoplasma haemocanis* (GenBank accession PX794531) with 99 % nucleotide identity to a strain previously reported in a feral dog from Mexico (GenBank MN294708). Five raccoons were coinfected with two pathogens, either Anaplasmataceae and CPPV-1, or Anaplasmataceae and *Mycoplasma* spp. All animals tested negative for *Rickettsia* spp. and *Trypanosoma* spp. Although several samples yielded amplification signals in qPCR, no amplicons were obtained in conventional or nested PCRs, except for the *Mycoplasma haemocanis*-positive sample. A summary of the detected pathogens, the diagnostic methods employed, and the level of molecular confirmation is provided in Table 1.

When stratifying pathogen detection by sex and age class, differences in prevalence were observed (Table 2). For Anaplasmataceae and CPPV-1 only males tested positive, while no infected females were detected. In contrast, *Mycoplasma* spp. was detected in both sexes. Juveniles showed a higher prevalence of Anaplasmataceae and CPPV-1, compared to

Table 1
Summary of detected pathogens, diagnostic methods used, and level of molecular confirmation.

Pathogen	Diagnostic method	Target gene/ region	Positive/ Total[a]	Level of confirmation
Anaplasmataceae (*Ehlichia* spp. and *Anaplasma* spp.)	qPCR	16S rRNA	6/19	Screening only (no confirmatory sequencing)
Anaplasma spp.	Conventional PCR	16S rRNA	0/6	No amplification detected
Anaplasma platys and *A. phagocytophilum*	Nested PCR	*groEL*	0/6	No amplification detected
Ehrlichia spp.	Nested PCR	16S rRNA	0/6	No amplification detected
CPPV-1	qPCR	VP2	6/14	Screening only (no confirmatory sequencing)
CPPV-1	Hemi-nested PCR	VP2	0/6	No amplification detected
Mycoplasma spp.	qPCR	16S rRNA	4/14	Screening only (no confirmatory sequencing)
Mycoplasma spp	Conventional PCR	16S rRNA	1/4	Sequenced (species confirmed: *M. haemocanis,* GenBank PX794531)

[a] Due to limited sample, not all individuals were tested for every assay; therefore, denominators vary by pathogen.

Table 2
Prevalence of selected pathogens in raccoons (*Procyon lotor*) from MANP by sex and age group.

Agent	Male +/total (%)	Females +/total (%)	Adults +/total (%)	Juveniles +/total (%)
Anaplasmataceae	6/14 (42.9)	0/5 (0)	4/15 (26.7)	2/4 (50.0)
CPPV-1	6/12 (50.0)	0/2 (0)	4/10 (40.0)	2/4 (50.0)
Mycoplasma spp.	3/12 (25.0)	1/2 (50.0)	3/10 (30.0)	1/4 (25.0)

Due to limited sample, not all individuals were tested for every assay; therefore, denominators vary by pathogen.

adults. These differences suggest potential age- and sex-associated variation in exposure risk or susceptibility.

Leukogram variations were frequently observed (14/19 raccoons), with leukocytosis and neutrophilia being the most common findings (10/19 each), followed by monocytosis (8/19). However, these changes were not consistently associated with PCR positivity; for example, 3/6 Anaplasmataceae-positive raccoons showed no significant hematological findings. Individual pathogen detection is provided in Supplementary Table S1, and individual hematological and biochemical values are shown in Supplementary Table S2 and S3. Biochemical deviations were common and were dominated by increased aspartate aminotransferase (AST) and creatine kinase (CK/CPK) activities, consistent with capture- and handling-associated muscle effects. These increases occurred in both pathogen-positive and pathogen-negative raccoons; therefore, no consistent infection-associated biochemical pattern was evident (Supplementary Table S3).

4. Discussion

This study provides molecular evidence of vector-borne and hemotropic pathogens circulating in raccoons (*Procyon lotor*) inhabiting one of Costa Rica's most heavily visited national parks and surrounding areas. Although the sample size was limited, the findings reveal the coexistence of multiple infectious agents within a population exposed to intense human and domestic animal contact, underscoring the role of raccoons as potential reservoirs at the wildlife–human interface (Keesing et al., 2010; Ellwanger and Chies, 2021).

While some pathogens, such as *Mycoplasma haemocanis*, were confirmed by sequencing, others, including members of the Anaplasmataceae family, were identified exclusively through qPCR screening. Stratified analyses by sex and age group revealed apparent differences in pathogen occurrence across host categories, with Anaplasmataceae and CPPV-1 more frequently observed in males and juveniles, whereas *Mycoplasma* spp. occurred across both sexes and age classes. These patterns may reflect differences in behavior (e.g., ranging or foraging), hormonal influences on immune function, or variation in vector exposure risk (Stringer et al., 2010; Wait et al., 2023). Although formal statistical testing was not feasible due to limited sample size, this stratification offers valuable baseline insights for future comparative and longitudinal studies.

