UNIVERSIDAD
NACIONAL
DE COLOMBIA

Effect of landscape structure on the diversity of microbiota and intestinal protozoa between wild and domestic mammals in some regions of Colombia

Néstor Javier Roncancio Duque

Universidad Nacional de Colombia

Facultad de Ciencias Agropecuarias, Doctorado en Ciencias Agrarias

Palmira, Colombia

2024

Effect of landscape structure on the diversity of microbiota and intestinal protozoa between wild and domestic mammals in some regions of Colombia

Néstor Javier Roncancio Duque

Tesis presentada como requisito parcial para optar al título de:

Doctor en Ciencias Agrarias

Directora:

Ph.D., MSc. Diana Carolina López Álvarez

Línea de Investigación:

Producción Animal Tropical

Grupo de Investigación:

Grupo de investigación en Diversidad Biológica

Universidad Nacional de Colombia

Facultad de Ciencias Agropecuarias, Doctorado en Ciencias Agrarias

Palmira, Colombia

2024

Declaración de obra original

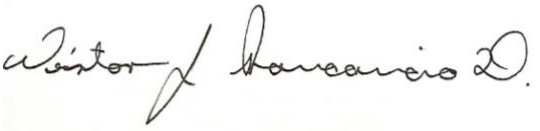
Yo declaro lo siguiente:

He leído el Acuerdo 035 de 2003 del Consejo Académico de la Universidad Nacional. «Reglamento sobre propiedad intelectual» y la Normatividad Nacional relacionada al respeto de los derechos de autor. Esta disertación representa mi trabajo original, excepto donde he reconocido las ideas, las palabras, o materiales de otros autores.

Cuando se han presentado ideas o palabras de otros autores en esta disertación, he realizado su respectivo reconocimiento aplicando correctamente los esquemas de citas y referencias bibliográficas en el estilo requerido.

No se hizo uso ningún material protegido por derechos de autor (por ejemplo, tablas, figuras, instrumentos de encuesta o grandes porciones de texto), todo el material usado se produjo en este trabajo

Por último, he sometido esta disertación a la herramienta de integridad académica, definida por la universidad.



Néstor Javier Roncancio Duque

Fecha 31/01/2024

Acknowledges

We thank Universidad Nacional de Colombia sede Palmira for the teaching assistance scholarship (Resolution 061/2021, Concejo de Facultad, Facultad de Ciencias Agropecuarias sede Palmira). We are grateful to the Andean Bear Conservation Alliance (ABCA), the Cleveland Metroparks Zoo Wildlife Conservation Program, and Rewild for their support of this doctoral thesis. We also acknowledge MINCIENCIAS for the scholarship: “Jóvenes investigadores e innovadores en las regiones para atención de demandas definidas por los CODECTI de los Departamentos de Chocó, Valle del Cauca, Cauca y Nariño – BPIN 2022000100068”. The *Saguinus leucopus* samples was collected within the framework of the White footed tamarin monitoring plan implementation of the Corporación Autonoma Regional de Caldas – Corpocaldas, funded by ISAGEN and Agreement EPM-UDEA No. 2021-000023 Acta de ejecución No. CT-2021-000023-A7. Estrategia para la conservación del titi gris (*Saguinus leucopus*) en el área de influencia de la central Porce III. We extend our thanks to the owners and managers of the properties where the samples were collected, as well as the National Natural Parks of Colombia. Finally, we thank to Professor Javier Antonio Benavidez for his good intentions and initial guidance in this process.

Resumen

Efecto de la estructura del paisaje sobre la diversidad de la microbiota y protozoarios intestinales entre mamíferos silvestres y domésticos en algunas regiones de Colombia.

Con relación a la microbiota intestinal, la hipótesis postula que un aumento en la diversidad microbiana contribuye significativamente a una mejor funcionalidad en varios procesos asociados con ella. La microbiota intestinal muestra una alta susceptibilidad a diversas formas de estrés, y el impacto de tales factores estresantes puede ser profundo, afectando tanto su composición como su función. Una comprensión integral de cómo distintas formas de estrés afectan a la microbiota intestinal es imperativa para el desarrollo de estrategias destinadas a preservar la salud gastrointestinal y, por ende, el bienestar general de los individuos. Por otro lado, los parásitos constituyen componentes integrales dentro de procesos naturales que podrían determinar la regulación poblacional y mantienen el equilibrio del ecosistema. Sin embargo, tanto los cambios naturales como los antropogénicos pueden perturbar estos procesos ecológicos. Entre las enfermedades infecciosas, aquellas inducidas por protozoos son contribuyentes destacados a la morbilidad y mortalidad humanas. Los cambios ambientales exacerban las interacciones entre la vida silvestre, los animales domésticos y los humanos, intensificando así las tasas de transmisión entre especies. Por tanto, el objetivo de este estudio fue investigar las asociaciones, incluyendo relaciones y similitudes, de la estructura del paisaje, influenciada por actividades humanas, la diversidad de la microbiota intestinal y la abundancia de protozoarios gastrointestinales entre mamíferos silvestres y domésticos en Colombia. La determinación taxonómica se hizo mediante metabarcoding con cebadores dirigidos al gen rRNA 18S y secuenciación Nanopore, con un enfoque principal en la detección de protozoos. Se recopilaron un total de 148 muestras de seis mamíferos silvestres y tres mamíferos domésticos en 29 paisajes focales en Colombia. También se empleó la microscopía para validar algunos agentes. Para describir epidemiológicamente las muestras, se estimaron la riqueza de taxones en la microbiota intestinal, así como la prevalencia, intensidad media y abundancia media de los protozoarios. Se utilizaron regresiones Beta y Poisson bayesianas para evaluar la relación entre

las métricas del paisaje y la disimilitud, la diversidad de la microbiota intestinal y de protozoarios, y la abundancia de protozoarios específicos. El estudio identificó varias especies no reportadas previamente en las seis especies silvestres hospedadoras evaluadas. Se observó una proporción predominante de los phylum Ascomycota, Pseudomonadota, Basidiomicota y Apicomplexa, reflejando por un lado una microbiota intestinal saludable, aunque también la posible predominancia de ciertos elementos negativos. Las comparaciones entre caninos y equinos, así como entre tapires y osos entre los mamíferos terrestres, indicaron una mayor similitud tanto en la microbiota intestinal como en los protozoarios. En primates, el mono aullador rojo mostró una proximidad más cercana a los bovinos y equinos que a otros primates. Los hallazgos indicaron que una mayor proporción de cobertura vegetal natural estaba relacionada con una mayor similitud en la microbiota intestinal entre mamíferos silvestres y domésticos. Además, mayores proporciones de cobertura vegetal natural, presencia de cuerpos de agua, número de parches de bosque e irregularidades en la forma de los bosques se asociaron con una mayor diversidad (tanto en riqueza como en equidad) en la microbiota intestinal y de protozoarios intestinales a diferentes escalas.

Palabras clave: Epidemiología del paisaje, índice de disimilaridad, Fragmentación, Ganado, Microbiota intestinal, Vida silvestre**.**

Abstract

Effect of landscape structure on the diversity of microbiota and intestinal protozoa between wild and domestic mammals in some regions of Colombia

Concerning the gut microbiota, the hypothesis posits that increased microbial diversity contributes significantly to enhanced functionality across various associated processes. The intestinal microbiota exhibits high susceptibility to diverse forms of stress, and the impact of such stressors can be profound, affecting both its composition and function. A comprehensive understanding of how distinct forms of stress influence the intestinal microbiota is imperative for the developing strategies aimed at preserving gastrointestinal health and, consequently, the overall well-being of individuals. Conversely, parasites constitute integral components within natural processes that could determine population regulation and maintain ecosystem balance. However, both natural and anthropogenic changes can disrupt these ecological processes. Among infectious diseases, those induced by protozoa are prominent contributors to human morbidity and mortality. The environmental changes exacerbate interactions among wildlife, domestic animals, and humans, thereby intensifying transmission rates between species. Thus, the objective of this study was to investigate the associations, including relationships and similarities, between landscape configuration influenced by human activities and the diversity of intestinal microbiota, as well as the abundance of gastrointestinal parasites, among wild and domestic mammals in Colombia. Taxonomic determination was achieved through metabarcoding with primers targeting the rRNA 18S gene and Nanopore sequencing, with a primary focus on detecting protozoa. A total of 148 samples were collected from six wildlife mammals and three domestic mammals across 29 focal landscapes in Colombia. Microscopy was also employed to validate certain agents. To describe the epidemiological landscape, taxa richness in gut microbiota, as well as the prevalence, mean intensity, and mean abundance of protozoa, were estimated. Bayesian Beta and Poisson regressions were employed to assess the relationship between landscape metrics and dissimilarity, gut and protozoa diversity, and the abundance of specific target protozoa. The study identified several new species within the six assessed host species. A predominant proportion of phyla Ascomycota, Pseudomonadota, Basidiomicota, and Apicomplexa were observed, reflecting a healthy intestinal microbiota and a potential predominance of certain negative elements. Comparisons between canines and equines, as well as between tapirs and bears among terrestrial mammals, indicated greater similarity in both gut microbiota and protozoa. In primates,

the red howler monkey exhibited closer proximity to bovines and equines than to other primates. The findings indicated that a higher proportion of natural vegetation coverage correlated with increased similarity in gut microbiota among wild and domestic mammals. Additionally, higher proportions of natural vegetation coverage, presence of water bodies, number of forest patches, and irregularities in forest shapes were associated with greater diversity (both richness and evenness) in gut microbiota and intestinal protozoa across different scales.

Keywords: Cattle, Dissimilarity index, Fragmentation, Gut microbiota, Landscape epidemiology, Wildlife**.**

Content

Pág.

List of figures

List of tables

Pág.

Introduction

Health in agriculture is the capacity to satisfy the daily requirements and to adapt to the environmental changes which implies the capacity to preserve in the system the productive parameters. When the disease appears, humans, animals, or plants decrease their productivity. In most cases, infective parasites and zoonotic diseases are the main focus (Eriksson & Lindstrom, 2008; Frankish et al., 1996). Parasites are integral components of natural processes that drive population regulation and ecosystem balance (Clayton & Moore, 1997; Delahay & Delahay, 2009). They have the potential to influence key population parameters such as birth and death rates (Nunn & Altizer, 2006). These ecological processes may be disrupted by both natural and anthropogenic changes. Furthermore, parasites could act synergistically with habitat reduction, poaching, and pollution, posing a threat to biodiversity conservation (Aguirre, 2009; Smith et al., 2009; Wisely et al., 2008). Parasites may also contribute to substantial economic losses due to decreased production, financial expenditures on control and treatment, and increased mortality in livestock, particularly cattle (Charlier et al., 2014; Rashid et al., 2019; Rodríguez-Vivas et al., 2017). In the face of potential environmental changes, parasites may alter their impact on wildlife, domestic animals, and human health by intensifying contact between hosts and infectious agents. In this context, a comprehensive prioritized analysis based on the "One Health" approach proves valuable for decision-making (Jenkins et al., 2015) and should be developed, considering informative proxies and indicators pertaining to the interactions between human, animal, and ecosystem health (Aguirre et al., 2002).

Mammals are an important group for epidemiological surveillance due to the impact that diseases can have on their endangered populations, and because of the risk of zoonotic transmission. As some primate species are used for bush meat, biomedical models, or, as pets, many mammals are in continuous close contact with humans, which increases the risk of cross-transmission and disease spread, highlighting the urgent need for mammal epidemiological surveillance (Artois et al., 2009; Chapman et al., 2005).

Thus, the main aim of this study is to determine the association (relationships and similarities) of livestock, local landscape, and human activity features with the occurrence probability of the pathogenic gastrointestinal protozoa in wild mammals in Colombia. Our hypothesis is the further away the livestock is from the sustainability standards, based on the greater the transformation in the landscape, the greater the richness and abundance of protozoa on the mammals. To test my hypothesis, I have formulated two specific aims: 1. to determine the relationship between the livestock features (Landscape metrics) and the presence and gastrointestinal protozoa burden in its domestic animals and associated environment, given that their epidemiology may pose a risk some wild mammals and 2. to determine the relationship between the local landscape features and the severity of human activities with the occurrence probability of the gastrointestinal protozoa in some wild mammals. The first specific aim is documented in chapter 3 titled “Relationship of the landscape configuration and the diversity of gut protozoa and microbiota in livestock associated with conservation areas in Colombia” here we describe epidemiologically the domestic species samples in function of the diversity gut microbiota and the prevalence, mean intensity and mean abundance of the protozoa detected, Apicomplexa, Tricomonadida, Trypanosomatidae, *Paramecium*, and *Giardia* clades and I evaluate the relationship between of landscape metrics in the bovine and equine gut microbiota and protozoa diversity (richness and evenness). To discuss the risk, the parasites found were contrasted with a systematic review of parasites in several wildlife mammals (Rondón et al., 2021; Solórzano-García & Pérez-Ponce de León, 2018; Uribe et al., 2021), one of them was developed in the present word and documented in the chapter one title “Parasites in bears (Ursidae): sampling gaps in the Spectacle Bear (*Tremarctos ornatus*)”. The second specific aim is documented in chapter 4 titled” Relationship of landscape configuration with the diversity of gut microbiota and protozoa in wild mammals and its similarity with livestock in conservation areas in Colombia”, here we describe epidemiological wildlife mammals sampled in the same way of the domestic in chapter 3. Additionally, we estimated the dissimilarity of gut microbiota and protozoa between all host species and assessed the relationship of the landscape metrics with those dissimilarities, finally we assessed the relationship between the local landscape features and the severity of human activities with the diversity of gut microbiota and protozoa, and mean abundance of the gastrointestinal protozoa in *Alouatta seniculus* in a multiscale approach that was the host species with enough sample size to this analysis.

Taxonomic determination was achieved through metabarcoding with primers targeting the rRNA 18S gene and Nanopore sequencing, with a primary focus on detecting protozoa. This approach had some challenges, mainly associated we the sample collection, that we should resolve, this

process was documented in chapter two titled “Comparison of concentration and quality of DNA of fecal samples of mammals between collected and transported in ethanol or lysis buffer”. As all chapters were written as independent papers, some texts are delivered repeatedly or have the same structure with numbers variation, mainly about the methods and some explication of similar results. The supplement information of the four chapters is in https://drive.google.com/drive/u/2/folders/12YwS-ACMZThxQv2bwdrM6JvjqUyxLj_6

References

Aguirre, A. A. (2009). Wild canids as sentinels of ecological health: A conservation medicine perspective. Parasites & Vectors, 2(Suppl 1), S7. https://doi.org/10.1186/1756-3305-2-S1-S7

Aguirre, A. A., Ostfeld, R. S., Tabor, G. M., House, C., & Pearl, M. C. (2002). Conservation medicine: Ecological health in practice. Oxford University Press.

Artois, M., Bengis, R., Delahay, R. J., Duchêne, M.-J., Duff, J. P., Ferroglio, E., Gortazar, C., Hutchings, M. R., Kock, R. A., Leighton, F. A., Mörner, T., & Smith, G. C. (2009). Wildlife Disease Surveillance and Monitoring. In R. J. Delahay, G. C. Smith, & M. R. Hutchings (Eds.), Management of Disease in Wild Mammals (pp. 187–213). Springer Japan. https://doi.org/10.1007/978-4-431-77134-0_10

Chapman, C. A., Gillespie, T. R., & Goldberg, T. L. (2005). Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews, 14(4), 134–144.

Charlier, J., Van Der Voort, M., Kenyon, F., Skuce, P., & Vercruysse, J. (2014). Chasing helminths and their economic impact on farmed ruminants. Trends in Parasitology, 30(7), 361–367. https://doi.org/10.1016/j.pt.2014.04.009

Clayton, D. H., & Moore, J. (1997). Host-parasite evolution: General principles and avian models. (No Title).

Delahay, R., & Delahay, R. J. (Eds.). (2009). Management of disease in wild mammals (1. ed). Springer.

Jenkins, E. J., Simon, A., Bachand, N., & Stephen, C. (2015). Wildlife parasites in a One Health world. Trends in Parasitology, 31(5), 174–180. https://doi.org/10.1016/j.pt.2015.01.002

Nunn, C., & Altizer, S. M. (2006). Infectious diseases in primates: Behavior, ecology and evolution. Oxford University Press, USA.

Rashid, M., Rashid, M. I., Akbar, H., Ahmad, L., Hassan, M. A., Ashraf, K., Saeed, K., & Gharbi, M. (2019). A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. Parasitology, 146(2), 129–141. https://doi.org/10.1017/S0031182018001282

Rodríguez-Vivas, R. I., Grisi, L., Pérez de León, A. A., Silva Villela, H., Torres-Acosta, J. F. de J., Fragoso Sánchez, H., Romero Salas, D., Rosario Cruz, R., Saldierna, F., & García Carrasco, D. (2017). Potential economic impact assessment for cattle parasites in Mexico. Review. Revista Mexicana de Ciencias Pecuarias, 8(1), 61. https://doi.org/10.22319/rmcp.v8i1.4305

Rondón, S., Cavallero, S., Renzi, E., Link, A., González, C., & D'Amelio, S. (2021). Parasites of Free-Ranging and Captive American Primates: A Systematic Review. Microorganisms, 9(12), 2546. https://doi.org/10.3390/microorganisms9122546

Smith, K. F., Behrens, M., Schloegel, L. M., Marano, N., Burgiel, S., & Daszak, P. (2009). Reducing the risks of the wildlife trade. Science, 324(5927), 594–595. https://doi.org/10.1126/science.1174460

Solórzano-García, B., & Pérez-Ponce de León, G. (2018). Parasites of Neotropical Primates: A Review. International Journal of Primatology, 39(2), 155–182. https://doi.org/10.1007/s10764-018-0031-0

Uribe, M., Payán, E., Brabec, J., Vélez, J., Taubert, A., Chaparro-Gutiérrez, J. J., & Hermosilla, C. (2021). Intestinal Parasites of Neotropical Wild Jaguars, Pumas, Ocelots, and Jaguarundis in Colombia: Old Friends Brought Back from Oblivion and New Insights. Pathogens, 10(7), 822. https://doi.org/10.3390/pathogens10070822

Wisely, S. M., Howard, J., Williams, S. A., Bain, O., Santymire, R. M., Bardsley, K. D., & Williams, E. S. (2008). An unidentified filarial species and its impact on fitness in wild populations of the black-footed ferret (Mustela nigripes). Journal of Wildlife Diseases, 44(1), 53–64

1. Parasites in bears (Ursidae): sampling gaps in the Spectacle Bear (*Tremarctos ornatus*)

Parásitos en osos (Ursidae): vacíos de muestreo en oso de anteojos (*Tremarctos ornatus*)

Néstor Roncancio-Duque

Médico Veterinario Zootecnista, MSc., PhD (c), Universidad Nacional de Colombia, njroncanciod@unal.edu.co, https://orcid.org/my-orcid?orcid=0000-0001-8575-8272

1.1 Abstract

Parasites are part of the natural processes that help regulate populations and maintain ecosystem balance. There is a growing recognition of parasites as important factors to the conservation of species, mainly those vulnerable to extinction in a changing environment. Bears are good biological models for monitoring infectious agents in wildlife, given their life cycle, broad home range, and severity of interactions with humans and their domestic animals as a result of their behavioral plasticity, intelligence, and omnivorous food habits. In the Andean region, the only bear species *Tremarctos ornatus* is listed as vulnerable. In order to determine the sampling gap and prioritize the approach for understanding parasite diversity in bears, I performed a systematic review and metanalysis of the documented parasites of bears across the world, and discuss the possibility of the parasites recorded in these other species being present in *T. ornatus* in the Andean region, specifically Colombia. In 283 relevant references, 647 records were found of 189 parasites in 37 countries. Of the bear species with parasites recorded, *U. americanus* had the most numerous and complete records. The tropical species *H. malayanus, M. ursinus,* and *T. ornatus* showed the smallest parasite diversity and unseen species estimate, despite being the

region where the greatest diversity of parasites was expected. Of interest are around 80 parasites that have been recorded in seven non-Colombian bear species, but are documented in other species in this country.

1.1.1 **Keywords:** epidemiological risk, infectious agents, *Tremarctos ornatus*, Ursids

1.2 Introduction

Parasites are part of the natural processes that help regulate populations and maintain ecosystem balance. They can affect population parameters such as birth and death rates, and some mathematical models suggest that they could play an important role in their hosts' population and evolutionary dynamics. There is a growing recognition that parasites play an important role in the biology and conservation of species, as they often lead to deleterious health effects, fitness reduction, and mortality (Aguirre et al., 2002; Smith et al., 2009; Wisely et al., 2008). These could cause local extinction in wildlife populations (Smith et al., 2006; Zhang et al., 2011), so any factor that can modify the ecosystem that is the natural reservoir of such infectious agents has the potential to disturb their epidemiology (Patz et al., 2003). As such, high rates of abnormal mortalities in wildlife must be further investigated to be confirmed epidemiologically and can to prevent further infections (Brena et al., 2018).

These ecological processes are altered by natural and anthropogenic changes, and parasites could become a threat to species conservation, together with habitat reduction, poaching and pollution. In response to the environmental changes, parasites could change their effect on wildlife, domestic and human health by increasing contact between hosts and infectious agents.

While infectious diseases are not considered an important global threat to wildlife viability (Smith et al., 2006), they are a common driver of population depletion (temporal or permanent) at the local scale, particularly of threatened, isolated, or small populations (García Marín et al., 2018; Ujvari & Belov, 2011). The mega-biodiversity of some countries such as Colombia introduces an additional epidemiological risk factor, because there are many more wild and domestic species that could serve as susceptible host and reservoirs for several infectious agents when the ecosystem dynamic is altered (Mackenstedt et al., 2015; Monsalve-Buriticá, 2019). A challenge to conservation medicine is to plan effective actions that mitigate the effect of emerging diseases that are currently driving biodiversity loss.

Bears could potentially perpetuate disease transmissions to human and livestock, given that they often explore anthropogenic habitats in agroecosystems due to their behavioral plasticity, intelligence, and omnivorous food habits (Gilbert, 1989; McCullough, 1982; Sasmal et al., 2019). Additionally, the current trend of increased interactions might make bears important vectors or intermediate hosts for several zoonotic pathogens (Baruch-Mordo et al., 2014; Bronson et al., 2014; Dubey & Jones, 2008), as they could predate or scavenge an infected host (Elbroch et al., 2015; Kindschuh et al., 2016; Lesmerises et al., 2015). The overlapping habitat use between bears and livestock sets up a potential risk for infectious disease transmission (Borka-Vitális et al., 2017; Westmoreland et al., 2016; Wu et al., 2018). Bears may be exposed to humans, livestock, and other wildlife pathogens through vectors, predation, scavenging, or environmental reservoirs (e.g., water) (Peña-Quistial et al., 2020; Stephenson et al., 2015). The impact of infectious diseases in bear conservation may act synergistically with other threats such as isolation and low population size, reducing populations even further and increasing their vulnerability to habitat reduction and degradation, dietary stress, hunting, and pathogens (Ishibashi et al., 2017; Schwab et al., 2011).

In high mountain Andean ecosystems, the spectacled bear is a good proxy for monitoring infectious agents in wildlife, given the species' life cycle, broad home range, and severity of interactions with humans and domesticated animals (Bard & Cain III, 2019; I. Goldstein et al., 2006). The spectacled bear (*Tremarctos ornatus*) is the only species of the Ursidae family in south America, and it is categorized as species vulnerable to extinction (VU) (Velez-Liendo & García-Rangel, 2018). Retaliatory killing by high mountain cattle ranchers and habitat degradation and reduction have been recognized as the main threats to the bear (I. R. Goldstein, 2002; Jorgenson & Sandoval-A, 2005; Kattan et al., 2004; Peyton, 1999). Recently, it was found that the presence of unaccompanied cattle (i.e., feral) reduces the probability of bear occupation (Parra-Romero et al., 2019). The interaction between wildlife and domesticated animals plays an important role in the transmission of different infectious agents. In the rural high mountain areas, domestic and feral animals excrete in areas where wildlife forage and prey, potentially leading to the transmission of different infectious agents (King et al., 2011).

In order to prioritize the monitoring effort, diagnosis methods and tools are key for knowing which parasites might be present in natural populations of the spectacled bear. This review discusses which pathogens have been found in the seven other species of bears and have been recorded too in any host in the Andean region, specifically Colombia, to infer which could be present in *T. ornatus*.

1.3 Materials and methods

1.3.1 Document review

The data was obtained from different sources available on the eight species of bears from across the globe. We searched for all relevant published studies. Searches were performed from 1808 until August, 2023 using Google Scholar (https://scholar.google.com), Scopus (https://www.scopus.com), and Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/). We restricted our search to documents in Spanish and English, but included some references in Chinese that had English abstracts. Search terms included: “Ursidae and nematodes”, “Ursidae and Protista”, “Ursidae and Protist”, “Ursidae and virus”, “Ursidae and bacteria”. We did not take into account “gut microbiota”, “intestinal flora”, “microbial population of gastrointestinal tract”, “gastrointestinal microbiota”. Duplicate articles found in more than one database or terms were excluded. Reviews and research articles, theses, and meeting abstract were included.

1.3.2 Parasite diversity

I estimated the specific richness index, “S”, of the ursid parasites determined to species level, as well as unique records of families and genera. Additionally, I estimated the expected parasite richness for each bear species using Chao, Jack1, Jack2, and Bootraps indices, considering the year as a sampling unit. As parasite species are expected to remain unseen or undetected in a collection of sample units, we used several popular ways of estimating the number of unseen species and add them to the observed species richness (Colwell & Coddington, 1994; Palmer, 1991). The incidence-based estimates use the species frequencies across a collection of years. I ran all analyses in the R package Vegan version 2.6-4 (Oksanen et al., 2013).

Agents found in other bear species that could be present in the Spectacled Bear. In order to understand more about the parasites found in the other seven bear species exclusive of *T. ornatus*, I reviewed in the same database the documented records of each parasite to the specific and generic level in the Andean Region in human, domestic or wildlife species. Search terms included “Species name or genera” and “Colombia”. If a record was not found, we changed “Colombia” to “Neotropical”, “Andes” or “South America”. We consider at least one reference enough to count the presence of a parasite.

1.4 Results

I found 283 relevant references of parasites in bears; 37 references were not found in original versions. The references were published between 1808 and 2023. Close to 647 records, 189 agents were determined to specific level, 27 agents to genera, and 21 only to family level (Supplement information 1). The recorded parasites were distributed across 121 genera, 95 families, and 12 kingdoms (Supplement information 1,Table 1). The documented records come from 37 different countries (Figure 1,Table 2).

Table 1. The distribution of parasites kingdoms documented in bears

Kingdom	**Frequency**
Animalia	430
Virus	76
Bacteria	70
Protista	51
Chromalveolata	12
Fungi	7
Diaphoretickes	4
Excavata	4
Alveolata	2
Coccidia	2
Amoebozoa	1
SAR	1

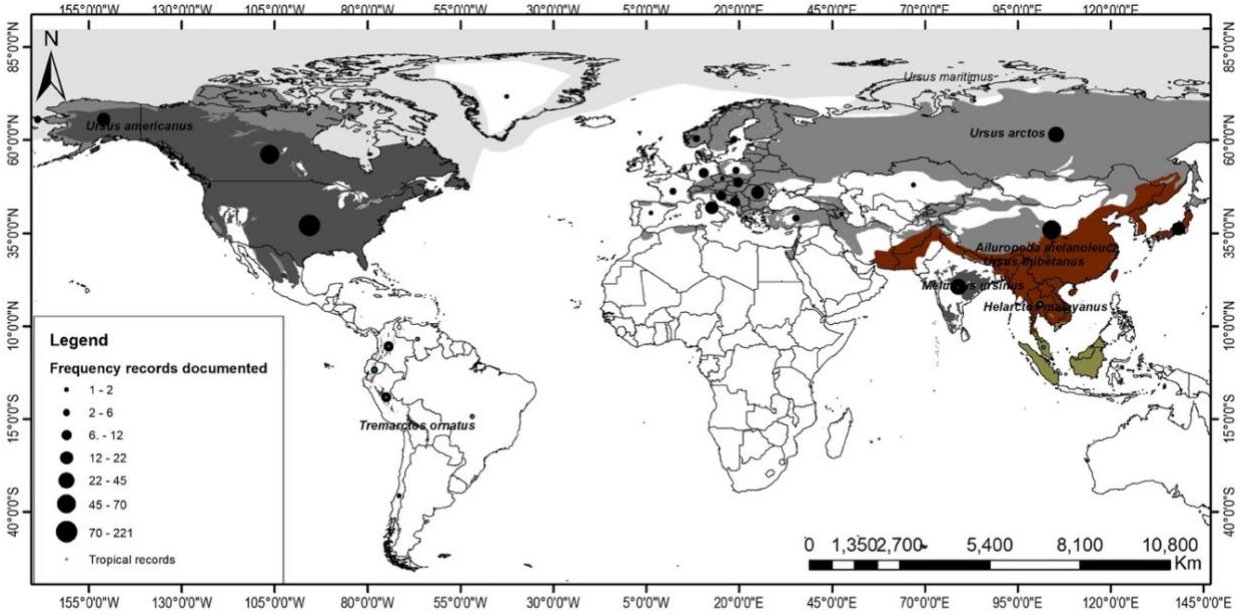


Figure 1. Density map of parasite records by bear species and country

Table 2. The distribution of country records of parasites in bears

ID	Country	Frequency
1	USA	221
2	Canada	70
3	China	69
4	Russia	45
5	India	32
6	Japan	22
7	Romania	19
8	Alaska	18
9	Italy	14
10	Colombia	12
11	Germany	11
12	Slovakia	11
13	Peru	10

14	Croatia	7
15	Yugoslavia	7
16	Norway	6
17	Thailand	6
18	Ecuador	4
19	France	4
20	Turkey	4
21	USA – Russia*	4
22	Poland	3
23	Sweden	3
24	Brazil	2
25	Chile	2
26	Greenland	2
27	Malaysia	2
28	Mexico	2
29	Netherlands	2
30	Spain	2
31	UK	2
32	Venezuela	2
33	Vietnam	2
34	Azerbaijan	1
35	Czech Republic	1
36	Denmark	1
37	Greece	1
38	Ireland	1
39	Kazajistan	1

*Bering strait

The Ursid species with the most records of parasites was *Ursus* americanus, but the diversity could be between 132 and 203 species. So, in species with a more sampling effort, this could be between 52 and 81% of completeness. The tropical species *Helarctos malayanus*, *Melursus ursinus*, and *T. ornatus* showed the smallest parasite diversity and unseen species estimates. This is probable due to their poor sampling coverage (Table 3). It is expected that there is a greater diversity of parasites in tropical regions (Diamond, 2016).

Table 3. Diversity analysis of parasites reported in ursid species.

Species	S	chao	chao.se	jack1	jack.se	jack2	boot	boot.se	n
A. melanoleuca	45	192	90	79	9	107	58	4	26
H. malayanus	11	59	30	21	4	29	15	2	8
M. ursinus	20	63	33	35	7	47	26	3	10
T. ornatus	25	60	25	40	9	51	32	5	7
U. americanus	107	187	28	166	15	203	132	9	46
U. arctos	92	345	101	162	17	221	120	8	39
U. maritimus	31	102	52	51	6	68	39	3	35
U. tibetanus	21	89	59	37	5	50	27	2	16

*Species richness

The parasites with the greatest number of records in bears were *Baylisascaris transfuga, Toxoplasma gondii, Trichinella sp., Trichinella spiralis, Canine morbillivirus, Dirofilaria ursi, Canine mastadenovirus A,* with 10 to 32 records. 134 agents were mentioned in a single record (Supplement information 2). At least 80 parasite species reported in the other seven bear species have been registered in other non-ursid Colombian species, with two others being reported from the Neotropical region and South America. Four agents not reported in *T. ornatus* have worldwide distributions (Supplement information 3).

1.5 Discussion

In spite of being a species located in a region of overall parasite richness, including several taxa reported in other bear species, tropical bears have less documented records of agents. This is evidently due to lack of sufficient sampling. In particular for *T. ornatus* of the 44 records (25

potential different taxa), only four determine the taxon at the species level, 31 reach the genus and the remaining nine just at the Family, Kingdom or Domain level (Mata et al., 2016; Navarro M. et al., 2015; Oniki-Willis & Willis, 2018; Zárate Rodriguez et al., 2022). This is a product of the fact that most of the research has been done with approaches based on microscopy, which in some cases only allow to identify “compatible structures” with some agents (Cruz Hurtado & Muñoz Huamaní, 2016; Figueroa, 2015; Quintero et al., 2023; Zárate Rodriguez et al., 2022) or with molecular approaches that do not reach sufficient taxonomic resolution for the different groups (Chica Cardenas, 2021). Of the 44 records in *T. ornatus*, 26 belong to the kingdom Animalia: 18 are nematodes, two trematodes, one cestode, and two arthropods. 15 records are Protists distributed in 10 genera. Two of the records that were able to be determined at the species level use specific molecular and immunological diagnostic approaches (Mata et al., 2016; Navarro M. et al., 2015).

Of the 85 infectious agents registered in the other bear species that have been found in Colombia in other hosts, 35 belong to the Animalia kingdom, 21 are bacteria, 14 viruses, 11 protists and 4 fungi. In the case of animal agents, at least 32 are endoparasites, as are protists, bacteria, fungi, and viruses. In this sense, the use of fecal samples, which in addition to being non-invasive, relatively cheap and easy to collect, once the accessibility to the sampling sites has been overcome, would allow having samples for the greatest number of agents of interest, including some hemotropic, which reach the digestive tract through different routes. However, the diagnosis on these samples demands the adequate means of collection and transport to guarantee the quality of the samples (Longmire et al., 1997; Wultsch et al., 2015), in addition to standardized extraction processes. Likewise, it is necessary to use Metabarcoding with a combination of markers, among which 16S, 18S and ITS are suggested (Francis & Šlapeta, 2022; Semblante et al., 2017; Stensvold et al., 2021), and with bioinformatic approaches that contrast the sequences with different specific databases (e.g., PlusPFP: Standard plus Refeq protozoa, fungi & plant, using kraken2 (Wood et al., 2019)). Thus, it would be possible to increase, cost-effectively, the sampling in this and other types of hosts and evaluate, within the framework of One Health, the similarity between different components of the assemblies. So far, the few comparison exercises evaluate maximum similarities at the genus level and thus it is only possible to speculate about the possible transmission of agents between different hosts (Zárate Rodriguez et al., 2022) but not to prove the hypothesis about the exchange of specific agents and to differentiate its epidemiology and the potential immunological responses stimulated in the different hosts.

All four groups of agents identified in other bear species, also found in or near Colombia, are potentially zoonotic. After the northern temperate zones, the tropics present some risk of zoonoses and carnivores (Order Carnivora), rodents and ungulates are particularly linked to the situation (Han et al., 2016). In this sense, increasing sampling efforts to complete the characterization of the diversity of infectious agents, with high taxonomic resolution, is key, not only to determine the richness of this component of biodiversity, but also to improve risk analysis generated by infectious diseases for wildlife, domestic animals and the human population (Shaheen, 2022).

In the Andean region, management efforts to conserve the Spectacle Bear could be faced with unknown associated epidemiological risks, potentially even affecting human welfare. The presence and load of infectious agents in wildlife and their interaction with humans, with high level taxonomic resolution, must be a research priority for conservation and public health stakeholders.

1.6 Acknowledgements

I thank Universidad Nacional de Colombia sede Palmira for the teaching assistance scholarship (Resolution 061/2021, Council of the Faculty of Agricultural Sciences Palmira). We are also grateful to the Andean Bear Conservation Alliance (ABCA), the Cleveland Metroparks Zoo Wildlife Conservation Program.

1.7 References

Aguirre, A. A., Ostfeld, R. S., Tabor, G. M., House, C., & Pearl, M. C. (2002). Conservation medicine: Ecological health in practice. Oxford University Press.

Bard, S. M., & Cain III, J. W. (2019). Pathogen prevalance in American black bears (Ursus americanus amblyceps) of the Jemez Mountains, New Mexico, USA. Journal of Wildlife Diseases, 55(4), 745–754.

Baruch-Mordo, S., Wilson, K. R., Lewis, D. L., Broderick, J., Mao, J. S., & Breck, S. W. (2014). Stochasticity in natural forage production affects use of urban areas by black bears: Implications to management of human-bear conflicts. PloS One, 9(1), e85122.

Borka-Vitális, L., Domokos, C., Földvári, G., & Majoros, G. (2017). Endoparasites of brown bears in Eastern Transylvania, Romania. Ursus, 28(1), 20–30.

Brena, P., Gauthier, D., Humeau, A., Baurier, F., Dej, F., Lemberger, K., Chollet, J.-Y., & Decors, A. (2018). How Can Computer Tools Improve Early Warnings for Wildlife Diseases? In How Information Systems Can Help in Alarm/Alert Detection (pp. 241–256). Elsevier.

Bronson, E., Spiker, H., & Driscoll, C. P. (2014). Serosurvey for selected pathogens in free-ranging American black bears (Ursus americanus) in Maryland, USA. Journal of Wildlife Diseases, 50(4), 829–836.

Chica Cardenas, L. A. (2021). Estimating the andean bear diet through DNA metabarcoding and its relationships to the gut microbiome [Universidad de los Andes]. https://repositorio.uniandes.edu.co/handle/1992/58061

Colwell, R. K., & Coddington, J. A. (1994). Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 345(1311), 101–118.

Cruz Hurtado, S. S. M., & Muñoz Huamaní, M. (2016). Identificación de parásitos gastrointestinales de carnívoros en cautiverio criados en el centro recreacional municipal del Cerrito de la Libertad de Huancayo.

Diamond, J. (2016). Sociedades comparadas: Un pequeño libro sobre grandes temas. Debate.

Dubey, J., & Jones, J. (2008). Toxoplasma gondii infection in humans and animals in the United States. International Journal for Parasitology, 38(11), 1257–1278.

Elbroch, L. M., Lendrum, P. E., Allen, M. L., & Wittmer, H. U. (2015). Nowhere to hide: Pumas, black bears, and competition refuges. Behavioral Ecology, 26(1), 247–254.

Figueroa, J. (2015). New records of parasites in free-ranging Andean bears from Peru. Ursus, 26(1), 21–27. https://doi.org/10.2192/URSUS-D-14-00034.1

Francis, E. K., & Šlapeta, J. (2022). A new diagnostic approach to fast-track and increase the accessibility of gastrointestinal nematode identification from faeces: FECPAKG2 egg nemabiome metabarcoding. International Journal for Parasitology, 52(6), 331–342. https://doi.org/10.1016/j.ijpara.2022.01.002

García Marín, J. F., Royo, L. J., Oleaga, A., Gayo, E., Alarcia, O., Pinto, D., Martínez, I. Z., González, P., Balsera, R., & Marcos, J. L. (2018). Canine adenovirus type 1 (CA dV-1) in free-

ranging European brown bear (Ursus arctos arctos): A threat for Cantabrian population? Transboundary and Emerging Diseases, 65(6), 2049–2056.

Gilbert, B. (1989). Behavioral plasticity and bear-human conflicts. 1–8.

Goldstein, I., Paisley, S., Wallace, R., Jorgenson, J. P., Cuesta, F., & Castellanos, A. (2006). Andean bear–livestock conflicts: A review. Ursus, 17(1), 8–15.

Goldstein, I. R. (2002). Andean bear-cattle interactions and tree nest use in Bolivia and Venezuela. Ursus, 369–372.

Han, B. A., Kramer, A. M., & Drake, J. M. (2016). Global Patterns of Zoonotic Disease in Mammals. Trends in Parasitology, 32(7), 565–577. https://doi.org/10.1016/j.pt.2016.04.007

Ishibashi, Y., Oi, T., Arimoto, I., Fujii, T., Mamiya, K., Nishi, N., Sawada, S., Tado, H., & Yamada, T. (2017). Loss of allelic diversity in the MHC class II DQB gene in western populations of the Japanese black bear Ursus thibetanus japonicus. Conservation Genetics, 18(2), 247–260.

Jorgenson, J. P., & Sandoval-A, S. (2005). Andean bear management needs and interactions with humans in Colombia. Ursus, 16(1), 108–116.

Kattan, G., Hernández, O. L., Goldstein, I., Rojas, V., Murillo, O., Gómez, C., Restrepo, H., & Cuesta, F. (2004). Range fragmentation in the spectacled bear *Tremarctos ornatus* in the northern Andes. Oryx, 38(2), 155–163.

Kindschuh, S. R., Cain III, J. W., Daniel, D., & Peyton, M. A. (2016). Efficacy of GPS cluster analysis for predicting carnivory sites of a wide-ranging omnivore: The American black bear. Ecosphere, 7(10), e01513.

King, J. S., Jenkins, D. J., Ellis, J. T., Fleming, P., Windsor, P. A., & Šlapeta, J. (2011). Implications of wild dog ecology on the sylvatic and domestic life cycle of Neospora caninum in Australia. The Veterinary Journal, 188(1), 24–33.

Lesmerises, R., Rebouillat, L., Dussault, C., & St-Laurent, M.-H. (2015). Linking GPS telemetry surveys and scat analyses helps explain variability in black bear foraging strategies. PLoS One, 10(7), e0129857.

Longmire, J. L., Maltbie, M., & Baker, R. J. (1997). Use of" lysis buffer" in DNA isolation and its implication for museum collections.

Mackenstedt, U., Jenkins, D., & Romig, T. (2015). The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. International Journal for Parasitology: Parasites and Wildlife, 4(1), 71–79.

Mata, A. P., Pérez, H. G., & Parra, J. G. (2016). Morphological molecular description of Baylisascaris venezuelensis, n. Sp. From a natural infection in the South American spectacled bear *Tremarctos ornatus* Cuvier, 1825 in Venezuela. Neotrop Helminthol, 10, 85–103.

McCullough, D. R. (1982). Behavior, bears, and humans. Wildlife Society Bulletin, 27–33.

Monsalve-Buriticá, S. (2019). Enfermedades emergentes y reemergentes de origen viral o bacteriano en Colombia. Fondo Editorial Biogénesis, 49–62.

Navarro M., D., Chávez V., A., Pinedo V., R., & Muñoz D., K. (2015). Factores de Riesgo Asociados a la Seroprevalencia de Toxoplasma gondii en Mamíferos del Orden Carnivora y Primates Mantenidos en Cautiverio. Revista de Investigaciones Veterinarias Del Perú, 26(3), 497. https://doi.org/10.15381/rivep.v26i3.11175

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). Package 'vegan'. Community Ecology Package, Version, 2(9), 1–295.

Oniki-Willis, Y., & Willis, E. O. (2018). Tick (Acarina) diversity from South American birds and mammals. Atualidades Ornitológicas, 206.

Palmer, M. W. (1991). Estimating species richness: The second-order jackknife reconsidered. Ecology (Durham), 72(4), 1512–1513.

Parra-Romero, A., Zamudio-López, J., Camargo-Cárdenas, J. E., Palacios-Medina, C. R., Torres, L., Castro, E., Espíndola, J., Meneses, H., Vera-Villamizar, L., Moreno-Gutiérrez, S., López-Velandia, O., Saenz, F., Rodríguez, M., Franco, N., Clavijo-Ríos, C., Rivera-Torres, C., López-Orjuela, H., Pachón-Bejarano, G., Jimenez-Palomo, G., … Márquez, R. (2019). Ocupación del oso andino (*Tremarctos ornatus*) en la región centro-norte de la Cordillera Oriental de Colombia. PNN de Colombia, CAR Cundinamarca, Corpoboyacá, Corporinoquía, Corpochivor, Cormacarena, Corpoguavio, ABCA y WCS.

https://www.researchgate.net/publication/362166694_Ocupacion_del_oso_andino_Tremarctos_ornatus_en_la_region_centro-norte_de_la_Cordillera_Oriental_de_Colombia

Patz, J., Githeko, A., McCarty, J., Hussein, S., Confalonieri, U., & De Wet, N. (2003). Climate change and infectious diseases. Climate Change and Human Health: Risks and Responses, 2, 103–132.

Peña-Quistial, M. G., Benavides-Montaño, J. A., Duque, N. J. R., & Benavides-Montaño, G. A. (2020). Prevalence and associated risk factors of Intestinal parasites in rural high-mountain communities of the Valle del Cauca—Colombia. PLoS Neglected Tropical Diseases, 14(10), e0008734.

Peyton, B. (1999). Spectacled bear conservation action plan. Bears: Status Survey and Conservation Action Plan, 157–164.

Quintero, L. R., Pulido-Villamarín, A., Parra-Romero, Á., Castañeda-Salazar, R., Pérez-Torres, J., & Vela-Vargas, I. M. (2023). Andean bear gastrointestinal parasites in Chingaza Massif, Colombia. Ursus, 2023(34e4). https://doi.org/10.2192/URSUS-D21-00020.1

Sasmal, I., Gould, N. P., Schuler, K. L., Chang, Y.-F., Thachil, A., Strules, J., Olfenbuttel, C., Datta, S., & DePerno, C. S. (2019). Leptospirosis in urban and suburban american black bears (ursus americanus) in western north carolina, usa. Journal of Wildlife Diseases, 55(1), 74–83.

Schwab, C., Cristescu, B., Northrup, J. M., Stenhouse, G. B., & Gänzle, M. (2011). Diet and environment shape fecal bacterial microbiota composition and enteric pathogen load of grizzly bears. PLoS One, 6(12), e27905.

Semblante, G. U., Phan, H. V., Hai, F. I., Xu, Z.-Q., Price, W. E., & Nghiem, L. D. (2017). The role of microbial diversity and composition in minimizing sludge production in the oxic-settling-anoxic process. Science of The Total Environment, 607–608, 558–567. https://doi.org/10.1016/j.scitotenv.2017.06.253

Shaheen, M. N. F. (2022). The concept of one health applied to the problem of zoonotic diseases. Reviews in Medical Virology, 32(4). https://doi.org/10.1002/rmv.2326

Smith, K. F., Acevedo-Whitehouse, K., & Pedersen, A. B. (2009). The role of infectious diseases in biological conservation. Animal Conservation, 12(1), 1–12.

Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology, 20(5), 1349–1357.

Stensvold, C. R., Jirků-Pomajbíková, K., Tams, K. W., Jokelainen, P., Berg, R. P. K. D., Marving, E., Petersen, R. F., Andersen, L. O., Angen, Ø., & Nielsen, H. V. (2021). Parasitic Intestinal Protists of Zoonotic Relevance Detected in Pigs by Metabarcoding and Real-Time PCR. Microorganisms, 9(6), 1189. https://doi.org/10.3390/microorganisms9061189

Stephenson, N., Higley, J. M., Sajecki, J. L., Chomel, B. B., Brown, R. N., & Foley, J. E. (2015). Demographic characteristics and infectious diseases of a population of American black bears in Humboldt County, California. Vector-Borne and Zoonotic Diseases, 15(2), 116–123.

Ujvari, B., & Belov, K. (2011). Major histocompatibility complex (MHC) markers in conservation biology. International Journal of Molecular Sciences, 12(8), 5168–5186.

Velez-Liendo, X., & García-Rangel, S. (2018). *Tremarctos ornatus*. The IUCN Red List of Threatened Species 2017: E.T22066A123792952. https://www.iucnredlist.org/species/22066/123792952

Westmoreland, L. S., Stoskopf, M. K., & Maggi, R. G. (2016). Prevalence of Anaplasma phagocytophilum in North Carolina eastern black bears (Ursus americanus). Journal of Wildlife Diseases, 52(4), 968–970.

Wisely, S. M., Howard, J., Williams, S. A., Bain, O., Santymire, R. M., Bardsley, K. D., & Williams, E. S. (2008). An unidentified filarial species and its impact on fitness in wild populations of the black-footed ferret (Mustela nigripes). Journal of Wildlife Diseases, 44(1), 53–64.

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 257. https://doi.org/10.1186/s13059-019-1891-0

Wu, J., Han, J.-Q., Shi, L.-Q., Zou, Y., Li, Z., Yang, J.-F., Huang, C.-Q., & Zou, F.-C. (2018). Prevalence, genotypes, and risk factors of Enterocytozoon bieneusi in Asiatic black bear (Ursus thibetanus) in Yunnan Province, Southwestern China. Parasitology Research, 117(4), 1139–1145.

Wultsch, C., Waits, L. P., Hallerman, E. M., & Kelly, M. J. (2015). Optimizing collection methods for noninvasive genetic sampling of neotropical felids. Wildlife Society Bulletin, 39(2), 403–412. https://doi.org/10.1002/wsb.540

Zárate Rodríguez, P. T., Collazos-Escobar, L. F., & Benavides-Montaño, J. A. (2022). Endoparasites Infecting Domestic Animals and Spectacled Bears (*Tremarctos ornatus*) in the Rural High Mountains of Colombia. Veterinary Sciences, 9(10), 537. https://doi.org/10.3390/vetsci9100537

Zhang, L., Yang, X., Wu, H., Gu, X., Hu, Y., & Wei, F. (2011). The parasites of giant pandas: Individual-based measurement in wild animals. Journal of Wildlife Diseases, 47(1), 164–171.

2. Comparison of DNA quantity and quality from fecal samples of mammals transported in ethanol or lysis buffer

Néstor Roncancio-Duque[1]*; Jeison Eduardo García-Ariza[1]; Nelson Rivera-Franco[2,3]; Andrés Mauricio Gonzalez-Ríos[2]; Diana López-Alvarez[1,2]*

[1] Facultad de Ciencias Agropecuarias, Grupo de Investigación en Diversidad Biológica. Universidad Nacional de Colombia, Sede Palmira. Colombia; njroncanciod@unal.edu.co, jeigarciaar@unal.edu.co, dilopezal@unal.edu.co

[2] Universidad del Valle, Facultad de Salud, Escuela de Ciencias Básicas, Grupo VIREM—Virus Emergentes y Enfermedad, Cali, Valle del Cauca, Colombia; rivera.nelson@correounivalle.edu.co, andres.mauricio.gonzalez@correounivalle.edu.co, dilopezal@unal.edu.co

[3] Department of Neurology. Johns Hopkins School of Medicine. Maryland, United States; rivera.nelson@correounivalle.edu.co

* Correspondence: njroncanciod@unal.edu.co, dilopezal@unal.edu.co

2.1 Abstract

Using fecal microbial community profiles through sequencing approaches helps to unravel the intimate interplay between health, wellness, and diet in wild animals with their environment. Ensuring the proper preservation of fecal samples before processing is crucial to ensure reliable results. In this study, we evaluated the efficiency of two different preservation methods, considering the following criteria: DNA yield, quality and integrity, and microbial community

structure based on Oxford Nanopore amplicon sequencing of the V3-V4 region of bacterial 16s rRNA and protozoa 18S rRNA genes. Eighteen mammalian fecal samples were collected transported in 99.8% ethanol and lysis buffer and processed between 55 and 461days post-collection. Wilcoxon tests were carried out for paired samples for quantitative measurements, while for the A260/280 ratio between both conservation media, was done descriptively, and the Bartlett test evaluated its dispersion. A Fisher test was performed to compare the number of positive reactions for DNA extraction or PCR amplification of the 16S and 18S rRNA genes between both storage media. The concentration of total DNA and amplicons, as well as the number of reads obtained in sequencing, was significantly higher in the samples preserved with lysis buffer compared to those with ethanol, with magnitudes up to three times higher, as well for integrity assessed by electrophoresis of total DNA and amplifications. The A260/280 values obtained using the lysis buffer were of optimal purity (mean: 1.92) and with little dispersion (SD: 0.27); on the other hand, the ethanol samples also presented an excellent average quality (mean: 1.94), but they were dispersed (SD: 1.10). For molecular studies using mammalian feces, the lysis Buffer reagent proved to be a reliable solution for their collection, conservation, and storage.

2.1.1 Keywords: biological sample, microbiota, preservation, stools

2.2 Introduction

In the field of life sciences, the analysis of fecal samples can be helpful in answering different type of research questions related to behavior, population ecology, health, well-being, and diet (Jedlicka et al., 2017; Plimpton et al., 2021). This analysis can be implemented through different approaches, including microscopy, spectroscopy, biochemical and hormone measurements, as well as molecular biology techniques (Acosta Z et al., 2015; Matysik et al., 2016; L. R. Morgan et al., 2021). Such studies offer insights into population genetics, molecular epidemiology, the characterization of gastrointestinal parasites and microbiota, and the detection of organic content in the samples (Ngcamphalala et al., 2019; Villamizar et al., 2019).

One of the advantages of using fecal samples is the relative ease of collection. This method is noninvasive, especially when researchers can directly observe the animal excreting the stool, as seen in the case of Atelidae primates (Rondón et al., 2021) or when the samples exhibit species-specific characteristics, as in Andean bear, tapirs, felines, and certain domestic animals

(Gonzales et al., 2016). Additionally, the relatively low cost associated with fecal sample collection sets it apart from more invasive methods, such as blood draws or tissue biopsies, which may require pre-medication or physical constraints (Aristizabal-Duque et al., 2018; Arnemo et al., 2006).

Collecting and storing fecal samples properly is essential to ensure the accuracy of biochemical or molecular measurements (Papaiakovou et al., 2018). However, obtaining high-quality and concentrated DNA from fecal matter samples is a complex task due to the presence of polymerase chain reaction (PCR) inhibitors, including urates, bile salts, complex polysaccharides, bilirubin, and byproducts of digested hemoglobin (Longmire et al., 1997). Furthermore, fecal samples are inherently contaminated with metabolites from the digestive process, and in the case of samples from wild animals, they may also be exposed to environmental contamination, degradation from exposure to solar radiation, humidity, and inadequate temperatures for preservation of macromolecules (Iker et al., 2013; Menu et al., 2018; Papaiakovou et al., 2018). Additionally, under field conditions, it is often challenging to maintain cold chains to preserve samples until they reach the laboratory (Camacho-Sanchez et al., 2013).

To address these challenges, preservation methods for molecular analyses must protect the target DNA from endogenous nucleases and degrading compounds. Some commercial companies have developed media for environmental DNA preservation, such as DNA stabilizer or RNALater, which have shown promise in improving results with these sample types (Choo et al., 2015; Gorzelak et al., 2015). However, these preservation buffers can be relatively expensive, and their availability may be limited, particularly in developing countries.

An alternative and cost-effective approach involves using ethanol and lysis buffer for collecting and transporting fecal samples (Longmire et al., 1997; Papaiakovou et al., 2018). In this study, we compare the concentration and quality of DNA obtained from samples collected and transported in both media. We then apply these samples to molecular analysis techniques, such as next-generation sequencing (NGS) using Oxford Nanopore Technology (ONT). Through bioinformatics analysis, we assess various molecular readouts, including DNA integrity, the number of reads obtained for each sample in both media, their conservation capacity, and the ability to establish the taxonomic classification of the microorganisms present (Antil et al., 2023). Our aim is to determine the reliability of these fecal sample preservatives and their ability to stabilize and protect genetic material.

2.3 Materials and Methods

2.3.1 Collection of fecal samples and storage

Eighteen fecal samples were collected from various species of wild and domestic mammals across different departments of Colombia (
Table 4). Each sample was divided into two equal parts, with one portion placed in molecular grade ethanol (99.8%) and the other in a lysis buffer. The lysis buffer was prepared with the following components: 0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, 0.5% SDS at pH 8 [13]. Both sets of samples were transported at ambient temperature to the laboratory and subsequently stored at -20°C until extraction. In most cases, the samples were frozen between eight and 20 days after collection. The time elapsed from the collection of each sample to the nucleic acid extraction process is provided in (
Table 4).

Table 4. Species and localities of the samples evaluated.

Code	Species	Storage duration (days)	Department	Latitude	Longitude	Elevation
42	Atelidae	67	Guaviare	2.441	-72.689	198
43	*Alouatta seniculus*	57	Caldas	5.189	-75.449	2114
44	*Bos primigenius*	57	Caldas	5.190	-75.449	2218
45	*Equus caballus*	57	Caldas	5.191	-75.451	2118
47	*Equus caballus*	57	Caldas	5.184	-75.449	2314
48	*Alouatta seniculus*	57	Caldas	5.189	-75.449	2110
49	*Alouatta seniculus*	55	Caldas	4.741	-75.595	1809
50	*Bos primigenius*	55	Risaralda	4.743	-75.602	1774

51	*Alouatta seniculus*	55	Quindío	4.755	-75.625	1942
52	*Alouatta seniculus*	55	Quindío	4.715	-75.624	1956
53	*Equus caballus*	55	Quindío	4.713	-75.633	1937
54	*Alouatta seniculus*	55	Quindío	4.710	-75.670	1687
55	*Alouatta seniculus*	55	Quindío	4.708	-75.673	1548
D001	*Tapirus pinchaque*	455	Valle	3.725	-75.951	3743
Eq001	*Equus caballus*	451	Valle	3.717	-75.988	3213
Oso001	*Tremarctos ornatus*	451	Valle	3.713	-75.987	3422
Oso002	*Tremarctos ornatus*	450	Valle	3.701	-75.962	3860
P001	*Puma concolor*	461	Valle	3.705	-75.960	3958

2.3.2 DNA extraction and 16s/18s rRNA library preparation

The commercial Kit DNeasy PowerSoil Pro® Kit was used for DNA extraction, according to the manufacturer's instructions with minor modifications (addition of 5 minutes of stirring to mix sample with the first component of the kit). The extracted DNA was diluted in 50 µl of kit resuspension solution. To assess the concentration and quality of extracted DNA, we utilized a Colibri system, (Titertek Berthold). High-quality genomic DNA typically exhibits an OD260/OD280 ratio within the range of 1.8 and 2.0. Values between 1.6 -1.8 are considered acceptable, while any measurement below 1.6 indicates potential contamination. To evaluate DNA integrity 2 µL of extracted DNA was visualized on a 1% agarose gel (w/v) containing GelRed (Biotium®). Electrophoresis was conducted in 0.5X TBE buffer at 100 V for 30 min, using a 1kb DNA ladder as a molecular weight marker (Thermo Scientific).

For molecular characterization studies, the viability of genomic DNA was determined by amplifying the V3-V4 hypervariable region of the 16S rRNA gene with locus-specific forward primer 341F (5´-CTAYGGGRBGCASCAG-3) and reverse primer 806R (5´-

GGACTACNNGGGTATCTAAT-3´) (Yu et al., 2005). Additionally, for 18S rRNA gene we utilized primer G3F1 (5' –GCCAGCAGCCGCGGTAATTC-3) and primer G3R1 (5' –ACATTCTTGGCAAATGCTTTCGCAG-3) (Krogsgaard et al., 2018). Endpoint polymerase chain reaction (PCR) was conducted using the Biorad CFX96 C1000 thermal cycler (Table S1 and Table S2). To ensure the reliability of the PCR results, each reaction included positive controls using *Pseudomonas* spp (16s rRNA) and stool from *Tapirus pinchaque* (D001, Table 1) genomic DNA. Negative controls were performed by using PCR-grade autoclaved water in place of template DNA for each PCR. To visualize the amplicons, we prepared 1.4% agarose gels using a 0.5X TBE buffer solution and stained them with GelRed; Electrophoresis was conducted at 90V for 40 minutes, using 100 bp Plus DNA Ladder (Thermo Scientific) as a molecular weight marker. Gels containing both total DNA and amplicons were visualized using a gel scanner (Nippon Genetics, FastGene FAS V model). To determine the concentration of amplicons, we employed a fluorometric method with the Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, United States) using the QubitTM dsDNA HS Assay Kit following the manufacturer's instructions (Freed & Silander, 2020).

2.3.3 Oxford Nanopore sequencing and bioinformatics

For sequencing of ~450bp-long amplified region was conducted using the MinION MK1B sequencing platform, managed by the MinKNOW software (van der Reis et al., 2023). The sequencing library was prepared with the Ligation Sequencing Kit SQK-LSK109 and using the Native Barcoding Expansion Kit 96 (EXP-NBD196) (Quick, 2023). The library pool was sequenced on an R-9.4.1 flow cell and run for 48h. Raw FAST5 files pass produced were basecalled under high-accuracy mode using the ONT basecaller Guppy v6.2.1. Subsequently, the resulting FASTQ files were employed for taxonomic assignment, a process carried out with Kraken2 (Wood et al., 2019; Wood & Salzberg, 2014), and the PlusPFP database (3/14/2023). The sequences obtained have been deposited in the NCBI under BioProject number PRJNA1036276, Biosample numbers SAMN38122182 to SAMN38122221, and accession numbers SRR26722958 to SRR26722997.

2.3.4 Statistical analysis

To compare the concentration of total DNA and amplicons, as well as the reads obtained in the sequencing, we performed a paired samples analysis by evaluating the assumptions of normality and homoscedasticity. The assessment of normality was conducted using Shapiro-Wilk test for datasets with less than 30 data points, while homoscedasticity evaluated with the Bartlett test. In cases where the concentration did not conform to a normal distribution in some samples ($p < 0.05$) or the homoscedasticity assumption was not met ($p < 0.05$), we conducted comparisons using the non-parametric Wilcoxon test for paired samples, with an alpha level of 0.05. The comparison of the 260/280 ratio was performed descriptively in relation to reference measurements. Data dispersion between ethanol and buffer was assessed using the Bartlett test. To determine the positivity of the extraction or amplification of the 16s rRNA and 18s rRNA genes between the buffer or ethanol media, we conducted a Fisher's exact test. This test compared the number of samples that exhibited a band or the absence of it in the agarose electrophoresis. All statistical analyzes were carried out in the Rcmdr 2.8-0 package with R 4.1.2 software (Fox et al., 2023).

2.4 Results

The concentration of total DNA and amplicons (18S rRNA and 16S rRNA), as well as 18S rRNA reads extracted from the samples transported in the lysis buffer were significantly higher than those obtained from the samples transported in ethanol ($p = 0.00067$, $p = 0.039$, $p = 0.039$, and $p = 0.011$, respectively) (Table 5). In the case of 16S rRNA readings, although no significant differences were observed ($p = 0.098$), it's worth noting that the median value was approximately two-fold higher when using the lysis buffer compared to ethanol (Figure 2).

Table 5. Concentration values from DNA extraction, PCR amplification and data obtained by ONT sequencing.

Code*	Species	Genomic ADN (ng/µL)	OD260/ OD280	Concentration Amplicon 18S rRNA (ng/uL)	Concentration amplicon 16s rRNA (ng/uL)	Reads for 18s rRNA**	Reads for 16s rRNA**
42-BF	Atelidae	54.3	1.9	7.3	NA	38,320	NA
42-OH	Atelidae	1.2	4.9	3.4	NA	28	NA

43-BF	*Alouatta seniculus*	8.9	2.2	9.7	8.9	207,967	291,884
43-OH	*Alouatta seniculus*	9.7	2.4	3.6	9.7	7	74,869
44-BF	*Bos primigenius*	49.6	1.8	8.9	49.6	291,275	177,149
44-OH	*Bos primigenius*	22.4	1.9	7.2	22.4	286,188	159,115
45-BF	*Equus caballus*	50.5	1.8	10.0	50.5	381,698	204,101
45-OH	*Equus caballus*	6.5	2.9	3.4	6.5	177	154,665
47-BF	*Equus caballus*	38.3	1.9	10.9	NA	319,354	NA
47-OH	*Equus caballus*	0.3	0.3	3.6	NA	42	NA
48-BF	*Alouatta seniculus*	17.7	1.8	7.2	NA	227,707	NA
48-OH	*Alouatta seniculus*	12.4	1.8	3.5	NA	291,880	NA
49-BF	*Alouatta seniculus*	206.4	1.9	14.2	NA	156,516	NA
49-OH	*Alouatta seniculus*	10.6	1.5	3.8	NA	69	NA
50-BF	*Bos primigenius*	21.3	1.7	3.2	21.3	18,085	67,914
50-OH	*Bos primigenius*	39.2	1.7	7.1	39.2	21,517	12,468
51-BF	*Alouatta seniculus*	226.8	1.9	13.3	226.8	405,748	1,158
51-OH	*Alouatta seniculus*	31.6	1.8	4.7	31.6	211,855	12,080
52-BF	*Alouatta seniculus*	170.1	1.9	12.0	170.1	317,394	294,428

52-OH	*Alouatta seniculus*	15.8	2.6	4.3	15.8	1,445	210,534
54-BF	*Alouatta seniculus*	10.3	2.6	NA	NA	NA	NA
54-OH	*Alouatta seniculus*	19.0	2.2	NA	NA	NA	NA
53-BF	*Alouatta seniculus*	23.7	2.0	7.1	NA	44,531	NA
53-OH	*Alouatta seniculus*	6.9	3.5	3.9	NA	24	NA
55-BF	*Alouatta seniculus*	22.5	2.3	6.8	22.5	4,529	347,702
55-OH	*Alouatta seniculus*	17.6	1.9	4.5	17.6	31,723	35,986
D1-BF	*Tapirus pinchaque*	23.4	1.6	6.4	NA	60,229	NA
D1-OH	*Tapirus pinchaque*	0.6	0.3	3.8	NA	77	NA
Eq1-BF	*Equus caballus*	42.0	1.7	5.6	42.0	77,208	145,101
Eq1-OH	*Equus caballus*	3.3	1.3	5.4	3.3	265	173,875
Os1-BF	*Tremarctos ornatus*	12.6	2.1	5.9	12.6	50,685	20,567
Os1-OH	*Tremarctos ornatus*	2.1	1.6	7.8	2.1	80	44,382
Os2-BF	*Tremarctos ornatus*	15.7	2.3	NA	NA	NA	NA
Os2-OH	*Tremarctos ornatus*	0.2	0.5	NA	NA	NA	NA
P1-BF	*Puma concolor*	51.5	1.5	2.0	NA	7,710	NA
P1-OH	*Puma concolor*	16.2	1.8	16.1	NA	64,781	NA

BF: buffer, Oh: ethanol

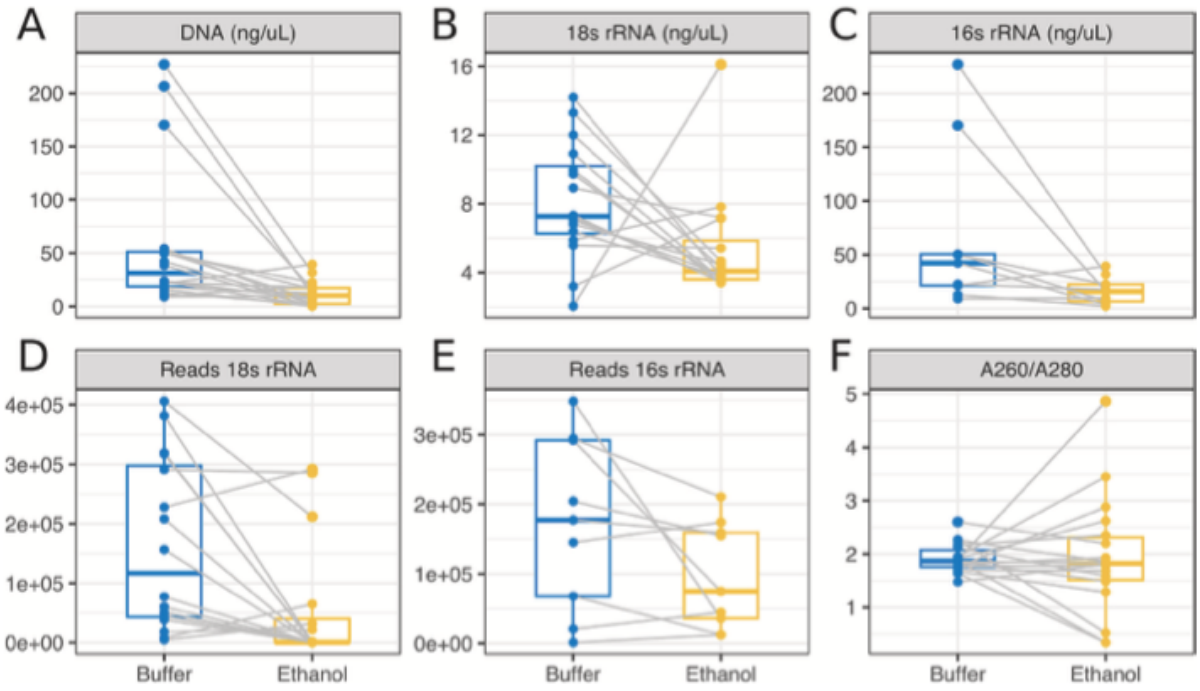


Figure 2. Boxplot with comparisons between the two transports media (A) genomic DNA concentration (B), 18S rRNA amplicon concentration (C),16S rRNA amplicon concentration, (D) reads ONT for 18S rRNA, (E) reads ONT for 16S rRNA, and (F), DNA quality. Lines connect each pair of samples, buffer – ethanol to each response variable analyzed. It shows the boxplot with samples in interquartile range and outliers. A, B, C, D and E show a depletion in the values to concentration and number of the reads between buffer and ethanol, F shows an increase in the variance.