Detection of Anaplasmataceae DNA in nearly one-third of the sampled animals aligns with previous reports from urban and peri-urban raccoons in Costa Rica and other regions, where *Ehrlichia canis*, *Anaplasma platys*, *A. phagocytophilum*, and related species have been identified in wildlife (Dolz et al., 2015; Lesiczka et al., 2023). In our study, 31.6 % of raccoons tested positive using a qPCR targeting the 16S rRNA gene of the Anaplasmataceae family, an assay that may detect a broader spectrum of species than the more specific molecular targets used in other surveys. By comparison, *Anaplasma phagocytophilum* was identified in only 15.7 % (16/102) of raccoons in southwest Germany (Reinhardt et al., 2023), with even lower prevalences reported in other European wildlife studies (e.g., 5.8 %; Lesiczka et al., 2023). Such differences may reflect variation in molecular targets, sample type or storage conditions, as well as ecological context. In Costa Rica, raccoons frequently co-occur with free-roaming dogs, recognized reservoirs of *Ehrlichia canis* and *Anaplasma platys*, and may be exposed to *Rhipicephalus sanguineus* sensu lato, a tick vector common in tropical regions but largely absent from European wildlife systems (Dantas-Torres, 2010; Gray et al., 2009; Otranto, 2018).

The absence of conventional PCR amplification despite qPCR positivity likely reflects low pathogen loads or partial DNA degradation, which are common in wildlife samples stored under field conditions. Similar findings have been reported in studies of hemotropic pathogens in raccoons and other carnivores (Maggi et al., 2013; Silaghi et al., 2016). Although qPCR screening revealed Anaplasmataceae DNA in a subset of raccoons, the absence of confirmatory amplicons using conventional and nested PCR precluded species-level identification and limited biological interpretation. These findings should therefore be interpreted as molecular evidence of exposure or low-level infection rather than active circulation. Low pathogen loads, DNA degradation, or the detection of residual or environmentally derived bacterial DNA may explain qPCR positivity without successful downstream amplification, as previously reported in wildlife surveillance studies (Maggi et al., 2013; Silaghi et al., 2016). While our data does not allow firm conclusions regarding pathogen dynamics, they confirm exposure within the population and may also indicate the presence of uncharacterized Anaplasmataceae lineages, possibly within the genus *Anaplasma* or other related genera, as described in wildlife and tick populations from other regions (Silaghi et al., 2016). Taken together, hematological variations were common across animals but were not consistently associated with qPCR positivity, suggesting nonspecific (e.g., capture-related) leukogram changes rather than pathogen-specific alterations. These results emphasize the need to combine molecular surveillance with ectoparasite screening to clarify the epidemiology of these bacteria in tropical ecosystems.

Additionally, the detection of CPPV-1 DNA in 42.9 % of the sampled individuals is notable and may reflect ongoing exposure or viral persistence within local carnivore communities. While raccoons are not considered primary hosts, the presence of viral DNA in blood supports

possible subclinical or latent infections, facilitating interspecies transmission (Balboni et al., 2018). Similar evidence of CPPV-1 DNA in peripheral blood has been reported in cats and dogs, supporting the hypothesis of hematogenous persistence (Ikeda et al., 2002; Obando-Corella et al., 2024). In Europe, CPPV-1 has been reported in invasive raccoons at relatively low prevalence. For example, Reinhardt et al. (2023) detected CPPV-1 in 7.8 % (8/102) of raccoons from southwest Germany using molecular methods. Similarly, Ndiana et al. (2021) reported an overall prevalence of 11.4 % (34/297) in wild carnivores from Italy, although species-specific data for raccoons were not provided, and detection in raccoons has also been documented in Poland without prevalence estimates (Lesiczka et al., 2023). In this context, the positivity observed in our study is notably high. In Costa Rica, stray and domestic dogs are frequently observed near Manuel Antonio National Park despite access restrictions and are well-recognized reservoirs of CPPV-1 (Vieira et al., 2015; Beus et al., 2024). Such spatial overlap likely facilitates viral maintenance and transmission at the human–wildlife interface. Similarly, hematological findings did not show a consistent pattern in CPPV-1–positive raccoons, supporting the interpretation that the observed leukogram variations were nonspecific.