The A260/280 ratio values for the samples collected in buffer had a mean of 1.92 with a standard deviation of 0.27, which places it between the values of optimal purity for DNA and with negligible dispersion. In contrast, although the samples transported in ethanol exhibited a mean value of 1.94, the deviation was 1.10, reflecting nearly four times greater dispersion compared to the lysis buffer. Only four samples showed optimal quality ranges (Figure 2, 260/280). Thus, the variances for both samples were different (Bartlett's K-squared = 25, p = 5.439E-07).

The agarose gels used to visualize the total DNA revealed the presence of high molecular weight bands, located above 10,000 bp, which corresponds to the maximum size of the molecular weight marker utilized. While some samples, such as 44BF, 45BF and 49BF, among others, exhibited a

characteristic “sweep” pattern indicative of degraded DNA, 15 out of the 18 samples collected in lysis buffer displayed a complete high-weight band. In contrast, such a band was only visible in four out of the 18 samples collected in ethanol (p -value Fisher's Exact Test = 0.00061) (Figure 3).

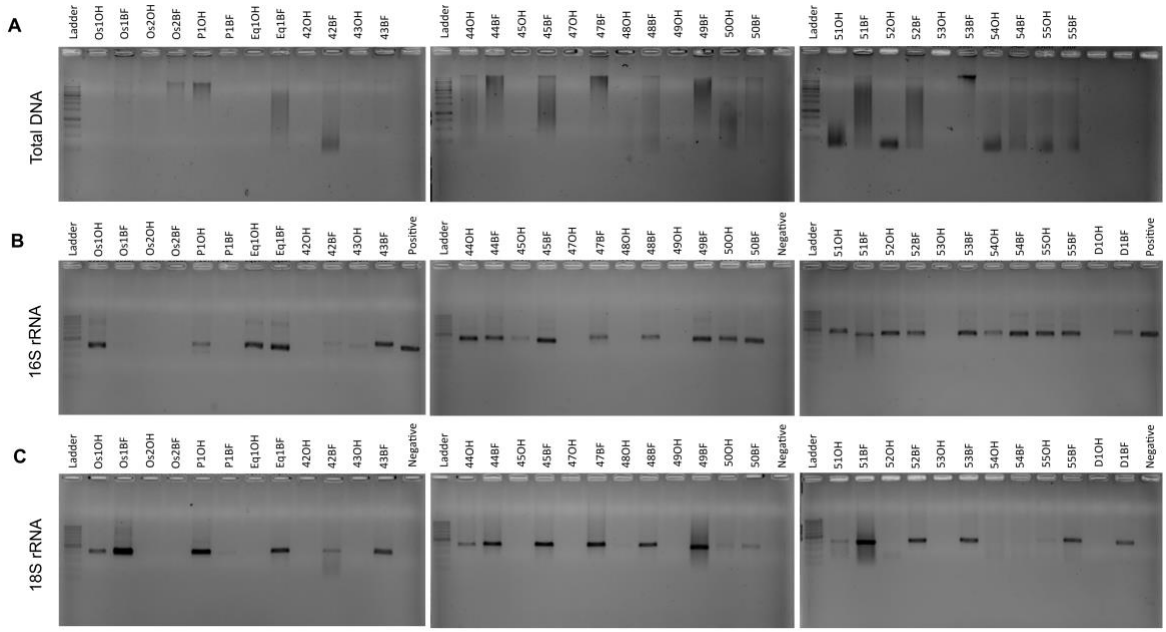


Figure 3. A. 1% agarose gel of total DNA, stained with GelRed. From left to right; 1: 1kb molecular weight marker; 2 – 13: samples. B. 1.4% agarose gel of 16S rRNA gene PCR products, stained with GelRed. From left to right: 1 = 100bp molecular weight marker, 2 – 13: samples; Positive: *Pseudomona*; Negative: water negative control. C. 1.4% agarose gel of 18S rRNA gene PCR products, stained with GelRed. From left to right; 1: 100bp molecular weight marker; 2 – 13: samples; Positive: positive control *Tapirus pinchaque*; Negative: negative control water.

The PCR of the V3-V4 subunit of the 16S rRNA gene presented a higher performance, although not significantly, in samples collected in lysis buffer, in which an amplicon of approximately 450 bp was visible in 16 out of the 18 samples, while in ethanol samples, it was observed in only 11 (p-value Fisher's Exact Test: 0.1212) (Figure 3). Furthermore, it´s worth noting that the amplified band of lysis buffer collected samples, such as 43BF, 45BF, 50BF and 54BF displayed greater intensity compared to their ethanol collected counterparts. This difference in intensity is likely attributed to variations in DNA concentration and integrity between the two sample sets. In the case of the PCR targeting the 18S gene, a higher performance was also observed in samples

collected in lysis buffer. Approximately 16 samples displayed an amplicon of around 450 bp, whereas in samples collected in ethanol, such a band was observed in only six out of the 18 samples (p-value Fisher's Exact Test: 0.0016) (Figure 3).

The processed raw reads using Kraken2, revealed that between 87% and 93% of the reads can be classified into some domain for both evaluated genes. Notably, the number of reads obtained for the samples preserved in lysis buffer was greater than those preserved in ethanol. When assessing the 16s rRNA gene, a higher percentage of classified reads corresponded to Bacteria in lysis buffer preserved samples. Similarly, in the case of the 18s rRNA gene, higher percentages were observed in the classification of reads for Fungi and Protozoa in the lysis buffer preserved samples. These findings provide strong evidence of greater genetic material preservation in the samples collected and preserved with lysis buffer, indicating its superior preservation capacity compared to ethanol (Figure 4, Table S3 and S4).

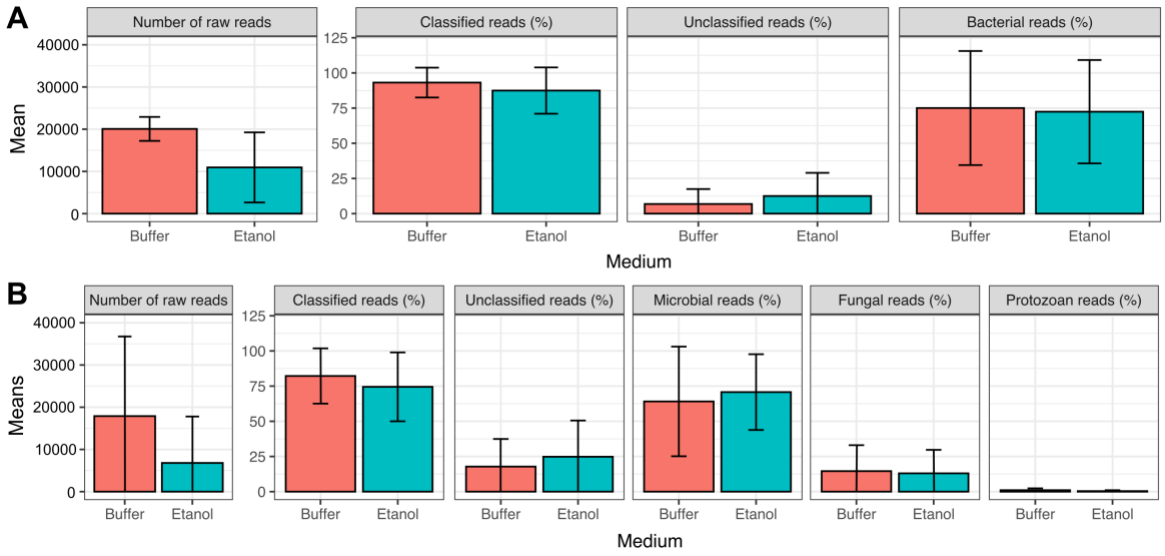


Figure 4. Data produced and classification by kraken2 for the samples in ethanol and lysis buffer where the 16s rRNA (A) or 18s rRNA (B) genes was amplified.

2.5 Discussion

Molecular studies of biological samples require appropriate techniques to obtain genomic DNA of high integrity and purity. Ensuring the correct preservation of samples for microbiome studies is essential to obtain accurate and reproducible results. Freezing samples at -80 °C is widely acknowledged as the optimal method for preserving nucleic acids and proteins over time, as it

effectively halts degradation without causing damage to the genome and proteome material of biological specimens (Burnham et al., 2023). However, in fieldwork, having the necessary equipment for freezing samples is often challenging.

An alternative solution to address this challenge is the preservation of samples in storage solutions. Various preservation media have been discussed in literature and are available in the biotechnology market. Their use is influenced by factors such as toxicity, resistance to inhibitors, preservation costs, transportation requirements, infectivity of the samples and laboratory expenses. Considering the non-significant difference in the amount of DNA obtained, the collection of samples in ethanol, as compared to other reagents like 5% potassium dichromate, RNA Later®, Paxgene®, Formalternate® (Ethylene glycol phenyl ether, Phenol, 1,2 - Propanediol), FTA cards, and drying samples with silica or a dehydrator, is often recommended as a pragmatic choice for preserving fecal samples collected in the field (Jin et al., 2016; Kumar & Bhadury, 2022; Papaiakovou et al., 2018). It is recognized that, the higher the concentration of ethanol, the faster the penetration of cell membranes and the deactivation of nucleases.

In our study, the fecal samples transported in the lysis buffer exhibited higher concentration of total DNA, amplicons, and a greater number of reads obtained during sequencing (Table 5, Figure 2). Furthermore, the samples collected in the buffer presented superior quality parameters and better gel resolution compared to those transported in ethanol. These qualities into improved performance in taxonomic identification. Previous studies have explored various methods to preserving fecal samples to obtain DNA. For instance, research assessing the use of dimethyl sufoxide saline buffer found that this reagent significantly outperformed ethanol, resulting in a 44% increase in amplification success and 17% improvement in genotyping accuracy (Wultsch et al., 2015), findings consistent with our study. Also well, other studies have reported similar results when examining rumen samples to assess microbiota; in these cases, ethanol yielded lower quality outcomes in comparison to two lysis buffers, namely Tris-NaCl-EDTA-SDS and guanidine hydrochlorate (Budel et al., 2022). Consistency in results across various studies has led to recommendations favoring the use of buffers for the long-term preservation of DNA extracted from fecal samples (Frantzen et al., 1998; Seutin et al., 1991). Nevertheless, it´s essential to recognize that outcomes may vary depending on factors such as the target species, sample type, environmental conditions, or even dietary habits (Wultsch et al., 2015). In our study, the lysis buffer method emerged as the most effective for a diverse array of species, encompassing varying habits and diets, including *Bos primigenius*, *Equus* caballus, *Alouatta seniculus* and *Tapirus pinchaque*, omnivory in *Tremarctos ornatus*, and carnivory in

Puma concolor (Table 4). This finding underscores the versatility of the buffer approach for the conservation and preservation fecal samples from different animal species.

In general, selecting an appropriate method for preserving fecal DNA is critical across all environments, particularly when working with low-quality and degraded samples (Wultsch et al., 2015). However, this consideration becomes more significant in tropical settings where DNA degradation occurs more rapidly. Therefore, optimizing sampling protocols is crucial. The fecal samples used in this study were collected in tropical areas of Colombia, characterized by an average temperature of 28°C and a relative humidity of 80-90% (Pabón et al., 2001). Elevated temperatures and increased humidity levels can both negatively impact the preservation of microorganisms in ethanol and lysis buffer. Under high temperatures, ethanol may evaporate, leading to a reduction in its concentration and subsequently diminishing its preserving effectiveness. Similarly, higher relative humidity can also exert a detrimental influence on microorganism preservation within ethanol and lysis buffer. Excessive moisture can condense on sample surfaces, causing dilution of the ethanol or lysis buffer, which may reduce their efficiency (C. A. Morgan et al., 2006). This study highlights the viability of using lysis buffer for molecular studies, including metabarcoding, in such environmental conditions, particularly in the tropical regions.

An additional noteworthy observation from our study was the duration between sample collection and long-term storage at freezing temperatures before processing for molecular analysis. Overall, there was no apparent impact of field storage duration on the amount of DNA extracted. Even when samples were stored for extended periods, such as 20 days or up to 461 days at -20ºC, the resulting DNA yields remained relatively consistent (Table 5).

To assess the reliability of two widely used genetic markers for metabarcoding (applicable to bacteria, protozoans and fungi) in stool samples after various storage durations, we conducted amplification experiments. Our results indicated a higher amplification reliability for 18S rRNA gene in samples preserved using the lysis buffer as opposed to ethanol. While for the 16S rRNA gene, we observed better performance with the lysis buffer, although it was not significantly different from the performance of samples stored in ethanol.

We utilized next-generation sequencing ONT, a proven and viable alternative for studying intestinal microbiota (Gill et al., 2006), to analyze the samples. Using the lysis buffer, we obtained twice the number of reads compared to samples preserved with ethanol for both genes

under study. However, it's important to note that various factors, such as temperature, storage time, and the concentration of preservation reagents, can influence the results both positively and negatively. For instance, previous research has shown that freezing can increase Firmicutes while benefiting Gram-positive bacteria at room temperature. Furthermore, different ethanol concentrations (e.g., 70% and 95%) can produce varying conservation effects. Notably, 95% ethanol demonstrates lower conservation stability, consistent with the results of our study.

Considering our findings, we recommend the use of a collection buffer as it requires only a small amount of fecal sample to yield a substantial amount of DNA. It consistently produces high-molecular-weight DNA suitable for various techniques. The buffer is well-suited for field conditions where samples may need to be stored for several weeks at ambient temperatures. These samples can be easily collected into vials pre-filled with lysis buffer. The buffer is also versatile and can be used with various tissues, such as cartilage, blood, striated or smooth muscle, hair follicles, and feathers. Importantly, it does not require refrigeration.

2.6 Conclusions

The present study assessed and compared the effects of two different stool sample preservation methods. Our experiment showed that the lysis buffer contributed to a higher quality and yield concentrations of DNA and amplification, with higher taxonomic assignment for microbiome composition. This finding offers a viable alternative to rapid freezing for subsequent fecal microbiome analysis, especially in situations where refrigeration and preserving a cold chain during transportation are logistically unfeasible. The collection of fecal samples is vital for investigating prospective associations between the fecal microbiota and the health conditions of wild animals and their environment.

Funding. The research was supported by National Institutes of Health (NIH, No. R01NS110122) to the Neuroviruses Emerging in the Americas Study (NEAS).
Institutional Review Board Statement: The animal study protocol was approved by Ethics Committee) of UNIVERSIDAD NACIONAL DE COLOMBIA (UN-17-03-23).

2.7 Acknowledgments

We thank the National University of Colombia - Palmira for the teaching assistance scholarship (Resolution 061/2021, Council of the Faculty of Agricultural Sciences Palmira headquarters). We

also thank the Andean Bear Conservation Alliance (ABCA), the Cleveland Metroparks Zoo Wildlife Conservation Program, and Rewild for supporting the doctoral thesis. We also thank MINCIENCIAS for the scholarship: “Jóvenes investigadores e innovadores en las regiones para atención de demandas definidas por los CODECTI de los Departamentos de Chocó, Valle del Cauca, Cauca y Nariño – BPIN 2022000100068”

Conflicts of Interest: The authors declare no conflict of interest.

2.8 References

Acosta Z, M., Tantaleán V, M., & Serrano-Martínez, E. (2015). Identificación de Parásitos Gastrointestinales por Coproscopía en Carnívoros Silvestres del Zoológico Parque de las Leyendas, Lima, Perú . In Revista de Investigaciones Veterinarias del Perú (Vol. 26, pp. 282–290). scielo .

Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., Gupta, R., Sood, U., Lal, R., & Toteja, R. (2023). DNA barcoding, an effective tool for species identification: A review. Molecular Biology Reports, 50(1), 761–775. https://doi.org/10.1007/s11033-022-08015-7

Aristizabal-Duque, S., Orozco Jimenez, L., Zapata Escobar, C., & Palacio-Baena, J. (2018). Conservation genetics of otters: Review about the use of noninvasive samples. Therya, 9, 85–93. https://doi.org/10.12933/therya-18-515

Arnemo, J. M., Ahlqvist, P., Andersen, R., Berntsen, F., Ericsson, G., Odden, J., Brunberg, S., Segerström, P., & Swenson, J. E. (2006). Risk of capture-related mortality in large free-ranging mammals: Experiences from Scandinavia. Wildlife Biology, 12(1), 109–113. https://doi.org/10.2981/0909-6396(2006)12[109:ROCMIL]2.0.CO;2

Budel, J. C. C., Hess, M. K., Bilton, T. P., Henry, H., Dodds, K. G., Janssen, P. H., McEwan, J. C., & Rowe, S. J. (2022). Low-cost sam-ple preservation methods for high-throughput processing of rumen microbiomes. Animal Microbiome, 4(1), 39. https://doi.org/10.1186/s42523-022-00190-z

Burnham, C. M., McKenney, E. A., Heugten, K. A., Minter, L. J., & Trivedi, S. (2023). Effect of fecal preservation method on captive southern white rhinoceros gut microbiome. Wildlife Society Bulletin, 47(2), e1436. https://doi.org/10.1002/wsb.1436

Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I., & Leonard, J. A. (2013). Preservation of RNA and DNA from mammal sam-ples under field conditions. Molecular Ecology Resources, 13(4), 663–673. https://doi.org/10.1111/1755-0998.12108

Choo, J., Leong, L., & Rogers, G. (2015). Sample storage conditions signficantly influence faecal microbiome profiles. Scientific Reports, 5, 16350. https://doi.org/10.1038/srep16350

Fox, J., Marquez, M., & Bouchet-Valat, M. (2023). Rcmdr: R Commander. R Package Version 2.9-0.

Frantzen, M. A., Silk, J. B., Ferguson, J. W., Wayne, R. K., & Kohn, M. H. (1998). Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology, 7(10), 1423–1428. https://doi.org/10.1046/j.1365-294x.1998.00449.x

Freed, N., & Silander, O. (2020). DNA quantification using the Qubit fluorometer v1.

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M., & Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. Science (New York, N.Y.), 312(5778), 1355–1359. https://doi.org/10.1126/science.1124234

Gonzales, F. N., Neira-Llerena, J., Llerena, G., & Zeballos, H. (2016). Small vertebrates in the spectacled bear_s diet (Tremarctos ornatus Cuvier, 1825) in the north of Peru . In Revista Peruana de Biología (Vol. 23, pp. 61–66). scielo .

Gorzelak, M. A., Gill, S. K., Tasnim, N., Ahmadi-Vand, Z., Jay, M., & Gibson, D. L. (2015). Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. PLOS ONE, 10(8), e0134802.

Iker, B. C., Bright, K. R., Pepper, I. L., Gerba, C. P., & Kitajima, M. (2013). Evaluation of commercial kits for the extraction and puri-fication of viral nucleic acids from environmental and fecal samples. Journal of Virological Methods, 191(1), 24–30. https://doi.org/10.1016/j.jviromet.2013.03.011

Jedlicka, J. A., Vo, A.-T. E., & Almeida, R. P. P. (2017). Molecular scatology and high-throughput sequencing reveal predominately herbivorous insects in the diets of adult and nestling Western Bluebirds (Sialia mexicana) in California vineyards. The Auk, 134(1), 116–127. https://doi.org/10.1642/AUK-16-103.1

Jin, S. S., Amnon, A., L., M. J., R., A. K., Zech, X. Z., Greg, H., & Rob, K. (2016). Preservation Methods Differ in Fecal Microbiome Stability, Affecting Suitability for Field Studies. mSystems, 1(3), 10.1128/msystems.00021-16. https://doi.org/10.1128/msystems.00021-16

Krogsgaard, L. R., Andersen, L. O. 'Brien, Johannesen, T. B., Engsbro, A. L., Stensvold, C. R., Nielsen, H. V., & Bytzer, P. (2018). Characteristics of the bacterial microbiome in association

with common intestinal parasites in irritable bowel syn-drome. Clinical and Translational Gastroenterology, 9(6), 161. https://doi.org/10.1038/s41424-018-0027-2

Kumar, G., & Bhadury, P. (2022). Effect of different fixatives on yield of DNA from human fecal samples. IOP SciNotes, 3(2), 24002. https://doi.org/10.1088/2633-1357/ac6d2e

Longmire, J., Maltbie, M., & Baker, R. J. (1997). Use of 'Lysis Buffer' in DNA isolation and its implication for museum collections /.

Matysik, S., Le Roy, C. I., Liebisch, G., & Claus, S. P. (2016). Metabolomics of fecal samples: A practical consideration. Trends in Food Science & Technology, 57, 244–255. https://doi.org/10.1016/j.tifs.2016.05.011

Menu, E., Mary, C., Toga, I., Raoult, D., Ranque, S., & Bittar, F. (2018). Evaluation of two DNA extraction methods for the PCR-based detection of eukaryotic enteric pathogens in fecal samples. BMC Research Notes, 11(1), 206. https://doi.org/10.1186/s13104-018-3300-2

Morgan, C. A., Herman, N., White, P. A., & Vesey, G. (2006). Preservation of micro-organisms by drying; a review. Journal of Mi-crobiological Methods, 66(2), 183–193. https://doi.org/10.1016/j.mimet.2006.02.017

Morgan, L. R., Marsh, K. J., Tolleson, D. R., & Youngentob, K. N. (2021). The Application of NIRS to Determine Animal Physiolog-ical Traits for Wildlife Management and Conservation. In Remote Sensing (Vol. 13, Issue 18). https://doi.org/10.3390/rs13183699

Ngcamphalala, P. I., Lamb, J., & Mukaratirwa, S. (2019). Molecular identification of hookworm isolates from stray dogs, humans and selected wildlife from South Africa. Journal of Helminthology, 94, e39. https://doi.org/10.1017/S0022149X19000130

Pabón, J., Zea, J., León, G., Hurtado, G., González, O., & Montealegre, J. (2001). La atmósfera, eltiempo y el clima. In P. Leyva (Ed.), El medio ambiente en Colombia. (pp. 35–91). Instituto de Hidrología, Meteorología y Estudios Ambientales, IDEAM.

Papaiakovou, M., Pilotte, N., Baumer, B., Grant, J., Asbjornsdottir, K., Schaer, F., Hu, Y., Aroian, R., Walson, J., & Williams, S. A. (2018). A comparative analysis of preservation techniques for the optimal molecular detection of hookworm DNA in a human fecal specimen. PLoS Neglected Tropical Diseases, 12(1), e0006130. https://doi.org/10.1371/journal.pntd.0006130

Plimpton, L. D., Henger, C. S., Munshi-South, J., Tufts, D., Kross, S., & Diuk-Wasser, M. (2021). Use of molecular scatology to assess the diet of feral cats living in urban colonies. Journal of Urban Ecology, 7(1), juab022. https://doi.org/10.1093/jue/juab022

Rondón, S., Cavallero, S., Renzi, E., Link, A., González, C., & D'Amelio, S. (2021). Parasites of Free-Ranging and Captive American Primates: A Systematic Review. Microorganisms, 9(12). https://doi.org/10.3390/microorganisms9122546

Seutin, G., White, B., & Boag, P. (1991). Seutin G, White BN, Boag PT.. Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69: 82-90. Canadian Journal of Zoology, 69, 82–90. https://doi.org/10.1139/z91-013

van der Reis, A. L., Beckley, L. E., Olivar, M. P., & Jeffs, A. G. (2023). Nanopore short-read sequencing: A quick, cost-effective and accurate method for DNA metabarcoding. Environmental DNA, 5(2), 282–296. https://doi.org/10.1002/edn3.374

Villamizar, X., Higuera, A., Herrera, G., Vasquez-A, L. R., Buitron, L., Muñoz, L. M., Gonzalez-C, F. E., Lopez, M. C., Giraldo, J. C., & Ramírez, J. D. (2019). Molecular and descriptive epidemiology of intestinal protozoan parasites of children and their pets in Cauca, Colombia: A cross-sectional study. BMC Infectious Diseases, 19(1), 190. https://doi.org/10.1186/s12879-019-3810-0

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 257. https://doi.org/10.1186/s13059-019-1891-0

Wood, D. E., & Salzberg, S. L. (2014). Kraken: Ultrafast metagenomic sequence classification using exact alignments. Genome Bi-ology, 15(3), R46. https://doi.org/10.1186/gb-2014-15-3-r46

Wu, C., Chen, T., Xu, W., Zhang, T., Pei, Y., Yang, Y., Zhang, F., Guo, H., Wang, Q., Wang, L., & Zhao, B. (2021). The maintenance of microbial community in human fecal samples by a cost effective preservation buffer. Scientific Reports, 11(1), 13453. https://doi.org/10.1038/s41598-021-92869-7

Wultsch, C., Waits, L. P., Hallerman, E. M., & Kelly, M. J. (2015). Optimizing collection methods for noninvasive genetic sampling of neotropical felids. Wildlife Society Bulletin, 39(2), 403–412. https://doi.org/10.1002/wsb.540

Yu, Y., Lee, C., Kim, J., & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnology and Bioengineering, 89(6), 670–679. https://doi.org/10.1002/bit.20347

3. Relationship of the landscape structure with the gut protozoa and microbiota diversity in livestock associated with conservation areas in Colombia: epidemiological risk to wildlife

Néstor Roncancio-Duque[1]*; Jeison Eduardo García-Ariza[1]; Nelson Rivera-Franco[2,3]; Juliana Peña Stadlin4; Diana López-Alvarez[1,2]*

[1] Facultad de Ciencias Agropecuarias, Grupo de Investigación en Diversidad Biológica. Universidad Nacional de Colombia, Sede Palmira. Colombia; njroncanciod@unal.edu.co, jeigarciaar@unal.edu.co, dilopezal@unal.edu.co

[2] Universidad del Valle, Facultad de Salud, Escuela de Ciencias Básicas, Grupo VIREM—Virus Emergentes y Enfermedad, Cali, Valle del Cauca, Colombia; rivera.nelson@correounivalle.edu.co, dilopezal@unal.edu.co

[3] Department of Neurology. Johns Hopkins School of Medicine. Maryland, United States; rivera.nelson@correounivalle.edu.co

[4] FZC Fundación Zoológica de Cali

* Correspondence: njroncanciod@unal.edu.co, dilopezal@unal.edu.co

3.1 Abstract

In the context of the sustainability of livestock, the three dimensions - environmental, economic, and social - intersect in the animal health component. Animal health is influenced, among other factors, by the risk of parasitosis and imbalances in the intestinal microbiota, which are also associated with various physiological processes in the organism. Additionally, through bidirectional phenomena, livestock can serve as reservoirs for parasites that impact ecosystem health by infesting wildlife. The diversity of intestinal microbiota depends on the biological diversity

of the areas they inhabit. This study assesses the diversity of intestinal microbiota and gastrointestinal protozoa that could affect wildlife, focusing on cattle and horses in productive systems located in conservation areas where they coexist with populations of wild mammals such as primates, pumas, Andean bears, and tapirs. Furthermore, the study evaluates the relationship between the level of fragmentation of natural vegetation cover and the diversity (Richness and evenness) of intestinal microbiota and protozoa in livestock (cattle and horses). Taxonomic determination was conducted using metabarcoding with primers for the rRNA 18S gene and Nanopore sequencing, primarily focused on detecting protozoa as proxies. Microscopy was also employed to verify some agents. Prevalence, mean intensity, and mean abundance were estimated to describe the epidemiology of the detected protozoa. Bayesian simple regressions were used to evaluate the relationship between landscape metrics and gut microbiota and protozoa diversity. Among the 30 samples that successfully amplified and provided sequences out of the 54 taken, 12, 13, and three protozoa were found in cattle, horses, and a canine sample, respectively. Except for *Trichomonas vaginalis*, all protozoa had documented records in at least one of the seven wild mammals they were compared (four primates, two carnivores, and one ungulate). A positive relationship was observed between the amount of native forest and the richness of intestinal microbiota and protozoa in cattle and horses. In conclusion, protozoa transmission is bidirectional risky from equine and bovine livestock to interacting wild mammals in conservation areas. Moreover, an increase in the amount of forest in the livestock enhances intestinal microbiota and protozoa, likely positively affecting livestock health.

3.1.1 Keywords: Protozoa, gut microbiota, fragmentation, livestock, wildlife mammals.

3.2 Introduction

Health in agriculture is the capacity to satisfy the daily requirements and to adapt to the environmental changes which implies the capacity to preserve in the system the productive parameters. In most cases, infective parasites and zoonotic diseases are the focus (Eriksson and Lindstrom, 2008; Frankish et al., 1996). Parasites may also drive important economic losses because of the decrease in production, the financial cost of control and treatment, and the mortality of livestock, particularly cattle (Echeverría et al., 2019; Kohler, 2004; Ryan et al., 2016). The economically important parasitic diseases are caused by protozoa and helminths (Rashid et al., 2019; Rodríguez-Vivas et al., 2017). In livestock, there are several risks linked to human behavior that have an impact on the epidemiology of infectious agents. Health status is a

cornerstone component in sustainable livestock, this depends on the synergic of the three dimensions of sustainability (environmental - social – economic) (Lovarelli et al., 2020). So, the effect of the parasites is bi-directional in all axes (Human – Animal - Wildlife) / (Wildlife - Animal - Human) and has a significant impact on the sustainability of the agricultural systems, human welfare, and the survival and reproductive success (Fitness) of the wildlife, mainly in conservation areas where they interact by acting as reservoirs. For these reasons, it is required the implementation of health plans for domestic animals as the conservation and management plans that threaten wildlife (Fisher et al., 2012; Lantz et al., 2018).

Concerning the gut, it is assumed that greater diversity will lead to better performance in various processes involving it (Danneskiold-Samsøe et al., 2019; Park et al., 2018). The microbiota in the gut participates in the breakdown of diet complex compounds (Sauvaitre et al., 2021; Song et al., 2021). The presence of beneficial microbiota helps train the immune system. During the early years of life, the colonization of the intestinal microbiota plays a crucial role in the development of the immune system. Additionally, the intestinal microbiota can modulate inflammatory responses and prevent inappropriate immune reactions (Yoo et al., 2020). The composition of the intestinal microbiota has been linked to metabolism and obesity (Aron-Wisnewsky et al., 2021). Changes in the microbiota have been observed to affect behavior and brain function, leading to research on the connection between gut health and neuropsychiatric disorders (Chen et al., 2021). A balanced microbiota can act as a barrier against harmful pathogens, preventing their colonization and growth (Calo-Mata et al., 2016). It has been suggested that the microbiota may affect the endocrine system, influencing hormone production that affects various bodily functions such as metabolism and blood sugar regulation (O'Callaghan et al., 2016).

The intestinal microbiota is highly sensitive to various types of stress, and these stresses can have significant impacts on its composition and function. Psychological stress has been associated with alterations in the diversity and balance of the microbiota. Physiological stress induced by imbalanced diets, exposure to pathogens, or excessive antibiotic use can also disrupt microbial homeostasis (He et al., 2021; Lange et al., 2016; Luo et al., 2021). Understanding how different forms of stress affect the intestinal microbiota is crucial for developing strategies that preserve gastrointestinal health and, by extension, the overall health of individuals.

In the environmental dimension, crucial aspects include land cover management (Sánchez-Romero et al., 2021). Sustainable management of these covers directly contributes to soil conservation, biodiversity, and water quality (Miralles-Wilhelm, 2021). In the social dimension, animal welfare is a key component, focusing on ensuring conditions that promote the physical and psychological health of animals in production systems (Broom, 2019; Perry et al., 2007). The

relationship between land cover management and animal welfare is fundamental, as adequately covered soil can provide thermal comfort, reduce stress, and prevent environment-related diseases (Mancera et al., 2018; Rulli et al., 2021).

Good agricultural and livestock practices, such as pasture rotation, planting forage crops, and implementing agroforestry systems, are key strategies to improve environmental sustainability and promote animal welfare (Broom et al., 2013). Additionally, adopting technologies that minimize environmental footprint, such as efficient waste management and the implementation of more efficient production systems, contributes to a holistic approach to sustainability in livestock production systems. In summary, the interconnection between environmental management and animal welfare is essential for achieving long-term sustainable livestock production systems from a health perspective (De Passillé and Rushen, 2005).

This study investigates the relationship between livestock features linked to the environmental dimension of system sustainability, and how these features influence the presence, burden, and diversity (richness and evenness) of gastrointestinal protozoa within the intestinal microbiota of domestic animals. We hypothesize that increased habitat fragmentation, characterized by less natural vegetation cover, greater isolation distances, and larger edge effects, will promote higher protozoan diversity but lower overall diversity of the intestinal microbiota in domestic animals.

3.3 Methods

3.3.1 Study Area

Samples were taken from 25 localities in nine departments. The analyses were conducted on 14 focal landscapes covering 224 km^2 each. These landscapes corresponded to those where positive results were obtained for at least one sample of the three included species of domestic mammals. The focal landscape's size was defined based on the largest area that included samples of wild and domestic species (Figure 5). It encompasses transformed ecosystems associated with selected conservation areas where samples of sampled wild mammals were taken (Chapter 4). The definition of the focal landscape size in landscape epidemiology is crucial for determining fragmentation statistics that are relevant for understanding epidemiological processes in a specific area (Jackson & Fahrig, 2012). Several criteria must be considered for this determination. Firstly, it is essential to consider the ecological dimensions of populations and the dynamics of the pathogens at play, suggesting the need for a scale that realistically captures interactions. Additionally, accessibility and topographical characteristics of the study area must

be considered, as they can influence the spread of diseases. The spatial resolution of available data and monitoring capacity are also determining factors in choosing the size of the focal landscape (Lausch, 2002). Therefore, it is necessary to adapt the scale of the focal landscape to the specific conditions of the epidemiological system in question, ensuring the relevance of fragmentation statistics for disease analysis at the local level (Ostfeld et al., 2005).

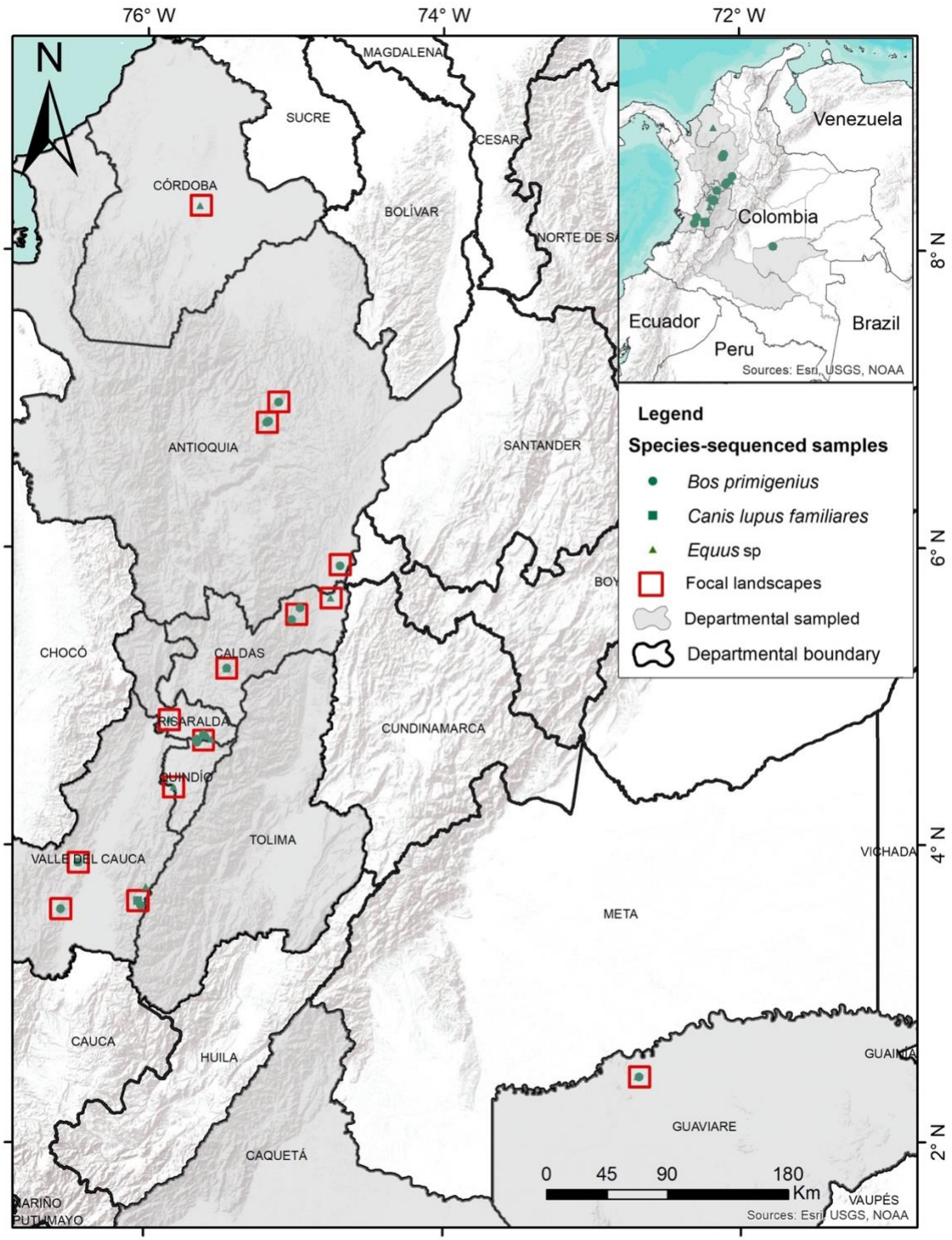


Figure 5. Study area. The map shows the focal landscapes (red boxes) sampled with the host domestic species that we were able to sample.

3.3.2 Collection of Fecal Samples and Storage

Fifty-four samples were collected from *Bos primigenius*, *Equus* sp., and *Canis lupus familiaris* in nine departments of Colombia (Table 6). The number of samples corresponds to the population in each site of each species of domestic species, assumed as an epidemiological unit—understood as an entity or group of individuals studied in a specific epidemiological context (Jakob-Hoff et al., 2014). In this sense, a sample could be representative of the unit (focal landscape). Each sample was taken with tweezers directly from the substrate (ground) carefully to take a piece that did not contact with any external material, prioritizing an internal portion of the deposition, immediately, each sample was deposited in a lysis buffer composed of 0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, and 0.5% SDS at pH 8 (Longmire et al., 1997). The samples were transported at ambient temperature to the laboratory and frozen at -20°C until extraction. Most samples were frozen between eight and 20 days after collection. The time from collection to extraction varied between samples.

Table 6. Number of samples collected, categorized by department and domestic host species.

Department/Species	*Bos primigenius*	*Canis lupus familiaris*	*Equus sp.*	**Grand Total**
Antioquia	7		4	**11**
Caldas	7		4	**11**
Caquetá	1		0	**1**
Cordoba	3		1	**4**
Guaviare	1		1	**2**
Quindío	4		1	**5**
Risaralda	2		4	**6**
Tolima			1	**1**
Valle del Cauca	6	2	5	**13**
Grand Total	**31**	**2**	**21**	**54**

3.3.3 DNA Extraction and 18S rRNA Library Preparation

For DNA extraction, the commercial Kit DNeasy PowerSoil Pro® Kit was used following the manufacturer's instructions with minor differences (addition of 5 minutes of stirring to mix sample with the first component of the kit). The extracted DNA was diluted in 50 µl of kit resuspension solution. The final yield and quality of extracted DNA were determined by Colibrí (Titertek Berthold) using 2 µl of DNA. Pure genomic DNA is indicated by an A260/A280 nm ratio between 1.8 and 2.0, while between 1.6-1.8 are acceptable, and any value less than 1.6 indicates contamination. DNA integrity was determined by visualizing 2 µL of extracted DNA on 1% agarose gel (w/v) containing GelRed (Biotium®). Subsequently, for the viability assessment of genomic DNA in molecular characterization studies, the 18S rRNA gene was targeted using primers G3F1 and G3R1 G3F1 (5' –GCCAGCAGCCGCGGTAATTC-3) and primer G3R1 (5' –ACATTCTTGGCAAATGCTTTCGCAG-3) (Krogsgaard et al., 2018). The protozoa targets were the agents including in Apicomplexa, Tricomonadida, Trypanosomatidae, *Paramecium*, and *Giardia* clades. Amplifications were done by endpoint polymerase chain reaction (PCR) using the Biorad CFX96 C1000 thermal cycler (Roncancio et al. 2024).

For visualization of the amplicons, 1.4% agarose gels were prepared and stained with GelRed. The gels of the total DNA and the amplicons were visualized in a gel photodocumenter (Nippon Genetics, FastGene FAS V model). Amplicon concentration was determined fluorometrically on the Qubit® 3.0 Fluorometer using the QubitTM dsDNA HS Assay Kit following the manufacturer's instructions (Freed & Silander, 2020).

3.3.4 Oxford Nanopore Sequencing and Bioinformatics

For sequencing the approximately 500 bp-long amplified regions, the MinION MK1B sequencing platform was utilized with the MinKNOW software. The sequencing library was prepared with the Ligation Sequencing Kit SQK-LSK109 and the Native Barcoding Expansion Kit 96 (EXP-NBD196). The library pool was sequenced on an R-9.4.1 flow cell for 48 hours. Raw FAST5 files produced were base called under high-accuracy mode using the ONT base caller Guppy v6.2.1. The FASTQ files were used for taxonomic assignment with Kraken2 and the PlusPFP database (3/14/2023). The results were subsequently filtered excluding clades with less than five reads and the Viridiplantae clade, corresponding to green plants, assuming that the presence of this clade was derived primarily from the diet, including water consumption.

3.3.5 Direct Observation: microscopy

To verify forms compatible with the protozoa target species intended for diagnosis, direct observation analyses were conducted using microscopy. This method also allowed the identification of forms compatible with other agents not diagnosable with the molecular markers used, providing additional information. Their utility for interaction and risk analyses is limited, as with these methodological approaches, most potential agents can only be determined at the genus level. The samples were collected in a 10% formalin fixing solution (PFA) and upon arrival at the laboratory, they were stored at a temperature of seven degrees Celsius. For parasitological diagnosis, the following approaches were employed:

Direct Coprology: A drop of saline solution and another of Lugol was applied to a microscope slide. An amount equivalent to the tip of a wooden applicator was added to both sides of the slide. Subsequently, homogenization was performed, and coverslips were applied.

Microscopic examination was conducted using an optical microscope at 5X magnification, with an assessment of all fields, 10X with an evaluation of all fields, and 40X with an evaluation of 40 fields. Special attention was given to suspicious structures during assessments at lower magnification.

Sheather Solution Flotation (Saturated Sucrose): The ovu-check® device was utilized. One gram of fecal matter was placed on the bottom lid, and the solution was added until reaching 1/3 of the container. The sample was homogenized with a wooden applicator, and a filter was added. The remaining solution was then added to complete the remaining 2/3. An embolus was left on the surface, and the solution was allowed to settle for 20 minutes. Afterward, it was covered with a cover slip. After 1 minute, the cover slip was removed, placed on a microscope slide, and read using the same methodology employed in direct coprology.

Ziehl-Neelsen: Specifically, to protozoa, the traditional method Ziehl-Neelsen was used, one the slide was mounted with the sample, we allowed the slide to air-dry at room temperature. Subsequently, once dry, it is heat-fixed using a hot plate for approximately 2 – 3 minutes. Direct flame fixation is avoided to prevent sample degradation. While the slide remains on the hot plate, phenol fuchsin is added for 5 minutes until vapor emission is observed. It is important not to let it boil, and additional staining is performed gradually if the dye appears to be drying. After 5 minutes, the slide is removed from the hot plate using forceps, and the dye is discarded into a container for chemical waste. Rinsing with R.O water is performed without direct water flow onto the sample until no color is observed in the effluent. Excess water is then removed. Subsequent

decolorization is achieved using acid-alcohol, followed by another wash with R.O water. Finally, Loeffler's methylene blue is added as a contrasting dye for 20 seconds to 1 minute.

3.3.6 Estimation of Covariates

Sustainable development in agriculture, forestry, and fisheries sectors, as defined as, conserving land, water, plant, and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable, and socially acceptable. The dimensions of sustainability—economic viability for farmers, environmental soundness, and social acceptability—have several indicators (Lovarelli et al., 2020; van der Linden et al., 2020). In this case, these indicators are associated with response variables related to the health status of livestock and its potential impact on wildlife. In this work, we focused on sustainability indicators related to the environmental dimension and based on land cover. We considered that less natural land cover is associated with more effects of size, isolation, and border, making livestock less sustainable. Covariates included landscape statistics related to land use coverages, bodies of water, roads, climatic variables, and altitude. Initially included covariates were River Length, Distance to Road, Forest Area, Forest Mean Shape Index (MSI), Forest Number of Patches (NumP), Forest Mean Patch Size, Pastures Area, Urbanized Zones Area, Precipitation, Temperature, and Elevation (Arce-Peña et al., 2019). The information used to estimate de covariates were the natural cover information 1:100.000 scale, digital elevation model, raster of temperature, precipitation, and shapes of simple drainage to the river and way (Fick & Hijmans, 2017; IDEAM, 2021; Instituto Geográfico Agustín Codazzi (Igac), 2023). The geoprocessing -clips, layer intercepts, zonal statistics- was done using ArcGis 10.7.1, and the landscape and cover metrics were estimated using Patch Analyst - Analysis by Region tools.

3.3.7 Statistical Analysis

An initial descriptive analysis was conducted based on species richness at various taxonomic levels. Additionally, for the considered protozoa, including the clades Apicomplexa, Tricomonadida, Trypanosomatidae, *Paramecium*, and *Giardia*, prevalence, mean intensity, and mean abundance were estimated. Prevalence refers to the proportion of cases of a specific disease or condition in a population at a given point in time. It is usually expressed as a percentage and represents the total number of cases relative to the total size of the at-risk population. Mean intensity refers to the average burden of a pathogen in infected individuals in a

population. It can be expressed in terms of the quantity of pathogens (parasites, bacteria, viruses, etc.) present in infected individuals. This measure helps understand the average severity of the disease in the affected population. Mean abundance refers to the average quantity of pathogens present in all individuals, whether infected or not, in a population. This measure provides information about the total burden of pathogens in the population, regardless of their infection status. To assess the relationship of covariates with the diversity of the intestinal microbiota and protozoa, richness and the inverse Simpson index were used as response variables for both sets. The analyses were performed using the Vegan package in R (Oksanen et al., 2013). The relationship was measured using a Bayesian Poisson simple regression method. Initially, a correlation test was conducted between all covariates to eliminate collinearity (autocorrelation). The Spearman coefficient of correlation was used for this assessment. These analyses were carried out in the R program, particularly in the PerformanceAnalytics package and Rcmdr (Fox et al., 2015; Peterson et al., 2018) (Supplement information 1). When two variables were associated (Spearman rank correlation coefficient: p-rs <0.05), one of them (less simple and precisely measurable) was eliminated (Sokal et al., 1995). The selected final variables are those that were not associated with others. As a result, six out of the 11 were considered covariates (River Length, Forest area, Forest MSI, Forest NumP, Urbanized zones area, and Precipitation). The Bayesian analysis was employed, utilizing an uninformative prior distribution for the precision of the explanatory variable effects and an uninformative prior distribution for the intercept (alpha) and slopes (beta). It was assumed that the posterior distributions of the intercept and the slope of each variable followed a normal distribution (McCarthy, 2007; Pfeiffer et al., 2008). Interactions (multiplicative effects) between the explanatory variables were not considered. To select the most parsimonious model, the Deviance Information Criterion (DIC) was employed. The estimation of intercepts and slopes was performed using Markov chains with 100,000 iterations, considering iterations from 10,001 to the final estimation. The DIC was estimated with an additional 100,000 iterations. The analyses were conducted using OpenBugs 3.2.2 software (Spiegelhalter et al., 2018).

3.4 Results

Of the 54 collected samples, sequencing was successful for 30 (16 bovine, 13 equine, and 1 canine). For bovines, the classification percentage ranged from 45.9% to 99.1%, with 10 out of 16 samples having determinations above 90%. For equines, the classification percentage ranged

from 53.6% to 99.7%, with 11 out of 13 samples above 93%. The canine sample had a classification percentage of 99.5% (Supplement information 2).

The diversity of the intestinal microbiota in bovines, after filtering the clades with more than 5 reads, was represented by four domains: Eukaryota (excluding Viridiplantae) with 97.08% of the reads, Bacteria with 2.89%, Archaea with 0.02%, and Viruses with 0.006%. This diversity was distributed across 25 phyla, 50 classes, 78 orders, 111 families, 133 genera, and 150 species. Similarly, in equines, the four domains were found with proportions of 99.21, 0.76, 0.006, and 0.006, respectively. This diversity was distributed across 23 phyla, 48 classes, 88 orders, 141 families, 174 genera, and 145 species. Just the species with more than five reads were considered for diversity (Species richness and evenness) analyses, comparisons, and regressions. The canine sample presented two domains: Eukaryota with 68.56% and Bacteria with 31.44%, distributed across 11 phyla, 26 classes, 33 orders, 43 families, 52 genera, and 61 species.

In bovines, the most represented phyla were Ascomycota, Basidiomycota, Apicomplexa, Pseudomonadota (Figure 6). The most abundant species were *Ustilaginoidea virens*, *Aspergillus puulaauensis, Thermothelomyces thermophilus, Thalassiosira pseudonana,* and *Fusarium poae*, with their relative abundances summing up to 53% of all OTUs reads with more than five reads (Supplement information 3). For equines, the most represented phyla were Ascomycota, Basidiomycota, Apicomplexa, and Pseudomonadota (Figure 7). The most abundant species were *Neurospora crassa, Thermothelomyces thermophilus, Ustilaginoidea virens, Malassezia restricta,* and *Puccinia striiformis*, with their relative abundances summing up to 68% (Supplement information 3). To the only one sample of *C. l. familiaris* the most represented phyla were Cyanobacteriota, Ascomycota, Basidiomycota, and Pseudomonadota. The most abundant species were *Nostoc* sp. C057, *Fusarium keratoplasticum*, *Halomonas* sp. JS92-SW72, *Fusarium poae*, but the first one had 58% of the reads (Supplement information 3).

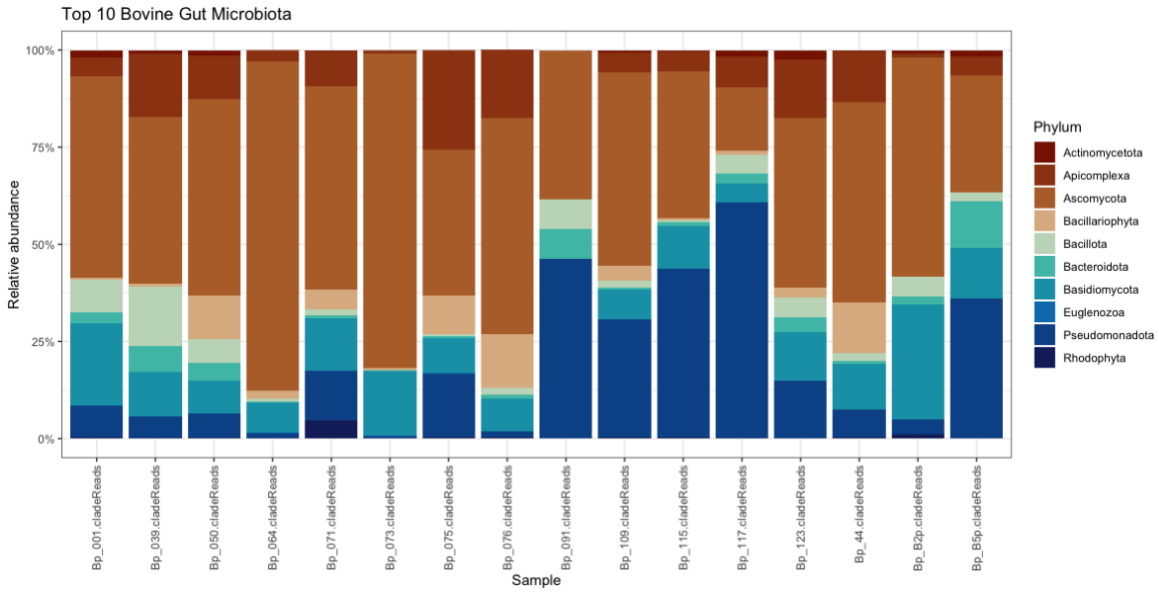


Figure 6. Relative abundance of microbiota phylum in bovine gut

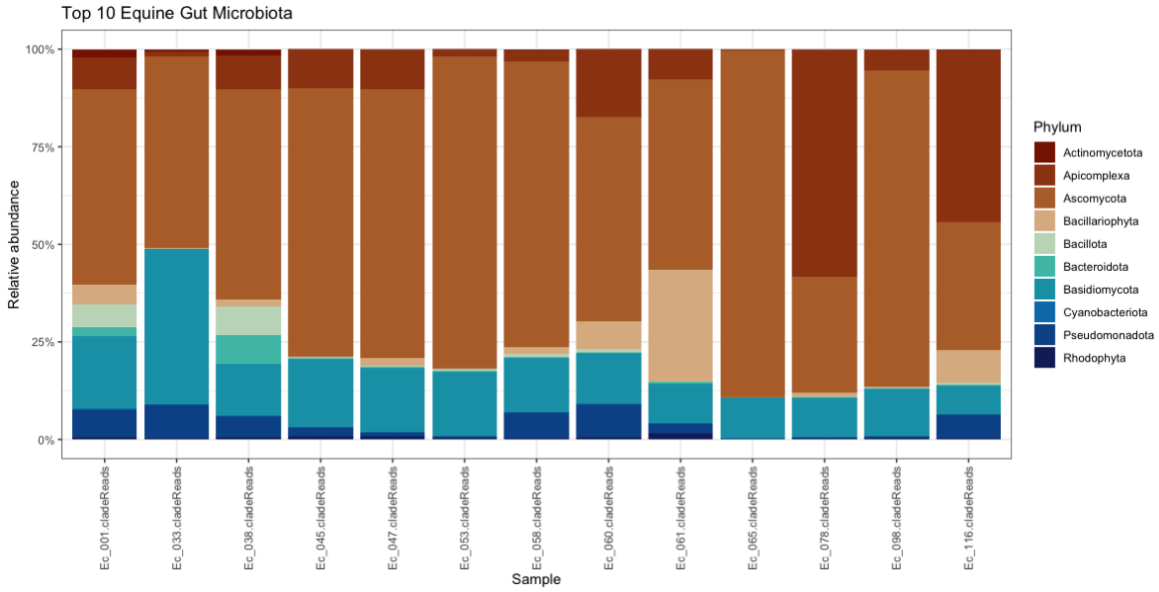


Figure 7. Relative abundance of microbiota phylum in equine gut

In cattle and horses, for the entire sample, *Toxoplasma gondii, Theileria species*, and *Babesia* showed the highest values in prevalence, mean intensity, and mean abundance (Table 7, and Table 8). In the single sample from the canine, the order of abundance (reads) was as follows: *Theileria orientalis* (97), *Toxoplasma gondii* (23), and *Babesia microti* (6).

Table 7. Bovine protozoa epidemiological descriptors. Prevalence is the proportion of cases of a pathogen in a population at a given point in time. It is expressed as a percentage. Mean intensity is the average burden of a pathogen in infected individuals in a population. It is expressed as the quantity of pathogens present in infected individuals. Mean abundance is the average quantity of pathogens present in all individuals.

Species	Prevalence			Mean intensity			Mean abundance		
	Mean	LlPI	ULPI	Mean	LlPI	ULPI	Mean	LlPI	ULPI
Theileria orientalis	0.59	0.35	0.80	156.67	148.60	165.00	94.00	89.15	99.00
Theileria equi	0.53	0.30	0.75	78.36	72.38	72.38	41.80	38.59	45.16
Babesia bigemina	0.65	0.41	0.85	12.00	9.94	14.24	8.00	6.63	9.50
Babesia microti	0.65	0.41	0.85	18.99	16.40	21.77	12.66	10.93	14.51
Babesia bovis	0.35	0.15	0.59	4.20	2.60	6.19	1.40	0.87	2.06
Plasmodium vivax	0.41	0.20	0.65	4.67	3.10	6.56	1.87	1.24	2.63
Plasmodium relictum	0.35	0.15	0.59	3.00	1.67	4.72	1.00	0.56	1.57
Plasmodium yoelii	0.35	0.15	0.59	1.40	0.57	2.62	0.47	0.19	0.87
Toxoplasma gondii	0.88	0.70	0.98	65.35	61.22	69.65	61.00	57.13	65.03
Besnoitia besnoiti	0.47	0.25	0.70	5.00	3.48	6.80	2.33	1.62	3.18
Cryptosporidium parvum	0.47	0.25	0.70	6.14	4.43	8.12	2.87	2.07	3.78
Trichomonas vaginalis	0.35	0.15	0.59	1.60	0.69	2.88	0.53	0.23	0.97

LLPI = Lower limit probability Interval, ULPI = Upper limit probability Interval, Colors highlighted biggest values in each parameter, red first, orange second, green third, and blue fourth.

Table 8. Equine protozoa epidemiological descriptors. Prevalence is the proportion of cases of a pathogen in a population at a given point in time. It is expressed as a percentage. Mean intensity is the average burden of a pathogen in infected individuals in a population. It is expressed as the quantity of pathogens present in infected individuals. Mean abundance is the average quantity of pathogens present in all individuals.

Species	Prevalence	Mean intensity	Mean abundance

	Mean	LIPI	ULPI	Mean	LIPI	ULPI	Mean	LIPI	ULPI
Theileria orientalis	0.79	0.55	0.95	615.30	600.10	631.00	512.75	500.10	525.60
Theileria equi	0.86	0.64	0.98	59.36	54.91	63.99	54.42	50.29	58.76
Babesia microti	0.86	0.64	0.98	70.64	65.76	75.75	64.75	60.28	69.40
Babesia bigemina	0.50	0.25	0.75	4.00	2.57	5.76	2.00	1.28	2.88
Babesia bovis	0.64	0.38	0.86	2.63	1.63	3.86	1.75	1.08	2.58
Plasmodium vivax	0.71	0.46	0.91	13.78	11.45	16.29	10.33	8.60	12.24
Plasmodium chabaudi	0.36	0.14	0.61	4.75	2.85	7.11	1.58	0.95	2.37
Plasmodium relictum	0.50	0.25	0.75	12.17	9.55	15.09	6.08	4.77	7.56
Toxoplasma gondii	0.93	0.75	1.00	188.25	180.60	196.10	188.25	180.60	196.10
Besnoitia besnoiti	0.43	0.19	0.68	15.00	11.82	18.57	6.25	4.92	7.75
Cryptosporidium parvum	0.50	0.25	0.75	12.83	10.15	15.84	6.42	5.07	7.92
Trichomonas vaginalis	0.21	0.11	0.45	4.00	1.73	7.23	0.67	0.29	1.20
Plasmodium coatneyi	0.14	0.02	0.36	12.00	6.19	19.67	1.00	0.52	1.65

LLPI = Lower limit probability Interval, ULPI = Upper limit probability Interval, Colors highlighted biggest values in each parameter, red first, orange second, green third, and blue fourth.

Distributing the sample across focal landscapes, not all of them yielded positive sequences for *Bos primigenius* and *Equus* sp. Out of the 14 local landscapes, each species showed results in 10, with some of them being different. In the case of cattle, a positive relationship was found between Forest Area, Number of Forest Patches, and Forest Mean Shape Index with the specific richness of the gut microbiota and protozoa. The best model, in both cases, included the Forest Area (Table 9). Similar to equines, the Forest Area was the main covariate that affected the gut microbiota and protozoa richness, in both cases positively. In no case, evenness was related to some of the covariates included.

Table 9. Significant regression models to assess the effect of landscape metrics in the bovine and equine gut microbiota and protozoa diversity (richness and evenness).

Model	beta	LIPI	ULPI	DIC	Δ DIC

BovGMRi_FA	0.36	0.31	0.42	374.50	0.0
BovGMRi_FNP	0.27	0.21	0.33	452.50	78.0
BovGMRi_Riv	0.28	0.22	0.35	459.00	84.5
BovGMRi_FMSI	-0.28	-0.37	-0.19	491.60	117.1
BovGMRi_UZ	-0.14	-0.25	-0.04	524.50	150.0
BovPRi_FA	0.39	0.17	0.61	67.37	0.0
BovPRi_FNP	0.36	0.13	0.59	70.06	2.7
BovPRi_FMSI	-0.39	-0.80	-0.03	75.04	7.7
EqGMRi_FA	0.30	0.23	0.38	139.30	0.0
EqGMRi_FNP	0.29	0.18	0.39	165.10	25.8
EqGMRi_Riv	0.36	0.21	0.52	171.00	31.7
EqPRi_Riv	0.43	0.07	0.81	52.43	0.0
EqPRi_FA	0.22	0.02	0.40	52.82	0.4

Bov=bovine, Eq=equine, GM= Gut microbiota, Ri=Richness, P=Protozoa, FA=Forest area, FNP= Forest Number of Patch, Riv=Length River, UZ= Urbanized zones, FMSI= Forest Mean shape index. LLPI = Lower limit probability Interval, ULPI = Upper limit probability Interval, DIC= Deviance Information Criteria, Δ DIC= delta DIC, difference between a model and most parsimonious model minor DIC. Gray highlighted models with positive relationships.

Through direct observation, the following were found in bovine samples: *Endolimax nana* (Q), *Entamoeba* (Q), Coccidia (Q), *Strongyloides* (H), *Iodamoeba*, *Entamoeba histolytica* (Q), *Giardia* (Q), *Saccharomyces* sp, and Cestode. For equines, *Strongyloides* (H), *Endolimax nana* (Q), Coccidia (Q), *Trichuris* (H), *Entamoeba* (Q), Ciliate (Q), *Oxyuris* (H), *Isospora* (Q), Nematode (H), *Saccharomyce*s, and fungal spores were found (Supplement information 4).

3.5 Discussion

The main phyla found in the samples were Ascomycota, Pseudomonadota, with Basidiomycota and Apicomplexa also present. The predominance of the Ascomycota phylum has been discovered to play a crucial role in the intestinal health of non-human mammals. Recent research suggests that evolutionary adaptation between these microorganisms and their hosts has led to

a beneficial symbiosis (Dworecka-Kaszak et al., 2016). Furthermore, it has been postulated that the predominant presence of Ascomycota could be associated with antagonistic properties toward potential pathogens, thereby strengthening the host's defenses (Limon et al., 2017). On the other hand, the abundance of Pseudomonadota highlights their crucial roles in intestinal homeostasis and might indicate an adaptive response to specific environmental conditions, such as exposure to pathogens or changes in the diet. Additionally, it has been suggested that certain members of Pseudomonadota may modulate the local immune response and actively participate in the degradation of complex compounds (Belvoncikova et al., 2022; Rizzetto et al., 2014).

Concerns about the wildlife species sampled in this research (Chapter 4) *Theileria* have been reported in Tapiridae (Da Silveira et al., 2017). However, its prevalence, mean intensity, and mean abundance are some of the greatest to three domestic hosts, which means that it is present in almost all sites. *Theileria* is a genus of protozoan parasites that infect various vertebrate hosts, including mammals and birds. The most well-known species within this genus are those that infect cattle, causing the disease known as tropical theileriosis. The epidemiology of *Theileria* involves a complex life cycle that typically includes both a vertebrate host and a tick vector. The protozoans are transmitted between hosts during the feeding process of infected ticks. In the case of cattle, for example, *Theileria* species are transmitted by ticks of the genus Rhipicephalus. The prevalence and distribution of *Theileria* depend on factors such as the presence of suitable tick vectors, host availability, and environmental conditions. Regions with a high density of ticks and susceptible hosts are more likely to have a higher prevalence of *Theileria* infections. While much research has focused on *Theileria* in domestic animals, there is also interest in understanding its occurrence in wildlife. *Theileria* has been found in various wild animal species, suggesting a broader ecological role. However, the epidemiology in wildlife populations is less well-studied compared to domestic animals (Mans et al., 2015).

Babesia has been documented in *Ursus arctos, U. thibetanus, U. americanus* (Bard & Cain, 2019; Ikawa et al., 2011; Jinnai et al., 2010; Skinner et al., 2017), but not in *Tremarctos ornatus*, Different species of *Babesia* have been found in *Puma concolor* (Yabsley et al., 2006) and *Alouatta seniculus* (de Thoisy et al., 2000). Here, *Babesia* had a prevalence of 86%, which means that it is relatively common, *Babesia* is a genus of protozoan parasites that infect red blood cells in various vertebrate hosts, including mammals, birds, and reptiles. The epidemiology of *Babesia* involves a life cycle that typically includes both a vertebrate host and a tick vector. Like *Theileria*, *Babesia* is transmitted between hosts during the feeding process of infected ticks. The primary vectors are often ticks of the *Ixodes* genus. The prevalence and distribution of *Babesia* infections are influenced by factors such as the presence of competent tick vectors, host reservoirs, and

environmental conditions. Regions with suitable tick habitats and a reservoir of infected vertebrate hosts are more likely to have a higher prevalence of *Babesia*. Certain species of *Babesia* are zoonotic. The epidemiology of *Babesia* is complex, and various species may have different host ranges and geographical distributions. Research in this field aims to understand the dynamics of *Babesia* infections, identify potential reservoir hosts, and develop strategies for the control and prevention of babesiosis in both human and animal populations (Laha et al., 2015).

Plasmodium, Toxoplasma gondii, and *Cryptosporidium* affect various species of Neotropical primates, including *Alouatta seniculus*, *Saguinus*, and *Aotus*. Similarly, *Toxoplasma* and *Cryptosporidium* have been documented in bears (Navarro M. et al., 2015; Rondón et al., 2021; Solórzano-García & Pérez-Ponce de León, 2018) and pumas (Hatam-Nahavandi et al., 2021; Li et al., 2020) but not in tapirs. *Toxoplasma* has the greatest prevalence of all the protozoa detected and *Cryptosporidium* was around in half of the samples. *Toxoplasma* and *Cryptosporidium* are parasitic protozoa with zoonotic significance, meaning they can infect both animals and humans. In the case of *Toxoplasma gondii*, infection primarily occurs through the ingestion of oocysts shed in the feces of felids, which act as definitive hosts. Transmission to wildlife species can occur through the ingestion of food or water contaminated with oocysts, posing potential health implications for wildlife (Aguirre et al 2019). Regarding *Cryptosporidium* spp., transmission is usually associated with water contaminated with oocysts. This transmission route is particularly relevant for wildlife relying on natural water sources. The presence of *Cryptosporidium* in aquatic environments can affect wildlife species using these resources for drinking or bathing (Gerace et al., 2019).