The detection of *Mycoplasma haemocanis* represents the first molecular record of this hemoplasma in raccoons worldwide. This bacterium is generally host-specific to canids and transmitted via blood-sucking arthropods such as fleas and ticks (Maggi et al., 2013; Beus et al., 2024). Its occurrence in raccoons from a protected area indicates potential spillover between domestic and wild carnivores sharing the same vector fauna. This detection contributes to the growing evidence that hemoplasmas can cross species boundaries under shared ecological pressures, particularly in disturbed environments where synanthropic wildlife overlaps with human settlements.

Nevertheless, studies on hemotropic *Mycoplasma* spp. in raccoons remain scarce. Previous research in North America has reported prevalences of up to 62.1 % in wild raccoons (*Procyon lotor*) (Volokhov et al., 2017), which is substantially higher than the 28 % observed in our study. In contrast, European surveys have documented intermediate prevalence levels that are consistent with our findings (Unterköfler et al., 2024). Although no consistent hematological alterations were associated with pathogen detection, mild leukogram changes and occasional hyperglobulinemia were observed in some individuals, findings that can be consistent with nonspecific or chronic antigenic stimulation. Similarly, previous studies have also described subclinical hemoplasma infections in raccoons without significant hematological effects (Maggi et al., 2013). Therefore, the absence of overt disease signs should not diminish the epidemiological importance of infected carriers, which may contribute to pathogen maintenance and onward transmission within wildlife communities and at the wildlife–domestic animal interface.

While our findings provide molecular evidence of pathogen exposure in raccoons from a high-tourism area, the zoonotic risk remains theoretical in the absence of data from humans or domestic animals in the study area. Nonetheless, these results highlight the potential for shared ecological niches to facilitate pathogen exchange at the wildlife–human interface. Continued surveillance of wildlife, vectors, and sympatric domestic animals in such settings is essential to support One Health–based risk assessments and inform preventive strategies (Keesing et al., 2010).

A major limitation of this study is the absence of ectoparasite sampling, which restricts our ability to directly assess vector involvement in pathogen transmission and renders inferences regarding vector-mediated spillover speculative. Future studies should prioritize the molecular screening of ectoparasites and sympatric host species to better elucidate pathogen transmission pathways at the wildlife–domestic interface, improve understanding of pathogen dynamics within tropical protected areas, and guide evidence-based management strategies.

Despite the limited sample size inherent to a small and protected raccoon population, this study provides valuable baseline data to support future longitudinal and comparative investigations. The confirmation of *Mycoplasma haemocanis* in raccoons highlights the permeability of the wildlife–domestic animal interface in ecotourism settings. Although the identification of vector-borne agents in raccoon blood samples is consistent with possible exposure to infected vectors, the lack of vector data limits the reconstruction of specific reservoir–vector–host cycles. Overall, these findings underscore the importance of continuous, integrative One Health monitoring to better understand and mitigate zoonotic pathogen circulation in tropical ecosystems.

CRediT authorship contribution statement

Jennifer Calderón-Bailey: Writing – review & editing, Writing – original draft, Resources, Investigation. **Ernesto Rojas-Sánchez:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **María Mata-Masís:** Writing – review & editing, Resources, Investigation. **Mauricio Jiménez-Soto:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Antony Solorzano-Morales:** Writing – review & editing, Resources, Investigation, Formal analysis. **María José Zuniga-Moya:** Writing – review & editing, Resources, Investigation. **Javier Varela-Amador:** Writing – review & editing, Resources, Investigation. **Karen Vega-Benavides:** Writing – review & editing, Resources, Investigation, Conceptualization. **Gaby Dolz:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Funding

This project was financed by project SIA-UNA 0048-18 “Diagnosis, Disease Control, and Management of Wild Animals” of the Hospital de Especies Menores y Silvestres (HEMS), by FUNDAUNA project 054530 “Diagnóstico e Investigación en Medicina Poblacional y Ecosistémica”, and Fondo Institucional de Desarrollo Académico “Health status of the grey-crowned Central American squirrel monkey (*Saimiri oerstedii citrinellus*), white-faced monkey (*Cebus imitator*) and raccoon (*Procyon lotor*) in Manuel Antonio National Park and surrounding areas, Costa Rica”.

Conflict of interest

The authors declare that there are no competing interests regarding the publication of this manuscript.

Acknowledgments

We thank the Costa Rican National System of Conservation Areas (SINAC) and staff at Manuel Antonio National Park for logistical support. We also acknowledge the veterinary and technical teams from Universidad Nacional who assisted in the capture and sampling of raccoons in the field, Valeria Villero, Guillermo Víquez, Paola Morán, Marian Meléndez, Mauricio Guerra, and Wilson Bonilla.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2026.101187.

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