Besnoitia species, including *B. besnoiti*, which was around in half of the samples, are known to infect a range of domestic and wild animals. However, it is poorly documented in Neotropical wildlife species. While much of the research has centered on domestic livestock, there is an increasing recognition of the potential risk posed to wildlife. Transmission of *Besnoitia* typically occurs through the ingestion of oocysts shed in the feces of infected animals, contributing to the environmental contamination of pastures and water sources. This transmission route raises concerns for various wild species that share habitats with infected domestic animals. Additionally, predation and scavenging behaviors in wildlife may contribute to the spread of *Besnoitia* within and between species (Olias et al., 2011).

Trichomonas is a genus of flagellate protozoans that are primarily parasites of vertebrates, including humans, cattle, birds, and cats. The primary focus of *T. vaginalis* transmission is within human populations, and its occurrence in wild animals is not well-documented. The potential for

cross-species transmission or the establishment of reservoirs in wildlife remains an area of emerging interest (BonDurant, 1997; Collántes-Fernández et al., 2018).

The risk to wildlife species is influenced by environmental, climatic, and behavioral factors. Changes in ecosystems, increased urbanization, and interaction with domestic animals may elevate wildlife exposure to these parasites. Furthermore, immunosuppression due to factors such as stress, malnutrition, or disease can increase individual susceptibility. Generally, the livestock is not sustainable socially, economically, and environmentally (Molina-B, 2019). In this sense, cattle become an important reservoir (Buret et al., 2019; Echeverría et al., 2019; Ryan et al., 2016).

Understanding the epidemiology of these parasites in the context of wildlife is essential for developing effective management and conservation strategies, as well as assessing the potential risk to public health associated with the interaction between wildlife, livestock, and humans. Ongoing research is necessary to enhance our understanding of these processes and to develop appropriate prevention and control measures. Additional primers specific must be used to expand the diagnostic spectrum (Avramenko et al., 2015).

The positive relationship observed between the forest area and number of the forest parches in focal landscapes and the richness of intestinal microbiota and gastrointestinal protozoa in bovine and equine livestock can be attributed to various ecological mechanisms. Native forests often harbor a rich array of plant species, insects, and wildlife. The diverse plant community contributes to a complex microbial environment in the soil, which, in turn, influences the gut microbiota of herbivores (Blum et al., 2019). Livestock grazing in areas with higher native forest cover may have increased exposure to a variety of microbes through foraging and environmental interactions (Phillips et al., 2018). The proximity of livestock to native forests can facilitate the transfer of microbes from the natural environment to the gastrointestinal tract of animals (Parajuli et al., 2020). Animals may consume vegetation, water, or soil containing a diverse range of microorganisms, influencing the composition and richness of their gut microbiota (Blum et al., 2019). Higher biodiversity in native forests may dilute the prevalence of specific pathogenic parasites. A more diverse ecosystem could reduce the concentration of parasites that specifically target livestock, leading to a lower risk of infection (Williams et al., 2021). The intricate web of interactions within diverse ecosystems may affect both beneficial microbes and potentially parasitic organisms (Leung et al., 2018). Certain microbial species may confer resistance or resilience to parasitic infections, influencing the overall health of the host animals (Kohl, 2012; Worsley et al., 2021). Native forests contribute to a more complex and varied environment compared to monoculture landscapes. This complexity may stimulate a more diverse microbial

community in the intestines of livestock, promoting overall gut health and resilience to gastrointestinal parasites (Berrilli et al., 2012; Stensvold & van der Giezen, 2018). The relationship with the length of water bodies can be explained by considering that rivers can provide water sources rich in essential minerals and other nutrients beneficial to the health of livestock. An adequate supply of good-quality water can promote the development and diversity of the intestinal microbiota. Proximity to water bodies often means access to richer and more diverse pastures. More varied pastures can provide a broader range of substrates for bacterial growth in the gastrointestinal tract of livestock. An environment that includes water can reduce stress in animals. Less stressed livestock is more likely to maintain a healthy microbiological balance in the intestinal tract (Arshad et al., 2021).

These explanations are based on general ecological principles, and the specific relationships may vary based on the characteristics of the landscape, climate, and management practices. Continued research, including longitudinal studies and molecular analyses, is essential to unravel the intricate dynamics between landscape features, microbial diversity, and gastrointestinal parasite abundance in livestock. However, to improve the sustainability standards in the environmental, economic, and social dimensions based on the animal health component, the livestock landscape configuration in Colombia must be changed from a matrix of grassland with forest patches to a matrix of native forest with grassland patches.

In conclusion, our study found mainly a positive relationship between the quantity of forest and the number of forest patches with the richness of gut microbiota and protozoa. It means that major natural land covers a major diversity of gut microbial components. The increase of protozoa diversity contrasts with our hypothesis but makes sense when it considers that a major diversity of protozoa has less burden on each one and less probability to generate an immunologic response to disease, which is a positive effect driven by a more natural a complex landscape context.

Funding: The research was supported by the National Institutes of Health (NIH, No. R01NS110122) to the Neuroviruses Emerging in the Americas Study (NEAS).

Institutional Review Board Statement: Andean Bear Conservation Alliance (ABCA), the Cleveland Metroparks Zoo Wildlife Conservation Program, and Rewild

3.6 Acknowledgments

We thank the National University of Colombia - Palmira for the teaching assistance scholarship (Resolution 061/2021, Council of the Faculty of Agricultural Sciences Palmira headquarters). We also thank the Andean Bear Conservation Alliance (ABCA), the Cleveland Metroparks Zoo Wildlife Conservation Program, and Rewild for supporting the doctoral thesis. We also thank Andrés Mauricio González-Ríos of MINCIENCIAS for the scholarship: “Jóvenes investigadores e innovadores en las regiones para atención de demandas definidas por los CODECTI de los Departamentos de Chocó, Valle del Cauca, Cauca y Nariño – BPIN 2022000100068”

Conflicts of Interest: The authors declare no conflict of interest.

Ethic statements: The animal study protocol was approved by the Ethics Committee) of UNIVERSIDAD NACIONAL DE COLOMBIA (UN-17-03-23).

3.7 References

Arce-Peña, N. P., Arroyo-Rodríguez, V., Dias, P. A. D., Franch-Pardo, I., & Andresen, E. (2019). Linking changes in landscape structure to population changes of an endangered primate. Landscape Ecology, 34(11), 2687–2701. https://doi.org/10.1007/s10980-019-00914-8

Aron-Wisnewsky, J., Warmbrunn, M. V., Nieuwdorp, M., & Clément, K. (2021). Metabolism and Metabolic Disorders and the Microbiome: The Intestinal Microbiota Associated With Obesity, Lipid Metabolism, and Metabolic Health—Pathophysiology and Therapeutic Strategies. Gastroenterology, 160(2), 573–599. https://doi.org/10.1053/j.gastro.2020.10.057

Arshad, M. A., Hassan, F., Rehman, M. S., Huws, S. A., Cheng, Y., & Din, A. U. (2021). Gut microbiome colonization and development in neonatal ruminants: Strategies, prospects, and opportunities. Animal Nutrition, 7(3), 883–895. https://doi.org/10.1016/j.aninu.2021.03.004

Avramenko, R. W., Redman, E. M., Lewis, R., Yazwinski, T. A., Wasmuth, J. D., & Gilleard, J. S. (2015). Exploring the Gastrointestinal “Nemabiome”: Deep Amplicon Sequencing to Quantify the Species Composition of Parasitic Nematode Communities. PLOS ONE, 10(12), e0143559. https://doi.org/10.1371/journal.pone.0143559

Bard, S. M., & Cain, J. W. (2019). PATHOGEN PREVALANCE IN AMERICAN BLACK BEARS (URSUS AMERICANUS AMBLYCEPS) OF THE JEMEZ MOUNTAINS, NEW MEXICO, USA. Journal of Wildlife Diseases, 55(4), 745. https://doi.org/10.7589/2018-12-286

Belvoncikova, P., Splichalova, P., Videnska, P., & Gardlik, R. (2022). The Human Mycobiome: Colonization, Composition and the Role in Health and Disease. Journal of Fungi, 8(10), 1046. https://doi.org/10.3390/jof8101046

Berrilli, F., Di Cave, D., Cavallero, S., & D'Amelio, S. (2012). Interactions between parasites and microbial communities in the human gut. Frontiers in Cellular and Infection Microbiology, 2. https://doi.org/10.3389/fcimb.2012.00141

Blum, W. E. H., Zechmeister-Boltenstern, S., & Keiblinger, K. M. (2019). Does Soil Contribute to the Human Gut Microbiome? Microorganisms, 7(9), 287. https://doi.org/10.3390/microorganisms7090287

BonDurant, R. H. (1997). Pathogenesis, Diagnosis, and Management of Trichomoniasis in Cattle. Veterinary Clinics of North America: Food Animal Practice, 13(2), 345–361. https://doi.org/10.1016/S0749-0720(15)30346-7

Broom, D. M. (2019). Animal welfare complementing or conflicting with other sustainability issues. Applied Animal Behaviour Science, 219, 104829. https://doi.org/10.1016/j.applanim.2019.06.010

Broom, D. M., Galindo, F. A., & Murgueitio, E. (2013). Sustainable, efficient livestock production with high biodiversity and good welfare for animals. Proceedings of the Royal Society B: Biological Sciences, 280(1771), 20132025. https://doi.org/10.1098/rspb.2013.2025

Buret, A. G., Motta, J.-P., Allain, T., Ferraz, J., & Wallace, J. L. (2019). Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: A role for iron? Journal of Biomedical Science, 26(1), 1. https://doi.org/10.1186/s12929-018-0495-4

Calo-Mata, P., Ageitos, J. M., Böhme, K., & Barros-Velázquez, J. (2016). Intestinal Microbiota: First Barrier Against Gut-Affecting Pathogens. In T. G. Villa & M. Vinas (Eds.), New Weapons to Control Bacterial Growth (pp. 281–314). Springer International Publishing. https://doi.org/10.1007/978-3-319-28368-5_12

Chen, S., Luo, S., & Yan, C. (2021). Gut Microbiota Implications for Health and Welfare in Farm Animals: A Review. Animals, 12(1), 93. https://doi.org/10.3390/ani12010093

Collántes-Fernández, E., Fort, M. C., Ortega-Mora, L. M., & Schares, G. (2018). Trichomonas. In M. Florin-Christensen & L. Schnittger (Eds.), Parasitic Protozoa of Farm Animals and Pets (pp. 313–388). Springer International Publishing. https://doi.org/10.1007/978-3-319-70132-5_14

Da Silveira, A. W., De Oliveira, G. G., Menezes Santos, L., da Silva Azuaga, L. B., Macedo Coutinho, C. R., Echeverria, J. T., Antunes, T. R., do Nascimento Ramos, C. A., & Izabel de Souza, A. (2017). Natural Infection of the South American Tapir (Tapirus terrestris) by Theileria equi. Journal of Wildlife Diseases, 53(2), 411–413. https://doi.org/10.7589/2016-06-149

Danneskiold-Samsøe, N. B., Dias de Freitas Queiroz Barros, H., Santos, R., Bicas, J. L., Cazarin, C. B. B., Madsen, L., Kristiansen, K., Pastore, G. M., Brix, S., & Maróstica Júnior, M. R. (2019). Interplay between food and gut microbiota in health and disease. Food Research International, 115, 23–31. https://doi.org/10.1016/j.foodres.2018.07.043

De Passillé, A., & Rushen, J. (2005). Food safety and environmental issues in animal welfare. Revue Scientifique et Technique-Office International Des Épizooties, 24(2), 757.

de Thoisy, B., Michel, J.-C., Vogel, I., & Vié, J.-C. (2000). A SURVEY OF HEMOPARASITE INFECTIONS IN FREE-RANGING MAMMALS AND REPTILES IN FRENCH GUIANA. Journal of Parasitology, 86(5), 1035–1040. https://doi.org/10.1645/0022-3395(2000)086[1035:ASOHII]2.0.CO;2

Dworecka-Kaszak, B., Dąbrowska, I., & Kaszak, I. (2016). The mycobiome – a friendly cross-talk between fungal colonizers and their host. Annals of Parasitology, 62(3), 175–184. https://doi.org/10.17420/ap6203.51

Echeverría, G., Reyna-Bello, A., Minda-Aluisa, E., Celi-Erazo, M., Olmedo, L., García, H. A., Garcia-Bereguiain, M. A., & de Waard, J. H. (2019). Serological evidence of Coxiella burnetii infection in cattle and farm workers: Is Q fever an underreported zoonotic disease in Ecuador? Infection and Drug Resistance, Volume 12, 701–706. https://doi.org/10.2147/IDR.S195940

Eriksson, M., & Lindstrom, B. (2008). A salutogenic interpretation of the Ottawa Charter. Health Promotion International, 23(2), 190–199. https://doi.org/10.1093/heapro/dan014

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology, 37(12), 4302–4315.

Fisher, M. C., Henk, Daniel. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. Nature, 484(7393), 186–194. https://doi.org/10.1038/nature10947

Fox, J., Bouchet-Valat, M., Andronic, L., Ash, M., Boye, T., Calza, S., Chang, A., Grosjean, P., Heiberger, R., & Pour, K. K. (2015). Package 'Rcmdr'.

Frankish, C. J., Green, L. W., Ratner, P. A., Chomik, T., & Larsen, C. (1996). Health impact assessment as a tool for population health promotion and public policy. A Report Submitted to the Health Promotion Division of Health Canada. Institute of Health Promotion Research, University of British Columbia.

Freed, N., & Silander, O. (2020). DNA quantification using the Qubit fluorometer v1 [Preprint]. https://doi.org/10.17504/protocols.io.bfy3jpyn

Gerace, E., Presti, V. D. M. L., & Biondo, C. (2019). Cryptosporidium infection: Epidemiology, pathogenesis, and differential diagnosis. European Journal of Microbiology and Immunology, 9(4), 119–123. https://doi.org/10.1556/1886.2019.00019

Hatam-Nahavandi, K., Calero-Bernal, R., Rahimi, M. T., Pagheh, A. S., Zarean, M., Dezhkam, A., & Ahmadpour, E. (2021). Toxoplasma gondii infection in domestic and wild felids as public health concerns: A systematic review and meta-analysis. Scientific Reports, 11(1), 9509. https://doi.org/10.1038/s41598-021-89031-8

He, Y., Maltecca, C., & Tiezzi, F. (2021). Potential Use of Gut Microbiota Composition as a Biomarker of Heat Stress in Monogastric Species: A Review. Animals, 11(6), 1833. https://doi.org/10.3390/ani11061833

IDEAM. (2021). Mapa de Coberturas de la Tierra. Metodología CORINE Land Cover adaptada para Colombia, escala 1:100 000 (Periodo 2018) [Map]. IDEAM–Instituto de Hidrología Meteorología y Estudios Ambientales. http://www.siac.gov.co/catalogo-de-mapas

Ikawa, K., Aoki, M., Ichikawa, M., & Itagaki, T. (2011). The first detection of Babesia species DNA from Japanese black bears (Ursus thibetanus japonicus) in Japan. Parasitology International, 60(2), 220–222. https://doi.org/10.1016/j.parint.2011.02.005

Instituto Geográfico Agustín Codazzi (Igac). (2023). Datos abiertos Igac.

Jackson, H. B., & Fahrig, L. (2012). What size is a biologically relevant landscape? Landscape Ecology, 27(7), 929–941. https://doi.org/10.1007/s10980-012-9757-9

Jakob-Hoff, R. M., MacDiarmid, S. C., Lees, C., Miller, P. S., Travis, D., & Kock, R. (2014). Manual of procedures for wildlife disease risk analysis (Vol. 2014). World Organisation for Animal Health Paris, France.

Jinnai, M., Kawabuchi-Kurata, T., Tsuji, M., Nakajima, R., Hirata, H., Fujisawa, K., Shiraki, H., Asakawa, M., Nasuno, T., & Ishihara, C. (2010). Molecular evidence of the multiple genotype infection of a wild Hokkaido brown bear (Ursus arctos yesoensis) by Babesia sp. UR1. Veterinary Parasitology, 173(1–2), 128–133. https://doi.org/10.1016/j.vetpar.2010.06.018

Kohl, K. D. (2012). Diversity and function of the avian gut microbiota. Journal of Comparative Physiology B, 182(5), 591–602. https://doi.org/10.1007/s00360-012-0645-z

Kohler, W. (2004). Infectious Diseases Transmissible from Animals to Humans. International Journal of Medical Microbiology, 293(7/8), 548.

Krogsgaard, L. R., Andersen, L. O., Johannesen, T. B., Engsbro, A. L., Stensvold, C. R., Nielsen, H. V., & Bytzer, P. (2018). Characteristics of the bacterial microbiome in association with common

intestinal parasites in irritable bowel syndrome. Clinical and Translational Gastroenterology, 9(6), e161. https://doi.org/10.1038/s41424-018-0027-2

Laha, R., Das, M., & Sen, A. (2015). Morphology, epidemiology, and phylogeny of Babesia: An overview. Tropical Parasitology, 5(2), 94.

Lange, K., Buerger, M., Stallmach, A., & Bruns, T. (2016). Effects of Antibiotics on Gut Microbiota. Digestive Diseases, 34(3), 260–268. https://doi.org/10.1159/000443360

Lantz, E. L., Lonsdorf, E. V., Heintz, M. R., Murray, C. M., Lipende, I., Travis, D. A., & Santymire, R. M. (2018). Non-invasive quantification of immunoglobulin A in chimpanzees (Pan troglodytes schweinfurthii) at Gombe National Park, Tanzania. American Journal of Primatology, 80(1), e22558. https://doi.org/10.1002/ajp.22558

Lausch, A. (2002). Applicability of landscape metrics for the monitoring of landscape change: Issues of scale, resolution and interpretability. Ecological Indicators, 2(1–2), 3–15. https://doi.org/10.1016/S1470-160X(02)00053-5

Leung, J. M., Graham, A. L., & Knowles, S. C. L. (2018). Parasite-Microbiota Interactions With the Vertebrate Gut: Synthesis Through an Ecological Lens. Frontiers in Microbiology, 9, 843. https://doi.org/10.3389/fmicb.2018.00843

Li, X., Nguyen, T., Xiao, C., Levy, A., Akagi, Y., Silkie, S., & Atwill, E. R. (2020). Prevalence and Genotypes of Cryptosporidium in Wildlife Populations Co-Located in a Protected Watershed in the Pacific Northwest, 2013 to 2016. Microorganisms, 8(6), 914. https://doi.org/10.3390/microorganisms8060914

Limon, J. J., Skalski, J. H., & Underhill, D. M. (2017). Commensal Fungi in Health and Disease. Cell Host & Microbe, 22(2), 156–165. https://doi.org/10.1016/j.chom.2017.07.002

Longmire, J. L., Maltbie, M., & Baker, R. J. (1997). Use of" lysis buffer" in DNA isolation and its implication for museum collections.

Lovarelli, D., Bacenetti, J., & Guarino, M. (2020). A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? Journal of Cleaner Production, 262, 121409. https://doi.org/10.1016/j.jclepro.2020.121409

Luo, J., Cheng, Y., Guo, L., Wang, A., Lu, M., & Xu, L. (2021). Variation of gut microbiota caused by an imbalance diet is detrimental to bugs' survival. Science of The Total Environment, 771, 144880. https://doi.org/10.1016/j.scitotenv.2020.144880

Mancera, K. F., Zarza, H., de Buen, L. L., García, A. A. C., Palacios, F. M., & Galindo, F. (2018). Integrating links between tree coverage and cattle welfare in silvopastoral systems evaluation. Agronomy for Sustainable Development, 38(2), 19. https://doi.org/10.1007/s13593-018-0497-3

Mans, B. J., Pienaar, R., & Latif, A. A. (2015). A review of Theileria diagnostics and epidemiology. International Journal for Parasitology: Parasites and Wildlife, 4(1), 104–118. https://doi.org/10.1016/j.ijppaw.2014.12.006

McCarthy, M. A. (2007). Bayesian Methods for Ecology. Cambridge University Press.

Miralles-Wilhelm, F. (2021). Nature-based solutions in agriculture: Sustainable management and conservation of land, water and biodiversity. Food & Agriculture Org.

Navarro M., D., Chávez V., A., Pinedo V., R., & Muñoz D., K. (2015). Factores de Riesgo Asociados a la Seroprevalencia de Toxoplasma gondii en Mamíferos del Orden Carnivora y Primates Mantenidos en Cautiverio. Revista de Investigaciones Veterinarias Del Perú, 26(3), 497. https://doi.org/10.15381/rivep.v26i3.11175

O'Callaghan, T. F., Ross, R. P., Stanton, C., & Clarke, G. (2016). The gut microbiome as a virtual endocrine organ with implications for farm and domestic animal endocrinology. Domestic Animal Endocrinology, 56, S44–S55. https://doi.org/10.1016/j.domaniend.2016.05.003

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). Package 'vegan'. Community Ecology Package, Version, 2(9), 1–295.

Olias, P., Schade, B., & Mehlhorn, H. (2011). Molecular pathology, taxonomy and epidemiology of Besnoitia species (Protozoa: Sarcocystidae). Infection, Genetics and Evolution, 11(7), 1564–1576. https://doi.org/10.1016/j.meegid.2011.08.006

Ostfeld, R., Glass, G., & Keesing, F. (2005). Spatial epidemiology: An emerging (or re-emerging) discipline. Trends in Ecology & Evolution, 20(6), 328–336. https://doi.org/10.1016/j.tree.2005.03.009

Parajuli, A., Hui, N., Puhakka, R., Oikarinen, S., Grönroos, M., Selonen, V. A. O., Siter, N., Kramna, L., Roslund, M. I., Vari, H. K., Nurminen, N., Honkanen, H., Hintikka, J., Sarkkinen, H., Romantschuk, M., Kauppi, M., Valve, R., Cinek, O., Laitinen, O. H., … Sinkkonen, A. (2020). Yard vegetation is associated with gut microbiota composition. Science of The Total Environment, 713, 136707. https://doi.org/10.1016/j.scitotenv.2020.136707

Park, H., Yeo, S., Arellano, K., Kim, H. R., & Holzapfel, W. (2018). Role of the gut microbiota in health and disease. Probiotics and Prebiotics in Animal Health and Food Safety, 35–62.

Perry, R. W., Thill, R. E., & Leslie Jr, D. M. (2007). Selection of roosting habitat by forest bats in a diverse forested landscape. Forest Ecology and Management, 238(1–3), 156–166.

Peterson, B. G., Carl, P., Boudt, K., Bennett, R., Ulrich, J., Zivot, E., Cornilly, D., Hung, E., Lestel, M., & Balkissoon, K. (2018). Package 'performanceanalytics'. R Team Cooperation, 3, 13–14.

Pfeiffer, D. U., Robinson, T. P., Stevenson, M., Stevens, K. B., Rogers, D. J., & Clements, A. C. A. (2008). Identifying factors associated with the spatial distribution of disease. Spatial Analysis in Epidemiology, 81–109. https://doi.org/10.1093/acprof:oso/9780198509882.003.0007

Phillips, J. N., Berlow, M., & Derryberry, E. P. (2018). The Effects of Landscape Urbanization on the Gut Microbiome: An Exploration Into the Gut of Urban and Rural White-Crowned Sparrows. Frontiers in Ecology and Evolution, 6, 148. https://doi.org/10.3389/fevo.2018.00148

Rashid, M., Rashid, M. I., Akbar, H., Ahmad, L., Hassan, M. A., Ashraf, K., Saeed, K., & Gharbi, M. (2019). A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. Parasitology, 146(2), 129–141. https://doi.org/10.1017/S0031182018001282

Rizzetto, L., De Filippo, C., & Cavalieri, D. (2014). Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease. European Journal of Immunology, 44(11), 3166–3181. https://doi.org/10.1002/eji.201344403

Roncancio-Duque, N., García-Ariza, J. E., Rivera-Franco, N., Gonzalez-Ríos, A. M., & López-Alvarez, D. (2024). Comparison of DNA quantity and quality from fecal samples of mammals transported in ethanol or lysis buffer. One Health, 18, 100731. https://doi.org/10.1016/j.onehlt.2024.100731

Rodríguez-Vivas, R. I., Grisi, L., Pérez de León, A. A., Silva Villela, H., Torres-Acosta, J. F. de J., Fragoso Sánchez, H., Romero Salas, D., Rosario Cruz, R., Saldierna, F., & García Carrasco, D. (2017). Potential economic impact assessment for cattle parasites in Mexico. Review. Revista Mexicana de Ciencias Pecuarias, 8(1), 61. https://doi.org/10.22319/rmcp.v8i1.4305

Rondón, S., Cavallero, S., Renzi, E., Link, A., González, C., & D'Amelio, S. (2021). Parasites of Free-Ranging and Captive American Primates: A Systematic Review. Microorganisms, 9(12), 2546. https://doi.org/10.3390/microorganisms9122546

Rulli, M. C., D'Odorico, P., Galli, N., & Hayman, D. T. S. (2021). Land-use change and the livestock revolution increase the risk of zoonotic coronavirus transmission from rhinolophid bats. Nature Food, 2(6), 409–416. https://doi.org/10.1038/s43016-021-00285-x

Ryan, U., Zahedi, A., & Paparini, A. (2016). Cryptosporidium in humans and animals—A one health approach to prophylaxis. Parasite Immunology, 38(9), 535–547. https://doi.org/10.1111/pim.12350

Sánchez-Romero, R., Balvanera, P., Castillo, A., Mora, F., García-Barrios, L. E., & González-Esquivel, C. E. (2021). Management strategies, silvopastoral practices and socioecological drivers in traditional livestock systems in tropical dry forests: An integrated analysis. Forest Ecology and Management, 479, 118506. https://doi.org/10.1016/j.foreco.2020.118506

Sauvaitre, T., Etienne-Mesmin, L., Sivignon, A., Mosoni, P., Courtin, C. M., Van de Wiele, T., & Blanquet-Diot, S. (2021). Tripartite relationship between gut microbiota, intestinal mucus and dietary fibers: Towards preventive strategies against enteric infections. FEMS Microbiology Reviews, 45(2), fuaa052. https://doi.org/10.1093/femsre/fuaa052

Skinner, D., Mitcham, J. R., Starkey, L. A., Noden, B. H., Fairbanks, W. S., & Little, S. E. (2017). PREVALENCE OF BABESIA SPP., EHRLICHIA SPP., AND TICK INFESTATIONS IN OKLAHOMA BLACK BEARS (URSUS AMERICANUS). Journal of Wildlife Diseases, 53(4), 781–787. https://doi.org/10.7589/2017-02-029

Sokal, R. R., Rohlf, F. J., & Rohlf, J. F. (1995). Biometry. Macmillan.

Solórzano-García, B., & Pérez-Ponce de León, G. (2018). Parasites of Neotropical Primates: A Review. International Journal of Primatology, 39(2), 155–182. https://doi.org/10.1007/s10764-018-0031-0

Song, Q., Wang, Y., Huang, L., Shen, M., Yu, Y., Yu, Q., Chen, Y., & Xie, J. (2021). Review of the relationships among polysaccharides, gut microbiota, and human health. Food Research International, 140, 109858. https://doi.org/10.1016/j.foodres.2020.109858

Stensvold, C. R., & van der Giezen, M. (2018). Associations between Gut Microbiota and Common Luminal Intestinal Parasites. Trends in Parasitology, 34(5), 369–377. https://doi.org/10.1016/j.pt.2018.02.004

van der Linden, A., de Olde, E. M., Mostert, P. F., & de Boer, I. J. M. (2020). A review of European models to assess the sustainability performance of livestock production systems. Agricultural Systems, 182, 102842. https://doi.org/10.1016/j.agsy.2020.102842

Williams, A. R., Myhill, L. J., Stolzenbach, S., Nejsum, P., Mejer, H., Nielsen, D. S., & Thamsborg, S. M. (2021). Emerging interactions between diet, gastrointestinal helminth infection, and the gut microbiota in livestock. BMC Veterinary Research, 17(1), 62. https://doi.org/10.1186/s12917-021-02752-w

Worsley, S. F., Davies, C. S., Mannarelli, M.-E., Hutchings, M. I., Komdeur, J., Burke, T., Dugdale, H. L., & Richardson, D. S. (2021). Gut microbiome composition, not alpha diversity, is associated with survival in a natural vertebrate population. Animal Microbiome, 3(1), 84. https://doi.org/10.1186/s42523-021-00149-6

Yabsley, M. J., Murphy, S. M., & Cunningham, M. W. (2006). Molecular Detection and Characterization of Cytauxzoon felis and a Babesia Species in Cougars from Florida. Journal of Wildlife Diseases, 42(2), 366–374. https://doi.org/10.7589/0090-3558-42.2.366

Yoo, J., Groer, M., Dutra, S., Sarkar, A., & McSkimming, D. (2020). Gut Microbiota and Immune System Interactions. Microorganisms, 8(10), 1587. https://doi.org/10.3390/microorganisms8101587

4. Effect of landscape structure on the diversity and similarity of intestinal microbiota and protozoa between wild mammals and livestock in conservation areas in Colombia

Efecto de la estructura del paisaje en la diversidad y similitud de la microbiota y protozoarios intestinales entre mamíferos silvestres y ganado en áreas de conservación Colombia

Néstor Roncancio-Duque[1]*; Jeison Eduardo García-Ariza[1]; Nelson Rivera-Franco[2,3]; Juliana Peña Stadlin[4]; Diana López-Alvarez[1,2]*

[1] Facultad de Ciencias Agropecuarias, Grupo de Investigación en Diversidad Biológica. Universidad Nacional de Colombia, Sede Palmira. Colombia; njroncanciod@unal.edu.co, jeigarciaar@unal.edu.co, dilopezal@unal.edu.co

[2] Universidad del Valle, Facultad de Salud, Escuela de Ciencias Básicas, Grupo VIREM—Virus Emergentes y Enfermedad, Cali, Valle del Cauca, Colombia; rivera.nelson@correounivalle.edu.co, dilopezal@unal.edu.co

[3] Department of Neurology. Johns Hopkins School of Medicine. Maryland, United States; rivera.nelson@correounivalle.edu.co

[4] FZC Fundación Zoológica de Cali

* Correspondence: njroncanciod@unal.edu.co, dilopezal@unal.edu.co

4.1 Abstract

Concerning the gut microbiota, the hypothesis posits that increased microbial diversity contributes significantly to enhanced functionality across various processes associated with it. The intestinal microbiota exhibits high susceptibility to diverse forms of stress, and the impact of such stressors can be profound, affecting both its composition and function. A comprehensive understanding of how distinct forms of stress influence the intestinal microbiota is imperative for the development of strategies aimed at preserving gastrointestinal health and, consequently, the overall well-being of individuals. Conversely, parasites constitute integral components within natural processes that facilitate population regulation and maintain ecosystem balance. However, both natural and anthropogenic changes can disrupt these ecological processes. Among infectious diseases, those induced by protozoa are prominent contributors to human morbidity and mortality. Most protozoal diseases are zoonotic, and environmental changes exacerbate interactions among wildlife, domestic animals, and humans, thereby intensifying transmission rates between species. Thus, the objective of this study was to investigate the associations, including relationships and similarities, between landscape configuration influenced by some human activities and the diversity of intestinal microbiota, as well as the abundance of gastrointestinal protozoa, among wild and domestic mammals in Colombia. Taxonomic determination was achieved through metabarcoding with primers targeting the rRNA 18S gene and Nanopore sequencing, with a primary focus on detecting protozoa. A total of 148 samples were collected from six wildlife mammals and three domestic mammals across 29 focal landscapes in Colombia. Microscopy was also employed to validate certain agents. To describe the epidemiological landscape, taxa richness in gut microbiota, as well as the prevalence, mean intensity, and mean abundance of protozoa, were estimated. Bayesian Beta and Poisson regressions were employed to assess the relationship between landscape metrics and dissimilarity, gut and protozoa diversity, and the abundance of specific target protozoa. The study identified several don't documented protozoa species within the six assessed host species. A predominant proportion of phyla Ascomycota, Pseudomonadota, Basidiomicota, and Apicomplexa were observed, reflecting not only a healthy intestinal microbiota but also a potential predominance of certain negative elements. Comparisons between canines and equines, as well as between tapirs and bears among terrestrial mammals, indicated greater similarity in both gut microbiota and protozoa. In primates, the red howler monkey exhibited closer proximity to bovines and equines than to other primates. The findings indicated that a higher proportion of natural vegetation coverage correlated with

increased similarity in gut microbiota among wild and domestic mammals. Additionally, higher proportions of natural vegetation coverage, presence of water bodies, number of forest patches, and irregularities in forest shapes were associated with greater diversity (both richness and evenness) in gut microbiota and intestinal protozoa across different scales.

4.1.1 Key words: Protozoa, microbiota, gut, dissimilarity index, multiscale landscape structure

4.2 Introduction

Concerning the gut, it is postulated that greater microbial diversity contributes to enhanced functionality in various processes related to it (Danneskiold-Samsøe et al., 2019; Park et al., 2018). Gut microbiota plays a crucial role in breaking down complex compounds and releasing nutrients (Sauvaitre et al., 2021; Song et al., 2021). Notably, the presence of beneficial bacteria helps train the developing immune system, and a lack of diversity during this critical period may be linked to an increased risk of autoimmune diseases and allergies (Yoo et al., 2020). Additionally, the intestinal microbiota can modulate inflammatory responses and mitigate inappropriate immune reactions.

Specific bacterial strains influence fat storage and appetite regulation (Aron-Wisnewsky et al., 2021). Alterations in the microbiota have been observed to affect behavior and brain function, (Chen et al., 2021). A balanced microbiota serves as a barrier against harmful pathogens (Calo-Mata et al., 2016). It has been proposed that the microbiota may influence the endocrine system, affecting hormone production (O'Callaghan et al., 2016).

The intestinal microbiota is highly sensitive to various forms of stress, and such stressors can significantly impact its composition and function. Psychological stress has been associated with alterations in the diversity and balance of the microbiota. Moreover, physiological stress can also disrupt microbial homeostasis (He et al., 2021; Lange et al., 2016; Luo et al., 2021). Understanding how different forms of stress affect the intestinal microbiota is crucial for developing strategies that preserve gastrointestinal health and, consequently, the overall health of individuals.

Parasites are integral components of natural processes that could determine population regulation and ecosystem balance (Clayton & Moore, 1997; R. Delahay & Delahay, 2009). They have the potential to influence key population parameters such as birth and death rates (Nunn & Altizer, 2006). Certain mathematical models propose that parasites play a significant role in the

dynamics of host populations and evolution (Begon & Townsend, 2021). However, these ecological processes may be disrupted by both natural and anthropogenic changes. Furthermore, parasites could act synergistically with habitat reduction, poaching, and pollution, posing a threat to biodiversity conservation (Aguirre, 2009; Smith et al., 2009; Wisely et al., 2008). Parasites may also contribute to substantial economic losses due to decreased production, financial expenditures on control and treatment, and increased mortality in livestock, particularly cattle (Charlier et al., 2014; Rashid et al., 2019; Rodríguez-Vivas et al., 2017). In the face of potential environmental changes, parasites may alter their impact on wildlife, domestic animals, and human health by intensifying contact between hosts and infectious agents.

The severity of interactions with parasites is a significant concern for public health and national economies (Jenkins et al., 2015). Parasites represent a crucial biological factor for species conservation due to their adverse effects on health conditions, reductions in fitness, and increased mortality (Aguirre, 2009; Smith et al., 2009; Wisely et al., 2008). Among infectious diseases, those caused by protozoa are major contributors to human morbidity and mortality in tropical, subtropical, and temperate zones (Fletcher et al., 2012, Kohler, 2004). Livestock, in general, is considered unsustainable from social, economic, and environmental perspectives (Molina Benavides et al., 2019). In this context, cattle emerge as significant parasite reservoirs (Buret et al., 2019; Echeverría et al., 2019; Ryan et al., 2016). The three primary pathogenic protozoa worldwide are *Cryptosporidium* sp., *Giardia* sp., and *Entamoeba histolytica* (Xiao & Fayer, 2008). Indicators such as presence, prevalence, and richness are crucial in understanding how animals harbor infectious agents and regulate them. Additionally, these factors can elucidate the host's immunological condition (Muehlenbein, 2006).

However, comprehending the emergence and causes of the spread of infectious diseases is essential for controlling and mitigating their adverse impacts on health, ecology, and the economy (Barrett et al., 2008; Kohler, 2004). This knowledge is relevant given the ongoing trends of globalization and climate change (Jensen et al., 2010; Miller et al., 2015; Simon et al., 2013; VanWormer et al., 2014). The emergence and transmission of a disease within wildlife populations that interacts with humans or livestock can have drastic effects on the viability of wildlife species. This scenario increases the frequency of interspecific transmission of pathogens, significantly impacting human health and the sustainability of livestock (Daszak et al., 2000; Fisher et al., 2012; Schurer et al., 2016; Thompson, 2013).

Moreover, the ongoing rate of environmental changes, such as climate change, habitat loss, species introduction, pollution, and ecosystem disturbances, has modified the dynamics of pathogen colonization and prevalence (Acevedo-Whitehouse & Duffus, 2009; Becker et al., 2015; Fountain-Jones et al., 2017). These alterations typically amplify the contact among wildlife, domestic animals, and humans, intensifying the transmission rates between species, the emergence of diseases, and zoonotic events (Gale et al., 2009; Miller et al., 2015). Wildlife can potentially serve as a bidirectional conduit for infectious agents between humans and domestic animal populations. Furthermore, heightened interactions among the three components (human, domestic animal, and wildlife), propelled by urban and agricultural expansion, alongside changes in the structure and composition of biological communities, increase the likelihood of agents typically found in wildlife becoming infectious to humans or livestock. Conversely, some diseases adapted to domestic settings (including potentially drug-resistant strains) can impact wildlife, utilizing it as a reservoir before reemerging with unknown epidemiology (Aguirre, 2009; Deem, 2015; Keesing et al., 2010). In this context, a comprehensive prioritized analysis based on the "One Health" approach proves valuable for decision-making (Jenkins et al., 2015) and should be developed, considering informative proxies and indicators about the interactions between human, animal, and ecosystem health (Aguirre et al., 2002).

Mammals are an important group for epidemiological surveillance due to the impact that diseases can have on their endangered populations, and because of the risk of zoonotic transmission. As some primate species are used for bush meat, biomedical models, or, as pets, many mammals are in continuous close contact with humans, which increases the risk of cross-transmission and disease spread, highlighting the urgent need for mammal epidemiological surveillance (Artois et al., 2009; Chapman et al., 2005).

In this research, our objective is to investigate the associations (relationships and similarities) among livestock, landscape structure determined by some human-derived activities, and the diversity of gut microbiota, as well as the abundance of certain pathogenic gastrointestinal protozoa in wild mammals in some regions in Colombia. To achieve this objective, we conducted an epidemiological description of the evaluated populations, determining richness in intestinal microbiota at various taxonomic levels. We also estimated the prevalence, mean intensity, and mean abundance of the considered protozoa. We assessed the dissimilarity between the microbiota and gastrointestinal protozoa among the evaluated wild mammals and associated cattle.

Furthermore, we explored the relationship between landscape metrics and the dissimilarity of gut microbiota and protozoa between domestic animals and wildlife mammals. Lastly, we determined the relationship between landscape configuration, employing a multiscale approach, and the diversity of gut microbiota and protozoa, along with the mean abundance of *Cryptosporidium parvum* and *Giardia intestinalis*, serving as proxies for protozoa in *Alouatta seniculus*, as this wild mammal species had a sufficient sample size (focal landscape) for analysis. We anticipate that greater area and reduced fragmentation in forest will result in a higher diversity of intestinal microbiota and lower diversity of protozoa and a mean abundance of *Cryptosporidium parvum* and *Giardia intestinalis*.

4.3 Methods

4.3.1 Study Area

Samples were taken from 29 localities in ten departments (Figure 8). In two different sampling, one associated with a high mountain ecosystem in the Central Andes Mountain chain in which we take samples of *Leopardus* sp., *Puma concocolor*, *Tapirus pinchaque,* and *Tremarctos ornatus*, and the other in Primates in several localities in Colombia focused on *Alouatta seniculus* and *Saguinus leucopus* with some opportunistic samples of *Aotus* species. To determine the relationship between landscape metrics and dissimilarity of the gut microbiota and protozoa between domestic animals and mammal wildlife, analyses were conducted on 11 focal landscapes covering 224 km^2 each. This area of focal landscapes corresponded to those where we got amplified samples for at least one sample of the three included species of domestic mammals and one sample of the eight wildlife mammals sampled. To determine the relationship between the local landscape features and the severity of human activities with the diversity of gut microbiota and protozoa, and the mean abundance of the gastrointestinal protozoa, that was done using a biological model *Alouatta seniculus* because it has enough focal landscape samples to run regression model with deplete the degrees of freedom, the multiscale approach was used, begging from 50 ha multiplying by two each scale until 6400 ha (eight scales). The samples were nested as the area of the local landscape expanded. The initial size was determined considering the mean home range for this species (Bustamante-Manrique, 2023). The definition of the focal landscape size in landscape epidemiology is crucial for determining fragmentation statistics that are relevant for understanding epidemiological processes in a specific area (Jackson & Fahrig,

2012). Several criteria must be considered for this determination. Firstly, it is essential to consider the ecological dimensions of populations and the dynamics of the pathogens at play, suggesting the need for a scale that realistically captures interactions. Additionally, accessibility and topographical characteristics of the study area must be considered, as they can influence the spread of diseases. The spatial resolution of available data and monitoring capacity are also determining factors in choosing the size of the focal landscape (Lausch, 2002). Therefore, it is necessary to adapt the scale of the focal landscape to the specific conditions of the epidemiological system in question, ensuring the relevance of fragmentation statistics for disease analysis at the local level (Ostfeld et al., 2005).

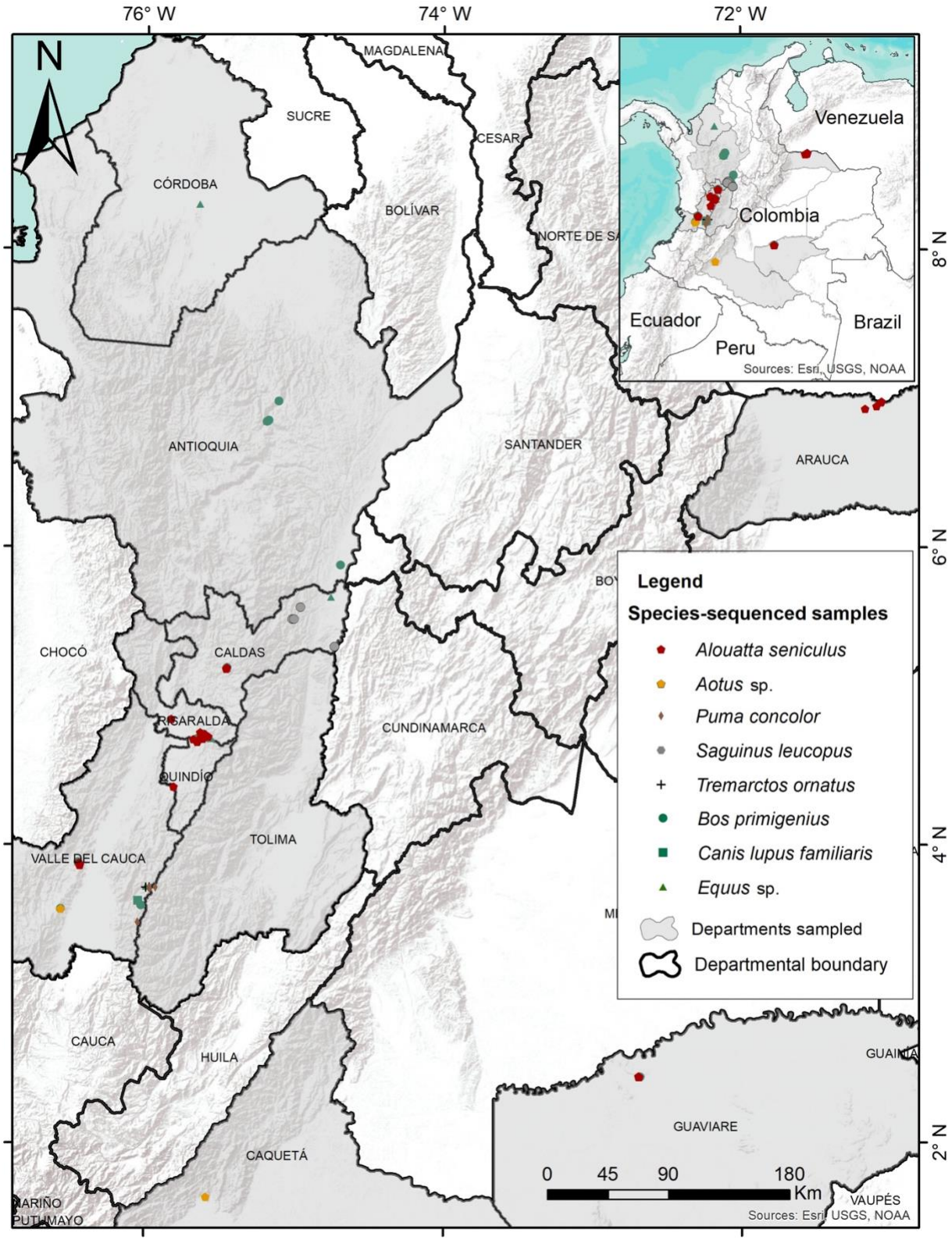

76° W
74° W
72° W
N
MAGDALENA
SUCRE
CESAR
CÓRDOBA
BOLÍVAR
NORTE DE SA
ANTIOQUIA
SANTANDER
ARAUCA
CHOCÓ
CALDAS
RISARALDA
CUNDINAMARCA
QUINDÍO
TOLIMA
VALLE DEL CAUCA
CAUCA
HUILA
CAQUETÁ
GUAINIA
GUAVIARE
VAUPÉS
NARIÑO
PUTUMAYO
8° N
6° N
4° N
2° N
Venezuela
Colombia
Ecuador
Peru
Brazil
Sources: Esri, USGS, NOAA
Legend
Species-sequenced samples
Alouatta seniculus
Aotus sp.
Puma concolor
Saguinus leucopus
Tremarctos ornatus
Bos primigenius
Canis lupus familiaris
Equus sp.
Departments sampled
Departmental boundary
0 45 90 180
Km
Sources: Esri, USGS, NOAA

Figure 8. Study area.

4.3.2 Collection of Fecal Samples and Storage

One hundred forty-eight fecal samples were collected from eight species of wildlife mammals and three domestic mammals in ten departments of Colombia (Table 10). The number of samples corresponds to the population in each site of each species, assumed as an epidemiological unit—understood as an entity or group of individuals studied in a specific epidemiological context (Jakob-Hoff et al., 2014). In this sense, a sample could be representative of the unit. Each sample was deposited in a lysis buffer composed of 0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, and 0.5% SDS at pH 8 (Longmire et al., 1997). The samples were transported at room temperature to the laboratory and frozen at -20°C until extraction. Most samples were frozen between eight and 20 days after collection. The time from collection to extraction varied between samples.

Table 10. Host species and departments sampled.

Department	Antioquia	Arauca	Caldas	Caquetá	Cordoba	Guaviare	Quindío	Risaralda	Tolima	Valle	Total
A. seniculus	5	4	8		3	4	13	6		6	49
A. griseimembra			1								1
A. lemurinus										1	1
A. vociferans				1							1
B. primigenius	7		7	1	3	1	4	2		6	31
C. l. familiares										2	2
Equus sp.	4		4		1	1	1	4	1	5	21
Leopardus sp.										3	3
P. concolor									2	4	6
S. leucopus			16								16
T. pinchaque									3	1	4
T. ornatus									2	11	13
Grand Total	16	4	36	2	7	6	18	12	8	39	148

4.3.3 DNA Extraction and 18S rRNA Library Preparation

For DNA extraction, the commercial Kit DNeasy PowerSoil Pro® Kit was used following the manufacturer's instructions with minor differences (addition of 5 minutes of stirring to mix the sample with the first component of the kit). The extracted DNA was diluted in 50 µl of kit resuspension solution. The final yield and quality of extracted DNA were determined by Colibrí (Titertek Berthold) using 2 µl of DNA. Pure genomic DNA is indicated by an A260/A280 nm ratio between 1.8 and 2.0, while between 1.6-1.8 are acceptable, and any value less than 1.6 indicates contamination. DNA integrity was determined by visualizing 2 µL of extracted DNA on 1% agarose gel (w/v) containing GelRed (Biotium®). Subsequently, for the viability assessment of genomic DNA in molecular characterization studies, the 18S rRNA gene was targeted using primers G3F1 and G3R1 G3F1 (5' –GCCAGCAGCCGCGGTAATTC-3) and primer G3R1 (5' –ACATTCTTGGCAAATGCTTTCGCAG-3) (Krogsgaard et al., 2018). The protozoa targets were the agents including in Apicomplexa, Tricomonadida, Trypanosomatidae, *Paramecium*, and *Giardia* clades. Amplifications were done by endpoint polymerase chain reaction (PCR) using the Biorad CFX96 C1000 thermal cycler (Roncancio et al. 2024).

For visualization of the amplicons, 1.4% agarose gels were prepared and stained with GelRed. The gels of the total DNA and the amplicons were visualized in a gel photodocumenter (Nippon Genetics, FastGene FAS V model). Amplicon concentration was determined fluorometrically on the Qubit® 3.0 Fluorometer using the QubitTM dsDNA HS Assay Kit following the manufacturer's instructions (Freed & Silander, 2020).

4.3.4 Oxford Nanopore Sequencing and Bioinformatics

For sequencing the approximately 500 bp-long amplified regions, the MinION MK1B sequencing platform was utilized with the MinKNOW software. The sequencing library was prepared with the Ligation Sequencing Kit SQK-LSK109 and the Native Barcoding Expansion Kit 96 (EXP-NBD196). The library pool was sequenced on an R-9.4.1 flow cell for 48 hours. Raw FAST5 files produced were base called under high-accuracy mode using the ONT base caller Guppy v6.2.1. The FASTQ files were used for taxonomic assignment with Kraken2 and the PlusPFP database (3/14/2023). The results were subsequently filtered excluding clades with less than five reads and the Viridiplantae clade, corresponding to green plants, assuming that the presence of this clade was derived primarily from the diet, including water consumption.

4.3.5 Direct Observation: microscopy (Supplement information 4)

To verify forms compatible with the parasite target species intended for diagnosis, direct observation analyses were conducted using microscopy. This method also allowed the identification of forms compatible with other agents not diagnosable with the molecular markers used, providing additional information. Their utility for interaction and risk analyses is limited, as with these methodological approaches, most potential agents can only be determined at the genus level. The samples were collected in a 10% formalin fixing solution (PFA) and upon arrival at the laboratory, they were stored at a temperature of 7 degrees Celsius. For parasitological diagnosis, the following approaches were employed:

Direct Coprology: A drop of saline solution and another Lugol was applied to a microscope slide. An amount equivalent to the tip of a wooden applicator was added to both sides of the slide. Subsequently, homogenization was performed, and coverslips were applied.

Microscopic examination was conducted using an optical microscope at 5X magnification, with an assessment of all fields, 10X with an evaluation of all fields, and 40X with an evaluation of 40 fields. Special attention was given to suspicious structures during assessments at lower magnification.

Sheather Solution Flotation (Saturated Sucrose): The ovu-check® device was utilized. One gram of fecal matter was placed on the bottom lid, and the solution was added until reaching 1/3 of the container. The sample was homogenized with a wooden applicator, and a filter was added. The remaining solution was then added to complete the remaining 2/3. An embolus was left on the surface, and the solution was allowed to settle for 20 minutes. Afterward, it was covered with a cover slip. After 1 minute, the cover slip was removed, placed on a microscope slide, and read using the same methodology employed in direct coprology.

Ziehl-Neelsen: Specifically, to protozoa, the traditional method Ziehl-Neelsen was used, one the slide was mounted with the sample, we allowed the slide to air-dry at room temperature. Subsequently, once dry, it is heat-fixed using a hot plate for approximately 2 – 3 minutes. Direct flame fixation is avoided to prevent sample degradation. While the slide remains on the hot plate, phenol fuchsin is added for 5 minutes until vapor emission is observed. It is important not to let it boil, and additional staining is performed gradually if the dye appears to be drying. After 5 minutes, the slide is removed from the hot plate using forceps, and the dye is discarded into a container

for chemical waste. Rinsing with R.O water is performed without direct water flow onto the sample until no color is observed in the effluent. Excess water is then removed. Subsequent decolorization is achieved using acid-alcohol, followed by another wash with R.O water. Finally, Loeffler's methylene blue is added as a contrasting dye for 20 seconds to 1 minute.

4.3.6 Estimation of Covariates

Sustainable development in agriculture, forestry, and fisheries sectors, as defined as, conserving land, water, plant, and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable, and socially acceptable. The dimensions of sustainability—economic viability for farmers, environmental soundness, and social acceptability—have several indicators (Lovarelli et al., 2020; van der Linden et al., 2020). In this case, these indicators are associated with response variables related to the health status of livestock and its potential impact on wildlife. In this work, we focused on sustainability indicators related to the environmental dimension and based on land cover. We considered that less natural land cover is associated with more effects of size, isolation, and border, making livestock less sustainable. Covariates included landscape statistics related to land use coverages, bodies of water, roads, climatic variables, and altitude (Arce-Peña et al., 2019). Initially, we considered 15 candidate covariates (Table 11).

Table 11. Initial covariates selected to evaluate the effect of the landscape configuration on the response variables defined.

Covariate
Distance to tracks (km)
Elevation (m)
Forest area (km^2)
Forest Mean Patch Size
Forest Mean Shape Index
Forest Number Patches
Habit (Arboreal=0, Terrestrial=1)

Number of samples
Pastures areas (km^2)
Precipitation (m^3)
River (km)
Temperature (^oC)
Urbanized zones (km^2)
Weigthed Cover Index (WCI)
Wildlife number of samples/Domestic number of samples

We introduced the Weighted Cover Index (WCI) to integrate an index that allowed us to measure the land cover composition and structure, not only the land cover structure as SI (Fahrig et al., 2011). To estimate the WCI, we qualified class types as follows (Table 12), based on the quality of wildlife mammals, if more mature or less transformed cover, best quality.

Table 12. Quality multipliers of land cover to estimate the Weighted Cover Index (WCI)

Cover	Quality Multiplier
1.1.1. Continuous urban fabric	1
1.1.2. Discontinuous urban fabric	1
1.2.1. Industrial or commercial areas	1
1.2.2. Road, railway, and associated land	1
1.2.4. Airports	1
1.2.5. Hydraulic works	1
1.3.1. Mining extraction areas	1
1.4.2. Recreational facilities	1
2.1.1. Other transitory crops	3
2.1.2. Cereals	3
2.2.1. Herbaceous permanent crops	3

2.2.2. Shrub permanent crops	3
2.2.3. Tree permanent crops	3
2.3.1. Clean pastures	2
2.3.2. Tree-covered pastures	2
2.3.3. Weedy pastures	2
2.4.1. Crop mosaic	3
2.4.2. Mosaic of grass and crops	2.5
2.4.3. Mosaic of crops, grass, and natural spaces	3.5
2.4.4. Mosaic of grass with natural spaces	3
2.4.5. Mosaic of crops with natural spaces	3.5
3.1.1. Dense forest	10
3.1.2. Open forest	9
3.1.3. Fragmented forest	8
3.1.4. Gallery and riparian forest	7
3.1.5. Forest plantation	3
3.2.1. Herbaceous vegetation	4
3.2.2. Shrubland	5
3.2.3. Secondary or transitional vegetation	6
3.3.1. Natural sandy areas	1
3.3.3. Bare and degraded lands	1
3.3.4. Burned areas	1
4.1.1. Swamp areas	1
4.1.3. Aquatic vegetation on water bodies	1
5.1.1. Rivers	1
5.1.2. Natural lagoons, lakes, and marshes	1
5.1.4. Artificial water bodies	1

The landscape index is calculated by multiplying the assigned value of each class type (e.g., dense forest, pasture) by its relative area within the focal landscape. These products are then summed to obtain the final index value. Consequently, landscapes dominated by classes with high assigned values (e.g., dense forest) will have higher index values (close to ten), while landscapes with a greater proportion of classes assigned lower values (e.g., pasture and crops) will have lower index values (between two and three) (Roncancio Duque, 2021).

To determine the relationship between landscape metrics and dissimilarity of the gut microbiota and protozoa between domestic and wildlife mammals the covariates were estimated to the each of 224 km^2 of focal landscape considering this objective. This focal landscape size was defined based on the largest area that included samples of wild and domestic species from the localities included. To determine the relationship between the focal landscape features and the severity of human activities with the diversity of gut microbiota and protozoa, and occurrence probability (mean abundance) of the gastrointestinal protozoa in *A. seniculus*, the covariates were estimated to each focal landscape to each one of the eight-scale analyzed. Here we do not consider habit and the relation of the number of the samples between domestic and wildlife mammals because are not relevant, and the precipitation and temperature because the resolution of the pixel is not accurate to this scale of analysis. The information used to estimate the covariates were the natural cover information 1:100.000 scale, Digital elevation model, raster of temperature, precipitation, and shapes of simple drainage to the river and way (Fick & Hijmans, 2017; IDEAM, 2021; IDEAM (Instituto de Hidrología, 2017). The geoprocessing -clips, layer intercepts, zonal statistics- was done using ArcGIS 10.7.1, and the landscape and cover metrics were estimated using Patch Analyst - Analysis by Region tools.

4.3.7 Statistical Analysis

An initial descriptive analysis was conducted to show the diversity based on specific richness at various taxonomic levels from species to phylum. Furthermore, for the considered protozoa, including the clades Apicomplexa, Tricomonadida, Trypanosomatidae, *Paramecium*, and *Giardia*, prevalence, mean intensity, and mean abundance were estimated. Prevalence, denoting the proportion of cases of a specific disease or condition in a population at a given point in time, is typically expressed as a percentage. It represents the total number of cases relative to the total size of the at-risk population. Mean intensity refers to the average burden of a disease in infected

individuals within a population, expressed in terms of the quantity of pathogens present. This measure aids in understanding the average severity of the disease in the affected population. Mean abundance, on the other hand, signifies the average quantity of pathogens present in all individuals, whether infected or not, in a population. This measure provides information about the total burden of pathogens in the population, irrespective of their infection status (Ostfeld et al., 2005).

To determine the dissimilarity between the microbiota and gastrointestinal protozoa among evaluated wild mammals and associated domestic counterparts, cluster dendrograms were constructed, and Bray-Curtis dissimilarity indices were estimated, considering taxa with more than five reads in the sample. Cluster analysis serves to evaluate the similarity between samples based on species composition. A common method involves using the Bray-Curtis index to quantify dissimilarity between assemblages. This index considers the presence and relative abundance of species in two samples, yielding a value ranging from 0 (identical assemblages) to 1 (completely different assemblages). By applying cluster analysis techniques to the Bray-Curtis dissimilarity matrix, samples with higher similarity are grouped, revealing patterns of assemblage structuring. To construct the dendrogram, three methods—single, average, and complete—were employed, and the optimal dendrogram was selected using the cophenetic distance method. This distance is calculated as the distance between two objects within the same cluster in the dendrogram. The distance from the first object to the second object passing through the joining node of the two objects is the cophenetic distance. A cophenetic matrix is a matrix that represents the cophenetic distances between all pairs of objects. This matrix can be correlated with the original dissimilarity matrix. The method with the highest cophenetic correlation can be regarded as having produced the best clustering model for the distance matrix (Borcard et al., 2011).

To determine the relationship between focal landscape features and the diversity of gut microbiota and protozoa, the mean abundance of target gastrointestinal protozoa detected in *A. seniculus Cryptosporidium parvum* and *Giardia intestinalis*, richness, and the inverse Simpson index were used as response variables. The analyses were performed using the Vegan package in R (Oksanen et al., 2013).

The relationship was assessed using simple beta regression methods for the dissimilarity index and simple Poisson regression for richness, inverse Simpson index, and mean abundance. Initially, a correlation test was conducted among all covariates to eliminate collinearity (autocorrelation). The Spearman coefficient of correlation was employed for this evaluation after

standardizing all covariates, given that the covariates were not normal and homoscedastic. These analyses were executed in the R program, specifically utilizing the PerformanceAnalytics package and Rcmdr (Fox et al., 2015; Peterson et al., 2018). When two covariates exhibited association (Spearman rank correlation coefficient: P-rs <0.05), one of them was excluded (Sokal et al., 1995). The final variables selected were those not associated with others. Consequently, for the dissimilarity regression, eight out of the 15 covariates were considered (Habit, WCI, Forest MSI, Forest NumP, Pastures area, Urbanized zones area, River Length, and Elevation). For the richness, inverse Simpson index, and mean abundance regression, three to five covariates were considered (Supplement information 1).

A Bayesian approach was employed, utilizing an uninformative prior distribution for the precision of the explanatory variable effects and an uninformative prior distribution for the intercept (alpha) and slopes (beta). It was assumed that the posterior distributions of the intercept and the slope of each variable followed a normal distribution (McCarthy, 2007; Pfeiffer et al., 2008). Interactions (multiplicative effects) between the explanatory variables were not considered. To select the most parsimonious model, the Deviance Information Criterion (DIC) was employed. The estimation of intercepts and slopes was performed using Markov chains with 100,000 iterations, considering iterations from 10,001 to the final estimation. The DIC was estimated with an additional 100,000 iterations. The analyses were conducted using OpenBugs 3.2.2 software (Spiegelhalter et al., 2018).

4.4 Results

4.4.1 General gut microbiota and protozoa diversity

Sequences were obtained from 73 out of 148 collected samples (Table 13). To *A. seniculus* the classification percentage ranged from 20.8% to 95%. For *S. leucopus*, the determination percentage ranged from 56.1% to 99.6%, with five out of six samples above 94.6%. The *Aotus* species samples had a classification percentage of 74.9 and 86.4%. The two samples of *T. ornatus* were 88.8 and 94.1 % of the classification percentage. *P. concolor* was from 88.1 and 97.8% and the two samples of *T. terrestris* were 58 and 94 %. For bovines, the classification percentage ranged from 45.9% to 99.1%, with 10 out of 16 samples having determinations above 90%. For equines, the determination percentage ranged from 53.6% to 99.7%, with 12 out of 13

samples above 93%. The canine sample had a classification percentage of 99.5% (Supplement information 2).

Table 13. Proportion of domain represented in the sequenced samples by host species.

Host/Domain	Archaea	Bacteria	Eukaryota	Viruses	Sequenced samples
A. lemurinus		53.93	46.04		1
A. seniculus	0.19	36.85	62.73	0.21	27
A. vociferans		16.46	83.54		1
B. primigenius	0.02	2.89	97.08	0.01	16
C. l. familiaris		31.44	68.56		1
Equus sp	0.01	0.76	99.21	0.01	13
O. leucopus		12.79	87.10	0.11	4
P. concolor		3.43	96.51	0.06	6
T. ornatus		0.55	99.43	0.02	2
T. pinchaque		10.26	89.74		2

The diversity distribution to each taxonomic level of the detected organisms in each host species is in Table 14.

Table 14. Gamma diversity (taxa richness) by host species to different taxonomic levels and some agent species with more than five reads used for epidemiological analyses (Supplement information 3).

Species	**Phylum**	**Classes**	**Order**	**Family**	**Genus**	**Species**
A. lemurinus	7	12	12	13	10	11
A. seniculus	40	76	143	287	571	693
A. vociferans	3	6	6	6	3	4

B. primigenius	25	50	78	111	133	150
C. l. familiaris	11	26	33	43	52	61
Equus sp.	23	48	88	141	174	145
S. leucopus	10	23	29	35	40	36
P. concolor	8	21	29	36	35	43
T. ornatus	9	22	32	43	44	49
T. pinchaque	11	25	41	61	64	70

In the case of *A. seniculus*, the main phyla observed were Apicomplexa, Ascomycota, and Pseudomonadota (Figure 9), with the most abundant protozoa identified as *Toxoplasma gondii*, *Plasmodium vivax*, *Babesia microti*, and *Plasmodium rectilineum* (Table 15). Conversely, *S. leucopus* exhibited Ascomyota and Pseudomanadota as prevailing phyla, hosting *Babesia microti* and *Toxoplasma gondii* as the most frequently detected protozoa. *A. lemurinus* displayed Pseudomonadota, and Ascomicota, with protozoa such as *Babesia bovis* and *Toxoplasma gondii* being the most represented in the sample. Similarly, the intestinal microbiota of *A. vociferans* featured Ascomicota, Campylobaterota, and Pseudomonadota as predominant phyla (Figure 10) (Supplement information 3).

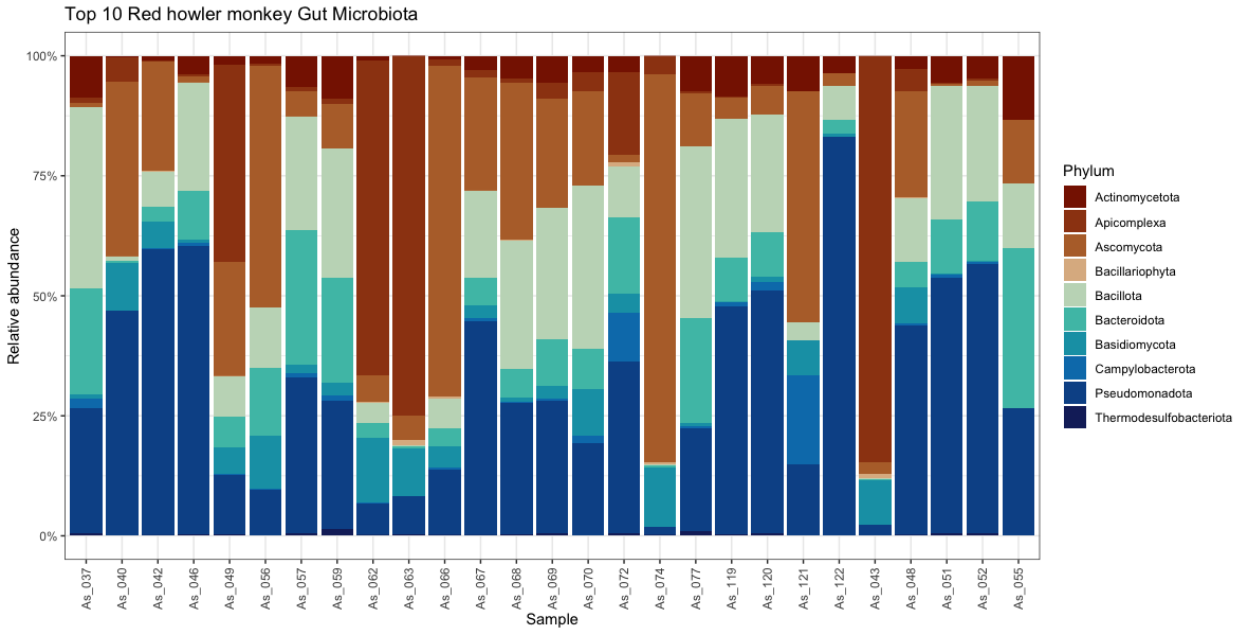


Figure 9. Phylum relative abundance to *Alouatta seniculus (As).*

Table 15. Epidemiological descriptors of protozoa to *Alouatta seniculus*

Species	Prevalence			Mean intensity			Mean abundance		
	Mean	LLPI	ULPI	Mean	LLPI	ULPI	Mean	LLPI	ULPI
Babesia bigemina	0.34	0.19	0.52	4	3	5	1	1	2
Babesia bovis	0.41	0.24	0.59	20	17	22	8	7	9
Babesia microti	0.59	0.41	0.76	51	48	55	30	28	33
Besnoitia besnoiti	0.41	0.24	0.59	9	8	11	4	3	5
Cryptosporidium parvum	0.34	0.19	0.52	5	4	7	2	1	2
Giardia intestinalis	0.17	0.06	0.33	1	1	3	0	0	0
Leishmania mexicana	0.24	0.11	0.41	1	0	2	0	0	0
Neospora caninum	0.28	0.13	0.45	2	1	3	0	0	1
Plasmodium berghei	0.28	0.13	0.45	1	1	2	0	0	1
Plasmodium chabaudi	0.38	0.22	0.56	5	3	6	2	1	2
Plasmodium coatneyi	0.31	0.16	0.49	4	3	6	1	1	2
Plasmodium cynomolgi	0.28	0.13	0.45	1	1	2	0	0	1
Plasmodium falciparum	0.31	0.16	0.49	1	0	2	0	0	1
Plasmodium malariae	0.45	0.27	0.63	3	2	4	1	1	2
Plasmodium reichenowi	0.28	0.13	0.45	1	0	2	0	0	0
Plasmodium relictum	0.55	0.37	0.72	30	27	33	17	15	18
Plasmodium sp. gorilla clade G2	0.24	0.11	0.41	2	1	3	0	0	1
Plasmodium vinckei	0.34	0.19	0.52	3	2	4	1	1	1
Plasmodium vivax	0.72	0.55	0.87	14	12	15	10	9	11
Plasmodium yoelii	0.41	0.24	0.59	2	2	3	1	1	1
Theileria annulata	0.24	0.11	0.41	1	0	2	0	0	0
Theileria equi	0.41	0.24	0.59	260	250	270	106	102	110

Theileria orientalis	0.52	0.34	0.69	5	4	7	3	2	3
Toxoplasma gondii	0.72	0.55	0.87	230	224	237	170	166	175
Trichomonas vaginalis	0.62	0.44	0.79	3	2	4	2	1	2
Trypanosoma brucei	0.14	0.04	0.28	2	1	4	0	0	0

LLPI = Lower limit probability Interval, ULPI = Upper limit probability Interval, Colors highlighted biggest values in each parameter, red first, orange second, green third, and blue fourth.

To *A. lemurinus* just we found the genome of *Babesia bovis* with 18 reads, *Babesia microti* 1, *Theileria orientalis* 1, and *Toxoplasma gondii* 8. To *A. vociferans* we did not find protozoa. To *S. leucopus* we found genome of *Trypanosoma brucei, Trichomonas vaginalis, Plasmodium coatneyi, P. relictum, P. falciparum, P. malariae, P. cynomolgi, P. vivax, P. chabaudi, Babesia microti, B. bigemina, B. bovis, Theileria orientalis, T. equi, Neospora caninum, Toxoplasma gondii. To B. microti* prevalence was 0.43 (PI95%= 0.23 - 0.64), mean intensity 2.88 (PI95%= 1.83 - 4.165), mean abundance 1.21 (PI95%= 0.768 - 1.758), *T. gondii* prevalence 0.62 (PI95%= 0.41 – 0.81), mean intensity 5.17 (PI95%= 3.96 - 6.54) and mean abundance 3.26 (PI95%= 2.5 - 4.13).

Furthermore, *T. ornatus* and *P. concolor* showcased a diversity of phyla in the intestinal microbiota, primarily represented by Ascomicota, Apicomplexa, and Basidiomycota (Figure 10). Genomic protozoa including *Theileria equi*, *Theileria orientalis*, *Toxoplasma gondii*, *Babesia bigemina*, and *Plasmodium rectilineum* were prevalent (

In bovine and equine samples, Ascomycota, Basidiomycota, and Apicomplexa were notably represented. Within the intestinal microbiota phyla, *Toxoplasma gondii*, *Theileria* species, and *Babesia* exhibited the highest values in terms of prevalence, mean intensity, and mean abundance. In the sole sample from *Canis lupus familiaris*, the most representative phyla included Cyanobacteriota, Ascomycota, Basidiomycota, and Pseudomonadota, with the highest abundances recorded for *Theileria orientalis*, *Toxoplasma gondii*, and *Babesia microti* (Chapter 3).

Table 16, Table 17). *T. pinchaque*, on the other hand, presented the phyla Ascomicota, Pseudomonadota, and Apicomplexa (Figure 10), with the common protozoa being *Theileria orientalis*, *Theileria equi*, *Toxoplasma gondii*, and *Babesia microti* in its samples (Table 18).

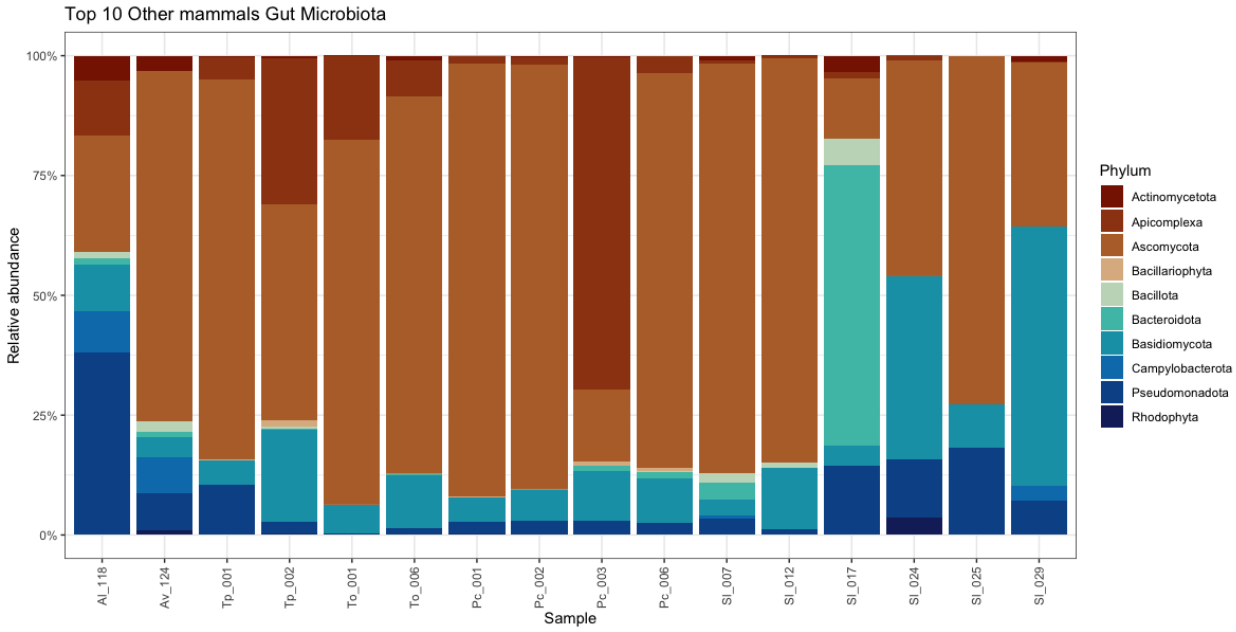


Figure 10. Phylum relative abundance to other host wild mammals. Al: *Aotus lemurinus*, Av: *Aotus vociferans*, Tp: *Tapirus pinchaque*, To: *Trematos ornatus*, Pc: *Puma concolor*, Sl: *Saguinus leucopus*

In bovine and equine samples, Ascomycota, Basidiomycota, and Apicomplexa were notably represented. Within the intestinal microbiota phyla, *Toxoplasma gondii*, *Theileria* species, and *Babesia* exhibited the highest values in terms of prevalence, mean intensity, and mean abundance. In the sole sample from *Canis lupus familiaris*, the most representative phyla included Cyanobacteriota, Ascomycota, Basidiomycota, and Pseudomonadota, with the highest abundances recorded for *Theileria orientalis*, *Toxoplasma gondii*, and *Babesia microti* (Chapter 3).

Table 16. Epidemiological descriptors of protozoa to *Tremarctos ornatus*

	Prevalence			Men intensity			Mean abundance		
Species	Mean	LLPI	ULPI	Mean	LLPI	ULPI	Mean	LLPI	ULPI
Babesia bigemina	0.50	0.10	0.91	20	12	30	10	6	15
Babesia microti	0.75	0.29	0.99	10	7	15	10	7	15

Cryptosporidium parvum	0.75	0.29	0.99	4	2	7	4	2	7
Plasmodium relictum	0.75	0.29	0.99	4	2	7	4	2	7
Plasmodium vivax	0.75	0.29	0.99	4	2	7	4	2	7
Theileria equi	0.75	0.29	0.99	1069	1024	1115	1069	1024	1115
Theileria orientalis	0.75	0.29	0.99	262	240	284	262	240	284
Toxoplasma gondii	0.75	0.29	0.99	261	240	285	261	240	285

Table 17. Epidemiological descriptors of protozoa to *Puma concolor*

Species	Prevalence			Mean intensity			Mean abundance		
	Mean	LLPI	ULPI	Mean	LLPI	ULPI	Mean	LLPI	ULPI
Babesia microti	0.83	0.48	0.99	6.25	4.06	8.93	6.25	4.06	8.93
Besnoitia besnoiti	0.50	0.15	0.85	3.50	1.41	6.54	1.75	0.71	3.26
Plasmodium relictum	0.33	0.05	0.72	13.98	7.65	22.14	3.50	1.91	5.58
Theileria equi	0.50	0.15	0.85	6.00	3.10	9.83	3.00	1.55	4.93
Theileria orientalis	0.67	0.29	0.95	3.67	1.83	6.16	2.75	1.38	4.61
Toxoplasma gondii	0.83	0.48	0.99	11.00	7.99	14.48	11.00	7.99	14.48

Table 18. Epidemiological descriptors of protozoa to *Tapirus pinchaque*

Species	Prevalence			Mean intensity			Mean abundance		
	Mean	LLPI	ULPI	Mean	LIPI	ULPI	Mean	LIPI	ULPI
Babesia microti	0.75	0.29	0.99	9	5	14	9	5	14
Theileria equi	0.75	0.29	0.99	57	47	69	57	47	69
Theileria orientalis	0.75	0.29	0.99	738	701	776	738	701	776
Toxoplasma gondii	0.75	0.29	0.99	37	29	46	37	29	46

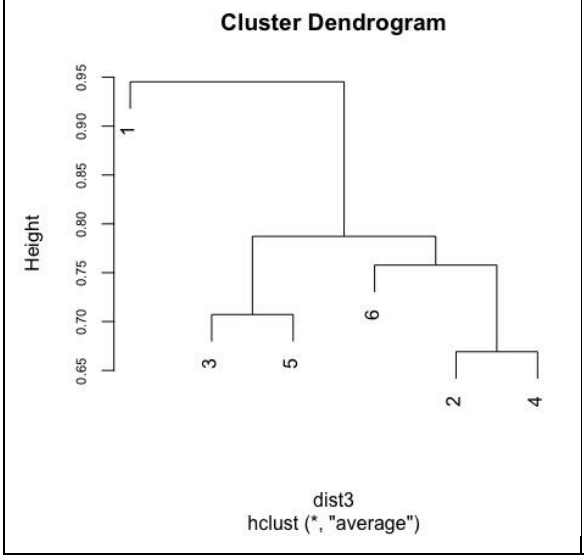
Table 19. Parasites were confirmed and additional records were by microscopy (Supplement information 4).

Kingdom	Phylum	Class	Order	Family	Species	*Bos primigenius*	*Equus sp*	*T. ornatus*	*T. pinchaque*	*A. seniculus*	*Leopardus sp.*
Animal	Cestodo					1					
Animal	Nematoda	Adenophorea	Enoplida	Capillariidae	*Capillaria* sp.						1
Animal	Nematoda	Adenophorea	Trichurida	Trichuridae	*Trichuris* sp.		1				
Animal	Nematoda	Chromadorea	Oxyurida	Oxyuridae	*Enterobius* sp.					1	
Animal	Nematoda	Chromadorea	Oxyurida	Oxyuridae	*Oxyuris* sp.		1				
Animal	Nematoda	Chromadorea	Oxyurida	Oxyuridae			1	1			
Animal	Nematoda	Chromadorea	Rhabditida	Strongylidae	Strongyloides sp.	1	1	1		1	1
Animal	Nematoda	Secernentea	Ascaridida	Ascarididae				1		1	1
Animal	Nematoda						1	1	1	1	
Fungi	Ascomycota	Hemiascomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	1	1				
Protist	Amoebozoa	Archamoebea	Mastigamoebida	Entamoebidae	*Entamoeba* sp.	1	1	1	1	1	1
Protist	Amoebozoa	Archamoebea	Mastigamoebida	Entamoebidae	*Iodamoeba* sp.	1					
Protist	Amoebozoa	Archamoebea	Mastigamoebida	Mastigamoebidae	*Endolimax nana*	1	1				
Protozoa	Bigyra	Blastocystea	Blastocystida	Blastocystea	Blastocystis sp.					1	
Protozoa	Metamonada	Fornicata	Diplomonadida		*Giardia sp*	1		1		1	1
Protozoa	Metamonada	Parabasalia	Trichomonadida		*Trichomonas* sp.					1	
Protozoa	Miozoa: Apicomplexa	Coccidiasina				1	1	1	1	1	
Protozoa	Miozoa: Apicomplexa	Conoidasida	Eucoccidiorida	Cryptosporidiidae	*Cryptosporidium* sp					1	
Protozoa	Miozoa: Apicomplexa	Conoidasida	Eucoccidiorida	Eimeriidae	*Isospora sp.*	1					
Protozoa								1	1		

4.4.2 Dissimilarity

High mountain

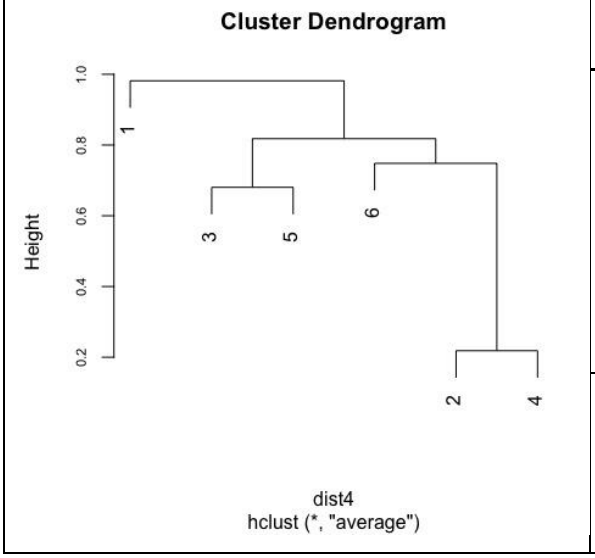
The diversity of gut microbiota among wild and domestic species in the central Andes revealed greater similarity between domestic canines and equines, sharing a 33% similarity. Closest to these, the puma exhibited a 19% similarity in intestinal microbiota with dogs and a 29% similarity with horses and mules (Figure 11). *T. pinchaque* primarily shared its intestinal microbiota with bears at a 29% similarity. The most distant organism in this landscape, in terms of intestinal microbiota, was the domestic bovine, with a maximum similarity of only 13% with the puma. Regarding protozoa, a similar pattern emerged, with a 78% similarity between domestic canines and equines, while similarities decreased among other species (Figure 12).



Bray curtis dissimilarity	Bp_ 1	Cf_ 2	Tp_ 3	Eq_ 4	To_ 5
Cf_2	0.97				
Tp_3	0.97	0.82			
Ec_4	0.95	0.67	0.73		
To_5	0.98	0.84	0.71	0.76	
Pc_6	0.87	0.81	0.74	0.71	0.84

Bp= *Bos primigenius*, Cf= *Canis lupus familiaris*, Tp= *Tapirus pinchaque*, Eq= *Equus* sp. To= *Tremarcots ornatus*, Pc= *Puma concolor*

Figure 11. Cluster dendrogram and matrix to gut microbiota dissimilarity between wild and domestic mammals in high mountain sampling.



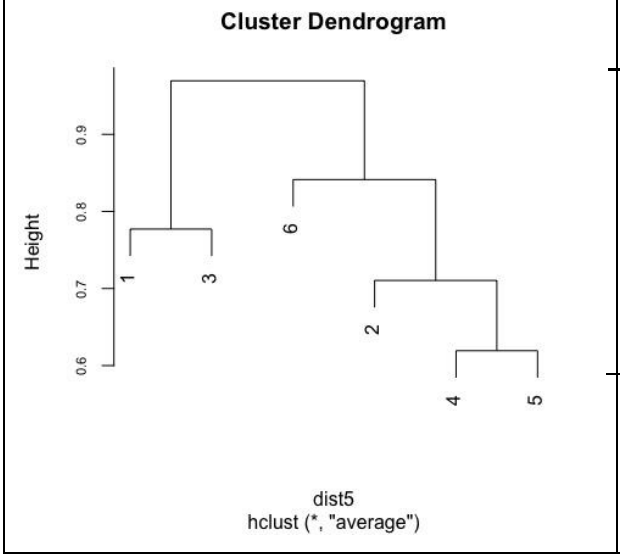
Bray curtis dissimilarity	Bp_1	Cf_2	Tp_3	Eq_4	To_5
Cf_2	0.98				
Tp_3	1.00	0.74			
Ec_4	0.99	0.22	0.65		
To_5	1.00	0.84	0.68	0.77	
Pc_6	0.94	0.74	0.94	0.76	0.96

Bp= *Bos primigenius*, Cf= *Canis lupus familiaris*, Tp= *Tapirus pinchaque*, Eq= *Equus* sp. To= *Tremarctos ornatus*, Pc= *Puma concolor*

Figure 12. Cluster dendrogram and matrix to gut protozoa dissimilarity between wild and domestic mammals in high mountain sampling.

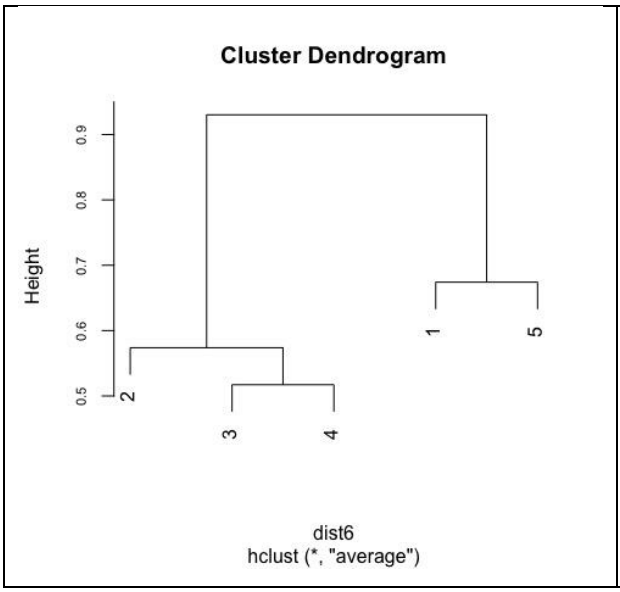
Primates

Concerning the primate-associated sampling, the highest similarity, both in intestinal microbiota and protozoa, was observed between domestic equines and bovines, with a greater resemblance in protozoa compared to the overall microbiota. Conversely, equines and bovines exhibited greater similarities with the red howler monkey than with other primates. In this regard, the howler monkey tends to cluster more closely with bovines than with other species, both in terms of microbiota and protozoa. The two nocturnal monkeys are proximate to each other in terms of microbiota. *S. leucopus* appears to be the species furthest away, both in terms of intestinal microbiota and protozoa (Figure 13, Figure 14).



Bray curtis dissimilarity	Al_1	As_2	Av_3	Bp_4	Ec_5
As_2	0.96				
Av_3	0.78	0.99			
Bp_4	0.98	0.65	0.99		
Ec_5	0.99	0.77	1.00	0.62	
Sl_6	0.89	0.77	0.96	0.83	0.92

Figure 13. Cluster dendrogram and matrix to gut microbiota dissimilarity between wild and domestic mammals in primates sampling.



Bray curtis dissimilarity	Al_1	As_2	Bp_3	Ec_4
As_2	0.92			
Bp_3	0.93	0.59		
Ec_4	0.98	0.56	0.52	
Sl_5	0.67	0.92	0.88	0.96

Figure 14. Cluster dendrogram and matrix to protozoa dissimilarity between wild and domestic mammals in primates sampling.

4.4.3 Relationship between landscape metrics and dissimilarity of the gut microbiota and protozoa between domestic animals and wildlife

Out of the initially considered 15 covariates, eight showed no significant associations with each other: Elevation, Forest Mean Shape Index, Forest Number Patches, Habit, Pastures areas, Urbanized zones, River, and Weighted Cover Index (Supplement information 1).

At the evaluated scale, a negative relationship was identified solely between the Weighted Cover Index and the dissimilarity of intestinal microbiota. Notably, this relationship proved to be the most parsimonious model for this response variable. Following this model, for intestinal microbiota dissimilarity, the subsequent model included the number of forest patches and the habit (terrestrial, arboreal). However, these models were significantly different from the first (Delta Dic > 2), and their relationships were not statistically significant (the probability interval included 0). Concerning protozoa dissimilarity, the most parsimonious model included pasture areas, followed by habit and the mean shape index. While these variables were not significantly different from each other, they also did not show a statistically significant relationship with this response variable (Table 20).

Table 20. Regression models to assess the effect of some landscape metrics in the dissimilarity of gut microbiota and protozoa diversity between the wild and domestic mammals sampled.

Model	Alpha			Beta			Phi				
	Mean	LIPI	ULPI	Mean	LIPI	ULPI	Mean	LIPI	ULPI	DIC	Delta DIC
GM_WCI	1.54	1.19	1.87	-0.35	-0.69	-0.01	25.40	9.34	49.76	-20.77	0.00
GM_FNP	1.51	1.12	1.89	-0.12	-0.48	0.29	18.25	6.77	35.60	-17.12	3.65
GM_Habit	1.51	1.12	1.90	-0.09	-0.45	0.43	18.21	6.72	35.72	-17.07	3.70
GM_FMSI	1.51	1.12	1.90	0.13	-0.25	0.53	18.17	6.71	35.52	-17.04	3.73
GM_E	1.51	1.12	1.90	-0.08	-0.48	0.35	17.85	6.65	34.70	-16.89	3.88
GM_Past	1.51	1.11	1.90	0.02	-0.37	0.42	17.50	6.51	34.06	-16.69	4.08
GM_Riv	1.51	1.11	1.90	0.00	-0.43	0.41	17.52	6.53	34.01	-16.64	4.13
GM_UA	1.52	1.12	1.91	0.02	-0.39	0.54	17.58	6.59	34.29	-16.62	4.15

P_PA	0.81	0.19	1.42	-0.54	-1.16	0.07	4.05	1.67	7.56	-2.13	0.00
P_Habit	0.89	0.14	1.50	0.07	-0.73	1.01	3.14	1.34	5.79	-1.13	1.01
P_FMSI	0.87	0.22	1.49	0.50	-0.18	1.22	3.80	1.58	7.10	-1.03	1.11
P_WCI	0.86	0.20	1.50	-0.31	-1.03	0.42	3.44	1.45	6.40	0.07	2.21
P_E	0.82	0.15	1.48	-0.21	-0.90	0.47	3.25	1.38	6.01	0.53	2.67
P_R	0.81	0.13	1.47	0.15	-0.47	0.77	3.18	1.35	5.89	0.65	2.79
P_UA	0.84	0.17	1.49	0.17	-0.52	0.94	3.20	1.36	5.92	0.72	2.85
P_FNP	0.83	0.15	1.49	-0.12	-0.80	0.60	3.19	1.34	5.91	0.78	2.92

GM: gut microbiota, P: Richness of gut protozoa, FNP: Forest number patches Past: Pastures area, FMSI: Forest mean shape index, WCI: weighted cover index, DisTr: Distance to track, Riv: length of river, UA: Urban areas, LLPI: Low limit probability interval, ULPI: Upper limit probability interval, DIC: Deviance information criteria, Δ DIC= delta DIC, difference between a model and most parsimonious model minor DIC. The red letter highlighted the significant regression and not statistically different models.

4.4.4 Relationship between the local landscape features and the severity of human activities with the diversity of gut microbiota and protozoa, and mean abundance of the gastrointestinal protozoa in *Alouatta seniculus*.

Regarding the diversity of intestinal microbiota, a significant relationship was observed in 29 out of the 33 evaluated regressions. The distance to roads negatively impacted richness at 50, 1600, 3200, and 6400 hectares, consistently across all models that included this variable. Similarly, the mean shape index of forests negatively influenced richness at seven scales, except at 1600 hectares where no significant relationship was found. On the other hand, the weighted cover index showed a positive effect on richness at 50, 800, 1600, and 6400 hectares, being negative at 200 and 3200 hectares, with no significant relationship at 100 and 400 hectares. The length of rivers exhibited a variable impact, affecting richness negatively at some scales and positively at others. The inclusion of pasture areas from 400 hectares onward had a positive impact on richness, while the number of forest patches from 1600 meters also showed a positive relationship across all evaluated scales (Table 21).

Concerning the evenness in the diversity of intestinal microbiota, a significant relationship was found in 31 out of the 33 evaluated regressions. The distance to roads and the quantity of pastures consistently showed a negative relationship at all scales. The shape and coverage of forests, as well as the presence of forest patches, had both positive and negative impacts at different scales. Moreover, evenness showed a positive relationship with the mean shape index and the weighted cover index at several scales, being negative at 3200 hectares. Additionally, a positive relationship was found with the number of forest patches at 1600 and 3200 hectares, with no significant relationship at 6400 hectares (Table 21).

Regarding gastrointestinal protozoa, richness was significantly related in 19 out of the 33 evaluated regressions. The distance to roads negatively impacted richness at all scales, while the weighted cover index had a positive effect at several scales and no relationship at others. The distance to rivers showed a variable impact. The pasture area had a positive influence from 400 hectares onward, while the number of forest patches from 1600 meters showed a positive relationship across all evaluated scales (Table 21).

Concerning evenness in the diversity of gastrointestinal protozoa, a negative relationship was found with the mean shape index of forests at 100, 200, and 400 hectares, as well as the weighted cover index at 800 and 1600 hectares. In other words, the effects of the included explanatory variables were only detected in five out of the 33 evaluated regressions (Table 21).

Regarding the mean abundances of *Giardia intestinalis*, a positive relationship was found with the weighted cover index at 50, 100, 200, 400, and 800 hectares. It also presented a positive relationship with the length of rivers at 50 hectares, while a negative relationship with the distance to roads at 100 hectares. At 3200 hectares, the mean shape index had a negative relationship with the relative abundance of Giardia intestinalis. Generally, the effects of the included explanatory variables were only detected in eight out of the 33 evaluated regressions. Concerning the relative abundance of *Cryptosporidium parvum*, no relationship was found in any of the evaluated regressions; in fact, some models did not run (Table 21).

The explanatory variable with the most weight in the modeling was the Weighted Cover Index, appearing in 26 models, followed by Forest Mean Shape Index in 18, Distance to Track in 16, Pastures Area in 12, Length of River in 11, and finally, Forest Number Patches in 8 (Table 21).

Table 21. Significant regression models to assess the effect of some landscape metrics in the epidemiological response variables in *Alouatta seniculus*. The gray resalted models have a positive effect, blank with negative.

Model	Scale	beta	LIPI	ULPI	DIC	Δ DIC
GM_R_DisTr		-0.67	-0.72	-0.63	5602	0
GM_R_FMSI		-0.15	-0.17	-0.13	6360	758
GM_R_WCI		0.04	0.02	0.05	6544	942
GM_R_Riv		-0.03	-0.04	-0.01	6553	951
GM_IS_Riv		-0.56	-0.65	-0.47	635.4	0
GM_IS_FMSI		0.61	0.50	0.72	660.2	25
GM_IS_WCI	50	0.41	0.33	0.49	689.4	54
GM_IS_DisTr		-0.64	-0.84	-0.45	749.7	114
P_R_DisTr		-0.56	-0.90	-0.25	174.8	0
P_R_WCI		0.19	0.05	0.32	179.4	5
P_R_Riv		0.17	0.03	0.30	181.4	7
AbM_G_WCI		0.98	0.45	1.59	52.75	0
AbM_G_Riv		0.52	0.05	1.02	62.26	10
GM_R_DisTr		-0.66	-0.71	-0.61	5658	0
GM_R_FMSI		-0.17	-0.19	-0.15	6203	545
GM_R_Riv		-0.04	-0.06	-0.02	6402	744
GM_IS_WCI		0.51	0.42	0.59	611.9	0
GM_IS_DisTr	100	-1.14	-1.41	-0.88	665.1	53
GM_IS_Riv		-0.47	-0.58	-0.36	670.4	59
GM_IS_FMSI		0.41	0.29	0.54	707.9	96
P_R_DisTr		-0.56	-0.93	-0.21	169.2	0
AbM_G_WCI		1.05	0.48	1.70	43.31	0

AbM_G_DisTr		-1.90	-3.76	-0.33	51.85	9
GM_R_FMSI		-0.21	-0.23	-0.19	5290	0
GM_R_Riv		-0.07	-0.10	-0.05	5573	283
GM_R_WCI		-0.03	-0.05	-0.01	5592	302
GM_IS_WCI		0.58	0.49	0.67	556.3	0
GM_IS_Riv	200	0.47	0.35	0.59	665.5	109
GM_IS_FMSI		0.16	0.04	0.28	718.6	162
P_R_WCI		0.14	0.01	0.27	158.5	0
P_IS_FMSI		-0.42	-0.72	-0.11	74.32	0
AbM_G_WCI		0.96	0.44	1.52	41.98	0
GM_R_Past		0.19	0.17	0.20	5507	0
GM_R_FMSI		-0.16	-0.18	-0.14	5606	99
GM_R_Riv		0.10	0.08	0.13	5772	265
GM_IS_WCI		0.60	0.52	0.68	507.7	0
GM_IS_Riv		0.49	0.36	0.61	653.7	146
GM_IS_Past	400	-0.49	-0.65	-0.33	666.3	159
GM_IS_FMSI		0.13	0.02	0.24	707.2	200
P_R_Riv		0.24	0.05	0.43	155.4	0
P_R_WCI		0.16	0.03	0.29	156.1	1
P_R_Past		0.15	0.00	0.30	157.7	2
P_IS_FMSI		-0.35	-0.62	-0.07	68.9	0
AbM_G_WCI		0.79	0.26	1.33	34.46	0
GM_R_WCI		0.13	0.11	0.15	5409	0
GM_R_Past	800	0.11	0.09	0.13	5491	82
GM_R_FMSI		-0.06	-0.09	-0.03	5584	175

GM_IS_Past		-0.27	-0.44	-0.11	361	0
GM_IS_FMSI		0.29	0.12	0.46	361.6	1
P_R_WCI		0.27	0.14	0.41	142.9	0
P_IS_WCI		-0.41	-0.70	-0.12	62.79	0
AbM_G_WCI		0.96	0.36	1.61	31.51	0
GM_R_DisTr		-0.77	-0.83	-0.72	3585	0
GM_R_FNP		0.16	0.14	0.18	4182	597
GM_R_WCI		0.09	0.07	0.11	4329	744
GM_IS_Past		-0.58	-0.80	-0.37	290.3	0
GM_IS_DisTr		-0.97	-1.34	-0.62	290.9	1
GM_IS_FMSI		0.33	0.17	0.48	307.4	17
GM_IS_FNP	1600	0.22	0.09	0.35	312	22
GM_IS_WCI		-0.12	-0.25	0.00	320	30
P_R_FNP		0.26	0.10	0.42	119.9	0
P_R_DisTr		-0.59	-1.02	-0.20	121.6	2
P_R_WCI		0.20	0.07	0.33	121.8	2
P_R_Past		0.19	0.00	0.37	126.2	6
P_IS_WCI		-0.53	-0.89	-0.19	51.15	0
GM_R_DisTr		-0.68	-0.74	-0.62	4286	0
GM_R_FNP		0.25	0.23	0.27	4301	15
GM_R_Past		0.11	0.08	0.14	4810	524
GM_R_FMSI	3200	-0.07	-0.09	-0.05	4817	531
GM_R_WCI		-0.02	-0.04	0.00	4853	567
GM_IS_Past		-1.59	-1.83	-1.36	371.9	0
GM_IS_WCI		0.54	0.47	0.61	392.4	21
GM_IS_DisTr		-1.71	-2.10	-1.35	489.3	117

GM_IS_FNP		0.42	0.32	0.51	529.8	158
GM_IS_FMSI		-0.14	-0.24	-0.04	596.9	225
P_R_FNP		0.23	0.08	0.38	125.5	0
P_R_DisTr		-0.54	-0.98	-0.13	127.8	2
P_R_Past		0.26	0.04	0.46	129	4
AbM_G_FMSI		-0.99	-2.09	-0.10	31.22	0
GM_R_DisTr		-0.93	-1.00	-0.86	1831	0
GM_R_WCI		0.31	0.28	0.33	1967	136
GM_R_FNP		0.26	0.23	0.28	2166	335
GM_R_Past		-0.10	-0.13	-0.07	2636	805
GM_R_FMSI		-0.06	-0.08	-0.04	2642	811
GM_IS_DisTr	6400	-1.85	-2.38	-1.36	226.7	0
GM_IS_Past		-0.71	-0.95	-0.48	256.5	30
GM_IS_FMSI		0.34	0.21	0.46	268.8	42
GM_IS_WCI		-0.26	-0.39	-0.12	283.3	57
P_R_WCI		0.34	0.17	0.50	79.31	0
P_R_DisTr		-0.86	-1.37	-0.39	82.24	3
P_R_FNP		0.22	0.05	0.38	88.81	10

GM_R: Richness of gut microbiota, GM_IS: Evenness (Inverse Simpson`s index), P_R: Richness of gut protozoa, P_IS: Evenness (Inverse Simpson`s index) of gut protozoa, AbM_G: Mean abundance of *Giardia intestinalis*. FNP: Forest number patches, Past: Pastures area, FMSI: Forest mean shape index, WCI: weighted cover index, DisTr: Distance to track, Riv: length of river, LLPI: Low limit probability interval, ULPI: Upper limit probability interval, DIC: Deviance information criteria, Δ DIC= delta DIC, difference between a model and most parsimonious model minor DIC

4.5 Discussion

Analyzing composition at the phylum level provides information about taxonomic richness and the stability of the microbiome, which is linked to resistance against environmental disturbances and pathogens (Rinninella et al., 2019). Different bacterial phyla are associated with specific metabolic functions, such as fiber fermentation and vitamin synthesis. Understanding the distribution of these phyla can help elucidate the metabolic and nutritional functions of the intestinal microbiome, crucial for understanding the relationship between diet, health, and disease (Rowland et al., 2018). Investigating the phylum-level composition of the intestinal microbiome can offer insights into how certain microbial communities may modulate the host's immune response (Mazmanian & Lee, 2014). Changes in the microbiome composition at the phylum level have been demonstrated to be associated with various diseases, including gastrointestinal disorders, obesity, diabetes, and infectious diseases (Ottman et al., 2012). Analyzing these changes can provide important clues about the etiology and progression of diseases, as well as potential biomarkers for early detection (Vijay & Valdes, 2022).

The main phyla found in the samples were Ascomycota, Pseudomonadota, Basidiomycota, and Apicomplexa. The predominance of the Ascomycota phylum has been discovered to play a crucial role in the intestinal health of non-human mammals. Recent research suggests that evolutionary adaptation between these microorganisms and their hosts has led to a beneficial symbiosis (Dworecka-Kaszak et al., 2016). Furthermore, it has been postulated that the predominant presence of Ascomycota could be associated with antagonistic properties toward potential pathogens, thereby strengthening the host's defenses (Limon et al., 2017). On the other hand, the abundance of Pseudomonadota highlights their crucial roles in intestinal homeostasis and might indicate an adaptive response to specific environmental conditions, such as exposure to pathogens or changes in the diet. Additionally, it has been suggested that certain members of Pseudomonadota may modulate the local immune response and actively participate in the degradation of complex compounds (Belvoncikova et al., 2022; Rizzetto et al., 2014).

The predominant presence of the Basidiomycota phylum in the intestinal microbiota is unusual and uncommon. If such predominance is observed, it could result from specific conditions or imbalances in the intestinal environment, such as fungal infections, environmental or dietary changes favoring the growth of certain fungi over bacteria, or health conditions like inflammatory bowel disease or other gastrointestinal pathologies (Pang et al., 2021; Rizzetto et al., 2014). The dominance of the Apicomplexa phylum in the intestinal microbiota would also be atypical and

uncommon. Apicomplexa comprises a group of obligate intracellular parasitic protozoa. These organisms generally do not form part of the normal intestinal microbiota, which is mainly composed of bacteria, with the occasional presence of fungi and other microorganisms. The presence of Apicomplexa protozoa could be due to environmental contamination or ingestion of contaminated food or water containing these pathogens (Van Voorhis et al., 2021). The occurrence of these taxa at such frequencies demands specific studies where the context is thoroughly analyzed, and factors such as the methodology used, the studied population, and experimental conditions are considered to interpret these findings accurately.

Nevertheless, considering the context of this research, focused on wildlife conservation and management, it is imperative to adopt a conservative approach regarding the presence and abundance of these organisms. This approach aims to minimize the risk of committing a Type II error concerning these hypotheses. In the realm of conservation, assuming a conservative stance ensures that potential ecological and microbiological contributions are not overlooked or underestimated. This cautious approach is essential for making informed decisions and implementing effective conservation strategies based on a comprehensive understanding of the intricate relationships between microbial communities and wildlife in their natural environments (Artois et al., 2009; Schurer et al., 2016).

In this study, probably, several first-time records of protozoan species were documented in different hosts at both the species and, in some cases, genus levels. For *A. seniculus*, species such as *Babesia bigemina, Babesia bovis, Babesia microti, Besnoitia besnoiti, Leishmania mexicana, Neospora caninum*, and various *Plasmodium* species, including *Plasmodium relictum* and *Plasmodium* sp. gorilla clade G2, were identified. Additionally, *Trypanosoma brucei* was reported as a new finding for *S. leucopus*, along with the presence of *Trichomonas vaginalis*, *Plasmodium coatneyi, P. relictum, P. falciparum, P. malariae, P. cynomolgi, P. vivax, P. chabaudi, Babesia microti, B. bigemina, B. bovis, Theileria orientalis, T. equi, Neospora caninum,* and *Toxoplasma gondii*. For *Aotus*, new records were established for the genus, including *Babesia bovis, B. microti,* and *Theileria* (Rondón et al., 2021; Solórzano-García & Pérez-Ponce de León, 2018). In *T. ornatus*, species such as *Babesia microti, Besnoitia besnoiti, Plasmodium relictum, Theileria equi,* and *Theileria orientalis* were identified. Additionally, *Cryptosporidium parvum* was reported just until genera, was determined until species (Roncancio in press Chapter 1). *T. pinchaque* reported *Babesia microti* and *Toxoplasma* as the first records for tapirs, highlighting that *Theileria equi*, previously documented in *Tapirus terrestris*, had not been recorded in *T.*

pinchaque before (Da Silveira et al., 2017). Lastly, in pumas, *Babesia microti, Besnoitia besnoiti, Plasmodium relictum, Theileria equi*, and *Theileria orientalis* were reported (Hatam-Nahavandi et al., 2021; Uribe et al., 2021, Li et al., 2020). Despite the limited sample size, the discovery of these findings reflects the broad information gap in this aspect for many species and their landscapes. This underscores the need for further research to enhance our understanding of protozoan diversity and distribution in various wildlife species and their ecosystems.

However, many of these results need to be reviewed with more in-depth studies. The use of metabarcoding and the analytical approach of Kraken2 may introduce uncertainty in taxonomic determination (Lu et al., 2022; Wood et al., 2019). Several factors contribute to this uncertainty, including the taxonomic complexity of protozoa (Imam, 2011). Protozoa can exhibit high taxonomic diversity, with closely related species that can increase the difficulty of distinguishing between similar sequences and affect the accuracy of taxonomic assignment. The use of specific protozoan reference sequences can also impact accuracy if the database is not well-curated or representative of the diversity in the sample (Krogsgaard et al., 2018; Stensvold et al., 2021). Intraspecific genetic variability is another factor to consider, as protozoa may have significant intraspecific genetic variability, posing challenges to precise identification, especially if reference sequences do not fully capture this variability (Seabolt et al., 2021). The choice of metabarcoding marker is crucial, as some markers may be more specific for certain groups of protozoa, while others may have higher taxonomic resolution (Stensvold et al., 2021). The presence of immature forms or cysts in fecal samples can also affect the representation of obtained sequences and the accuracy of taxonomic determination. Given these complexities, caution should be exercised in interpreting metabarcoding results, and additional validation and complementary approaches are recommended to enhance the reliability of taxonomic assignments in protozoa studies.

In this context, the almost constant presence of *Plasmodium relictum* is noteworthy. This protozoan is well-known for causing malaria in birds, and there have been no reports of *Plasmodium relictum* infecting mammals naturally. This protozoan is primarily transmitted through mosquitoes and has a specific relationship with birds *Plasmodium relictum* may be found in mammalian fecal samples as a result of ingesting infected birds. The presence of genetic material (DNA) or infective forms of the protozoa in mammalian feces could be attributed to the ingestion of birds carrying *Plasmodium relictum*. In the life cycle of these protozoa, birds are often the definitive hosts, and mosquitoes act as vectors for transmission between birds. However, some *Plasmodium* species can infect different types of hosts, and there is observed flexibility in their

host range, albeit generally with lower efficiency (Rondón et al., 2019). Detecting *Plasmodium relictum* in mammalian fecal samples may indicate the presence of the protozoa, but it is also essential to consider the possibility that genetic material or infective forms may originate from birds that have been consumed by these mammals. Confirmation of infection and a complete understanding of its life cycle typically require more detailed and specific analyses. Therefore, further investigations and specific studies are necessary to confirm the presence of *Plasmodium relictum* in mammals and to elucidate the dynamics of its transmission and potential impact on both avian and mammalian hosts (Nourani et al., 2020).

The higher similarity in gut microbiota and intestinal protozoa between dogs and equines suggests a shared microbial community, possibly influenced by their domestication and shared living environments. The similarity in gut microbiota and intestinal protozoa of the puma with dogs and horses could be attributed to shared ecological niches, interactions, or dietary overlaps in wild or domestic settings, driven by common sources of exposure or transmission. The tapir exhibits a relatively high similarity in intestinal microbiota with bears, indicating a potential ecological connection or dietary overlap in their natural habitats (Guzmán et al. in prep). On the other hand, the domestic bovine shows a lower resemblance to other large mountain mammals in terms of gut microbiota. This implies significant differences in their microbial communities, possibly due to distinct diets or environments influenced by close human management, including prophylactic measures such as deworming.

To the primates associated sampling, the cluster configuration could be due to the diet and interactions. Cattle, like equines, are herbivores that consume large amounts of fiber and vegetation. Red howler monkeys preferentially feed on fruits, but they are facultative folivores when fruit is scarce. In these fragmented landscapes, monkeys may be forced to consume more leaves than fruits, and to that extent, their diet would be more like that of domestic herbivores (Giraldo et al., 2007; Palma et al., 2011). The adaptation to an herbivorous diet can lead to a similar microbial composition. On the other hand, given that, in the sampled areas, red howler monkeys cohabitate with cattle and equines, it is possible that they share environments and, therefore, are exposed to similar sources of microorganisms and protozoa present in the environment and cross-contamination. On the other hand, the similarity between nocturnal monkeys and Saguinus may be explained by their similarities in diet; both are consumers of soft fruits, invertebrates, and small vertebrates (Bustamante-Manrique et al., 2021; Montilla et al.,

2021; Roncancio Duque et al., 2012). However, it is necessary to consider that the sample size may generate bias in the result.

The negative relationship between the weighted class index and the dissimilarity of the gut microbiota between domestic and wild animals, its mean, also more natural plant covers more similarity, can be explained by the complex interaction among the environment, diet, and animal microbiota (Qin et al., 2020). Areas with lower natural vegetation cover, typically associated with more urbanized or human-altered environments, tend to favor the presence of domestic animals and reduce the diversity of wild species (Fehlmann et al., 2020). This shift in animal assemblages can directly impact the gut microbiota of the animals inhabiting those areas. The diet of domestic animals, influenced by food availability in more human-modified environments, tends to be more uniform compared to that of wild animals, which rely on a variety of natural resources (Roncancio et al Chapter 3). Dietary variability is directly related to microbial diversity in the intestinal tract (Leeming et al., 2019).

Furthermore, the loss of natural vegetation cover can lead to increased exposure of domestic animals to pathogens and antimicrobial agents present in anthropogenic environments (Jin et al., 2017). This constant exposure can influence the selection and composition of the intestinal microbiota, resulting in greater similarity among the microbiota of domestic animals and lower diversity compared to the microbiota of wild animals (Blum et al., 2019; Parajuli et al., 2020; Phillips et al., 2018). In summary, landscape alteration due to the loss of natural vegetation cover leads to changes in animal assemblage composition, associated diets, and exposure to pathogens. These factors contribute to the observed negative relationship in the dissimilarity of the intestinal microbiota between domestic and wild animals in specific environments (Biek & Real, 2010; Williams et al., 2021).

The negative relationship between distance to roads and the irregularity of forest fragments with intestinal microbiota richness and overall protozoa diversity could be explained by the fact that shorter distances to roads might provide red howler monkeys with easier access to a variety of food sources. Additionally, more irregular forest shapes could be associated with greater plant and food resource diversity. Forest irregularity may promote increased mobility and heterogeneity in microenvironments within the habitat, leading to greater exposure to different microorganisms (Solórzano-García et al., 2023). Similarly, proximity to roads and forest shape irregularity might encourage interaction with other animal species and microorganisms present in the environment (Klain et al., 2023).

Regarding the decrease in intestinal microbiota diversity with distance from water bodies, this could be explained by the fact that proximity to rivers is often associated with a greater variety of microenvironments, such as riparian zones and gallery forests, affecting habitat mobility and connectivity for red howler monkeys Henriquez. Reduced connectivity could limit individual dispersal and, consequently, genetic mixing and microorganism transfer between populations (Bonte et al., 2012). Additionally, a reduction in interaction with aquatic environments, where specific water-related microorganisms may be encountered, could contribute to decreased microbiota diversity (Solórzano-García et al., 2023).

The positive effect of the weighted index of coverages, including more natural vegetation such as forests, and the number of forest patches on the diversity of microbiota and intestinal protozoa in red howler monkeys may be attributed to these coverages providing a wide variety of plants, fruits, and insects, offering a more diverse diet to the primates (Giraldo et al., 2007; McKenzie et al., 2017; Palma et al., 2011). Forests, especially those in good conservation status, tend to be heterogeneous environments with diverse microhabitats, such as canopy areas, clearings, and riverbanks (Solórzano-García et al., 2023). Habitat heterogeneity enables red howler monkeys to access different ecological niches and promotes positive interactions with other species, including birds, mammals, and microorganisms.

Well-preserved forests provide a more natural and less disturbed environment, reducing environmental stress and facilitating habitat connectivity. This allows red howler monkeys greater mobility and access to different areas (Martínez-Mota et al., 2018). The increase in natural vegetation coverages can enhance the diversity of the red howler monkey's intestinal microbiota by providing a more varied diet, a more complex and less disturbed habitat, as well as increased connectivity with various environments and resources (Kowalewski et al., 2015; Palma et al., 2011). Conversely, increasing the number of grasslands decreases diversity through inverse mechanisms.

Regarding the relative abundance of *G. intestinalis* and *C. parvum*, the results are inconclusive, and it is necessary to increase the sample size. In these types of organisms and variables, many zero values are common, making it challenging to obtain robust models (Solórzano-García et al., 2017).

Even though infectious diseases are not considered an important global threat to wildlife viability (Smith et al., 2006), it is a common factor in population reduction (temporal or permanent) on a local scale, particularly to threatened, isolated, or low populations (García Marín et al., 2018;

Ujvari & Belov, 2011). On the other hand, the mega biodiversity in Colombia introduces an additional epidemiological risk factor: there are several wildlife and domestic species that could play a role such as susceptible hosts and reservoirs to a few infectious agents when the ecosystem dynamics have been altered (Mackenstedt et al., 2015; Monsalve-Buriticá, 2019).
Additionally, its current increased interactions probably make this mammal an important vector or intermediate host to several zoonotic pathogens (Baruch-Mordo et al., 2014; Bronson et al., 2014; Dubey & Jones, 2008). The impact on wildlife mammals of infectious disease can act synergistically with other threats like isolation and low population size, reducing even further their numbers and increasing their vulnerability to habitat reduction and degradation, dietary stress, hunting, and pathogens (Ishibashi et al., 2017; Schwab et al., 2011). The interaction between wildlife and domestic life cycles plays an important role in the transmission of different infectious agents.
In conclusion, we found a negative relationship between the Weighted Cover Index, its mean, the quality of the structure, and the composition of the natural plant cover with the dissimilarity of gut microbiota between domestic and wildlife mammals. Therefore, when the natural plan cover is more mature or less transformed the similarity of gut microbiota is major between both hosts. Additionally, we found generally a positive effect of the weighted index of coverages, including more natural vegetation such as forests, and the number of forest patches on the diversity of microbiota and intestinal protozoa in red howler monkeys that may be attributed to these coverages providing a wide variety of plants, fruits, and insects, offering a more diverse diet to the primates.

Funding: The research was supported by the National Institutes of Health (NIH, No. R01NS110122) to the Neuroviruses Emerging in the Americas Study (NEAS).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee) of UNIVERSIDAD NACIONAL DE COLOMBIA (UN-17-03-23).

4.6 Acknowledgments

We thank the National University of Colombia - Palmira for the teaching assistance scholarship (Resolution 061/2021, Council of the Faculty of Agricultural Sciences Palmira headquarters). We also thank the Andean Bear Conservation Alliance (ABCA), the Cleveland Metroparks Zoo

Wildlife Conservation Program, and Rewild for supporting the doctoral thesis. We also thank Andrés Mauricio González-Ríos of MINCIENCIAS for the scholarship: “Jóvenes investigadores e innovadores en las regiones para atención de demandas definidas por los CODECTI de los Departamentos de Chocó, Valle del Cauca, Cauca y Nariño – BPIN 2022000100068”. The *Saguinus leucopus* samples were taken in the framework of the White footed tamarin monitoring plan implementation of the Corporación Autonoma Regional de Caldas – Corporcaldas, funded by ISAGEN.

Conflicts of Interest: The authors declare no conflict of interest.

4.7 References

Acevedo-Whitehouse, K., & Duffus, A. L. J. (2009). Effects of environmental change on wildlife health. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1534), 3429–3438. https://doi.org/10.1098/rstb.2009.0128

Aguirre, A. A. (2009). Wild canids as sentinels of ecological health: A conservation medicine perspective. Parasites & Vectors, 2(Suppl 1), S7. https://doi.org/10.1186/1756-3305-2-S1-S7

Aguirre, A. A., Ostfeld, R. S., Tabor, G. M., House, C., & Pearl, M. C. (2002). Conservation medicine: Ecological health in practice. Oxford University Press.

Arce-Peña, N. P., Arroyo-Rodríguez, V., Dias, P. A. D., Franch-Pardo, I., & Andresen, E. (2019). Linking changes in landscape structure to population changes of an endangered primate. Landscape Ecology, 34(11), 2687–2701. https://doi.org/10.1007/s10980-019-00914-8

Aron-Wisnewsky, J., Warmbrunn, M. V., Nieuwdorp, M., & Clément, K. (2021). Metabolism and Metabolic Disorders and the Microbiome: The Intestinal Microbiota Associated With Obesity, Lipid Metabolism, and Metabolic Health—Pathophysiology and Therapeutic Strategies. Gastroenterology, 160(2), 573–599. https://doi.org/10.1053/j.gastro.2020.10.057

Artois, M., Bengis, R., Delahay, R. J., Duchêne, M.-J., Duff, J. P., Ferroglio, E., Gortazar, C., Hutchings, M. R., Kock, R. A., Leighton, F. A., Mörner, T., & Smith, G. C. (2009). Wildlife Disease Surveillance and Monitoring. In R. J. Delahay, G. C. Smith, & M. R. Hutchings (Eds.), Management of Disease in Wild Mammals (pp. 187–213). Springer Japan. https://doi.org/10.1007/978-4-431-77134-0_10

Barrett, L. G., Thrall, P. H., Burdon, J. J., & Linde, C. C. (2008). Life history determines genetic structure and evolutionary potential of host–parasite interactions. Trends in Ecology & Evolution, 23(12), 678–685. https://doi.org/10.1016/j.tree.2008.06.017

Baruch-Mordo, S., Wilson, K. R., Lewis, D. L., Broderick, J., Mao, J. S., & Breck, S. W. (2014). Stochasticity in natural forage production affects use of urban areas by black bears: Implications to management of human-bear conflicts. PloS One, 9(1), e85122.

Becker, D. J., Streicker, D. G., & Altizer, S. (2015). Linking anthropogenic resources to wildlife–pathogen dynamics: A review and meta-analysis. Ecology Letters, 18(5), 483–495. https://doi.org/10.1111/ele.12428

Begon, M., & Townsend, C. R. (2021). Ecology: From individuals to ecosystems. John Wiley & Sons.

Belvoncikova, P., Splichalova, P., Videnska, P., & Gardlik, R. (2022). The Human Mycobiome: Colonization, Composition and the Role in Health and Disease. Journal of Fungi, 8(10), 1046. https://doi.org/10.3390/jof8101046

Biek, R., & Real, L. A. (2010). The landscape genetics of infectious disease emergence and spread. Molecular Ecology, 19(17), 3515–3531. https://doi.org/10.1111/j.1365-294X.2010.04679.x

Blum, W. E. H., Zechmeister-Boltenstern, S., & Keiblinger, K. M. (2019). Does Soil Contribute to the Human Gut Microbiome? Microorganisms, 7(9), 287. https://doi.org/10.3390/microorganisms7090287

Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V. M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T. G., Chaput-Bardy, A., Clobert, J., Dytham, C., … Travis, J. M. J. (2012). Costs of dispersal. Biological Reviews, 87(2), 290–312. https://doi.org/10.1111/j.1469-185X.2011.00201.x

Borcard, D., Gillet, F., & Legendre, P. (2011). Numerical ecology with R (Vol. 2). Springer.

Bronson, E., Spiker, H., & Driscoll, C. P. (2014). Serosurvey for selected pathogens in free-ranging American black bears (Ursus americanus) in Maryland, USA. Journal of Wildlife Diseases, 50(4), 829–836.

Buret, A. G., Motta, J.-P., Allain, T., Ferraz, J., & Wallace, J. L. (2019). Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: A role for iron? Journal of Biomedical Science, 26(1), 1. https://doi.org/10.1186/s12929-018-0495-4

Bustamante-Manrique, S. (2023). Efeito da restrição de hábitat no comportamento de bugios (Alouatta spp.).

Bustamante-Manrique, S., Botero-Henao, N., Castaño, J. H., & Link, A. (2021). Activity budget, home range and diet of the Colombian night monkey (*Aotus lemurinus*) in peri-urban forest fragments. Primates, 62(3), 529–536. https://doi.org/10.1007/s10329-021-00895-w

Calo-Mata, P., Ageitos, J. M., Böhme, K., & Barros-Velázquez, J. (2016). Intestinal Microbiota: First Barrier Against Gut-Affecting Pathogens. In T. G. Villa & M. Vinas (Eds.), New Weapons to Control Bacterial Growth (pp. 281–314). Springer International Publishing. https://doi.org/10.1007/978-3-319-28368-5_12

Chapman, C. A., Gillespie, T. R., & Goldberg, T. L. (2005). Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews, 14(4), 134–144.

Charlier, J., Van Der Voort, M., Kenyon, F., Skuce, P., & Vercruysse, J. (2014). Chasing helminths and their economic impact on farmed ruminants. Trends in Parasitology, 30(7), 361–367. https://doi.org/10.1016/j.pt.2014.04.009

Chen, S., Luo, S., & Yan, C. (2021). Gut Microbiota Implications for Health and Welfare in Farm Animals: A Review. Animals, 12(1), 93. https://doi.org/10.3390/ani12010093

Clayton, D. H., & Moore, J. (1997). Host-parasite evolution: General principles and avian models. (No Title).

Da Silveira, A. W., De Oliveira, G. G., Menezes Santos, L., da Silva Azuaga, L. B., Macedo Coutinho, C. R., Echeverria, J. T., Antunes, T. R., do Nascimento Ramos, C. A., & Izabel de Souza, A. (2017). Natural Infection of the South American Tapir (Tapirus terrestris) by Theileria equi. Journal of Wildlife Diseases, 53(2), 411–413. https://doi.org/10.7589/2016-06-149

Danneskiold-Samsøe, N. B., Dias de Freitas Queiroz Barros, H., Santos, R., Bicas, J. L., Cazarin, C. B. B., Madsen, L., Kristiansen, K., Pastore, G. M., Brix, S., & Maróstica Júnior, M. R. (2019). Interplay between food and gut microbiota in health and disease. Food Research International, 115, 23–31. https://doi.org/10.1016/j.foodres.2018.07.043

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging Infectious Diseases of Wildlife—Threats to Biodiversity and Human Health. Science, 287(5452), 443–449. https://doi.org/10.1126/science.287.5452.443

Deem, S. L. (2015). Conservation medicine to one health: The role of zoologic veterinarians. In Fowler's Zoo and Wild Animal Medicine, Volume 8 (pp. 698–703). Elsevier.

Delahay, R., & Delahay, R. J. (Eds.). (2009). Management of disease in wild mammals (1. ed). Springer.

Dubey, J., & Jones, J. (2008). Toxoplasma gondii infection in humans and animals in the United States. International Journal for Parasitology, 38(11), 1257–1278.

Dworecka-Kaszak, B., Dąbrowska, I., & Kaszak, I. (2016). The mycobiome – a friendly cross-talk between fungal colonizers and their host. Annals of Parasitology, 62(3), 175–184. https://doi.org/10.17420/ap6203.51

Echeverría, G., Reyna-Bello, A., Minda-Aluisa, E., Celi-Erazo, M., Olmedo, L., García, H. A., Garcia-Bereguiain, M. A., & de Waard, J. H. (2019). Serological evidence of Coxiella burnetii infection in cattle and farm workers: Is Q fever an underreported zoonotic disease in Ecuador? Infection and Drug Resistance, Volume 12, 701–706. https://doi.org/10.2147/IDR.S195940

Fahrig, L., Baudry, J., Brotons, L., Burel, F. G., Crist, T. O., Fuller, R. J., Sirami, C., Siriwardena, G. M., & Martin, J. L. (2011). Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters, 14(2), 101–112. https://doi.org/10.1111/j.1461-0248.2010.01559.x

Fehlmann, G., O'riain, M. J., FÜrtbauer, I., & King, A. J. (2020). Behavioral Causes, Ecological Consequences, and Management Challenges Associated with Wildlife Foraging in Human-Modified Landscapes. BioScience, biaa129. https://doi.org/10.1093/biosci/biaa129

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology, 37(12), 4302–4315.

Fisher, M. C., Henk, Daniel. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. Nature, 484(7393), 186–194. https://doi.org/10.1038/nature10947

Fletcher, S. M., Stark, D., Harkness, J., & Ellis, J. (2012). Enteric Protozoa in the Developed World: A Public Health Perspective. Clinical Microbiology Reviews, 25(3), 420–449. https://doi.org/10.1128/CMR.05038-11

Fountain-Jones, N. M., Craft, M. E., Funk, W. C., Kozakiewicz, C., Trumbo, D. R., Boydston, E. E., Lyren, L. M., Crooks, K., Lee, J. S., VandeWoude, S., & Carver, S. (2017). Urban landscapes can change virus gene flow and evolution in a fragmentation-sensitive carnivore. Molecular Ecology, 26(22), 6487–6498. https://doi.org/10.1111/mec.14375

Fox, J., Bouchet-Valat, M., Andronic, L., Ash, M., Boye, T., Calza, S., Chang, A., Grosjean, P., Heiberger, R., & Pour, K. K. (2015). Package 'Rcmdr'.

Freed, N., & Silander, O. (2020). DNA quantification using the Qubit fluorometer v1 [Preprint]. https://doi.org/10.17504/protocols.io.bfy3jpyn

Gale, P., Drew, T., Phipps, L. P., David, G., & Wooldridge, M. (2009). The effect of climate change on the occurrence and prevalence of livestock diseases in Great Britain: A review. Journal of Applied Microbiology, 106(5), 1409–1423. https://doi.org/10.1111/j.1365-2672.2008.04036.x

García Marín, J. F., Royo, L. J., Oleaga, A., Gayo, E., Alarcia, O., Pinto, D., Martínez, I. Z., González, P., Balsera, R., & Marcos, J. L. (2018). Canine adenovirus type 1 (CA dV-1) in free-ranging European brown bear (Ursus arctos arctos): A threat for Cantabrian population? Transboundary and Emerging Diseases, 65(6), 2049–2056.

Giraldo, P., Gómez-Posada, C., Martínez, J., & Kattan, G. (2007). Resource Use and Seed Dispersal by Red Howler Monkeys (*Alouatta seniculus*) in a Colombian Andean Forest. Neotropical Primates, 14(2), 55–64. https://doi.org/10.1896/044.014.0202

Hatam-Nahavandi, K., Calero-Bernal, R., Rahimi, M. T., Pagheh, A. S., Zarean, M., Dezhkam, A., & Ahmadpour, E. (2021). Toxoplasma gondii infection in domestic and wild felids as public health concerns: A systematic review and meta-analysis. Scientific Reports, 11(1), 9509. https://doi.org/10.1038/s41598-021-89031-8

He, Y., Maltecca, C., & Tiezzi, F. (2021). Potential Use of Gut Microbiota Composition as a Biomarker of Heat Stress in Monogastric Species: A Review. Animals, 11(6), 1833. https://doi.org/10.3390/ani11061833

IDEAM. (2021). Mapa de Coberturas de la Tierra. Metodología CORINE Land Cover adaptada para Colombia, escala 1:100 000 (Periodo 2018) [Map]. IDEAM–Instituto de Hidrología Meteorología y Estudios Ambientales. http://www.siac.gov.co/catalogo-de-mapas

IDEAM (Instituto de Hidrología, M. y E. A. de C. (2017). Resultados Monitoreo de la deforestación 2017.

Imam, T. (2011). The complexities in the classification of protozoa: A challenge to parasitologists. Bayero Journal of Pure and Applied Sciences, 2(2), 159–164. https://doi.org/10.4314/bajopas.v2i2.63805

Ishibashi, Y., Oi, T., Arimoto, I., Fujii, T., Mamiya, K., Nishi, N., Sawada, S., Tado, H., & Yamada, T. (2017). Loss of allelic diversity in the MHC class II DQB gene in western populations of the Japanese black bear Ursus thibetanus japonicus. Conservation Genetics, 18(2), 247–260.

Jackson, H. B., & Fahrig, L. (2012). What size is a biologically relevant landscape? Landscape Ecology, 27(7), 929–941. https://doi.org/10.1007/s10980-012-9757-9

Jakob-Hoff, R. M., MacDiarmid, S. C., Lees, C., Miller, P. S., Travis, D., & Kock, R. (2014). Manual of procedures for wildlife disease risk analysis (Vol. 2014). World Organisation for Animal Health Paris, France.

Jenkins, E. J., Simon, A., Bachand, N., & Stephen, C. (2015). Wildlife parasites in a One Health world. Trends in Parasitology, 31(5), 174–180. https://doi.org/10.1016/j.pt.2015.01.002

Jensen, S. K., Aars, J., Lydersen, C., Kovacs, K. M., & Åsbakk, K. (2010). The prevalence of Toxoplasma gondii in polar bears and their marine mammal prey: Evidence for a marine transmission pathway? Polar Biology, 33(5), 599–606. https://doi.org/10.1007/s00300-009-0735-x

Jin, Y., Wu, S., Zeng, Z., & Fu, Z. (2017). Effects of environmental pollutants on gut microbiota. Environmental Pollution, 222, 1–9. https://doi.org/10.1016/j.envpol.2016.11.045

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., Mitchell, C. E., Myers, S. S., Bogich, T., & Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature, 468(7324), 647–652. https://doi.org/10.1038/nature09575

Klain, V., Mentz, M. B., Bustamante-Manrique, S., & Bicca-Marques, J. C. (2023). Landscape structure has a weak influence on the parasite richness of an arboreal folivorous–frugivorous primate in anthropogenic landscapes. Landscape Ecology, 38(5), 1237–1247. https://doi.org/10.1007/s10980-023-01603-3

Kohler, W. (2004). Infectious Diseases Transmissible from Animals to Humans. International Journal of Medical Microbiology, 293(7/8), 548.

Kowalewski, M. M., Garber, P. A., Cortés-Ortiz, L., Urbani, B., & Youlatos, D. (Eds.). (2015). Howler monkeys: Behavior, ecology and conservation. Springer.

Krogsgaard, L. R., Andersen, L. O., Johannesen, T. B., Engsbro, A. L., Stensvold, C. R., Nielsen, H. V., & Bytzer, P. (2018). Characteristics of the bacterial microbiome in association with common intestinal parasites in irritable bowel syndrome. Clinical and Translational Gastroenterology, 9(6), e161. https://doi.org/10.1038/s41424-018-0027-2

Lange, K., Buerger, M., Stallmach, A., & Bruns, T. (2016). Effects of Antibiotics on Gut Microbiota. Digestive Diseases, 34(3), 260–268. https://doi.org/10.1159/000443360

Lausch, A. (2002). Applicability of landscape metrics for the monitoring of landscape change: Issues of scale, resolution and interpretability. Ecological Indicators, 2(1–2), 3–15. https://doi.org/10.1016/S1470-160X(02)00053-5

Leeming, E. R., Johnson, A. J., Spector, T. D., & Le Roy, C. I. (2019). Effect of Diet on the Gut Microbiota: Rethinking Intervention Duration. Nutrients, 11(12), 2862. https://doi.org/10.3390/nu11122862

Limon, J. J., Skalski, J. H., & Underhill, D. M. (2017). Commensal Fungi in Health and Disease. Cell Host & Microbe, 22(2), 156–165. https://doi.org/10.1016/j.chom.2017.07.002

Longmire, J. L., Maltbie, M., & Baker, R. J. (1997). Use of" lysis buffer" in DNA isolation and its implication for museum collections.

Lovarelli, D., Bacenetti, J., & Guarino, M. (2020). A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? Journal of Cleaner Production, 262, 121409. https://doi.org/10.1016/j.jclepro.2020.121409

Lu, J., Rincon, N., Wood, D. E., Breitwieser, F. P., Pockrandt, C., Langmead, B., Salzberg, S. L., & Steinegger, M. (2022). Metagenome analysis using the Kraken software suite. Nature Protocols, 17(12), 2815–2839. https://doi.org/10.1038/s41596-022-00738-y

Luo, J., Cheng, Y., Guo, L., Wang, A., Lu, M., & Xu, L. (2021). Variation of gut microbiota caused by an imbalance diet is detrimental to bugs' survival. Science of The Total Environment, 771, 144880. https://doi.org/10.1016/j.scitotenv.2020.144880

Mackenstedt, U., Jenkins, D., & Romig, T. (2015). The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. International Journal for Parasitology: Parasites and Wildlife, 4(1), 71–79.

Martínez-Mota, R., Pozo-Montuy, G., Bonilla Sánchez, Y. M., & Gillespie, T. R. (2018). Effects of anthropogenic stress on the presence of parasites in a threatened population of black howler monkeys (Alouatta pigra). Therya, 9(2), 161–169. https://doi.org/10.12933/therya-18-572

Mazmanian, S. K., & Lee, Y. K. (2014). Interplay between Intestinal Microbiota and Host Immune System. Journal of Bacteriology and Virology, 44(1), 1. https://doi.org/10.4167/jbv.2014.44.1.1

McCarthy, M. A. (2007). Bayesian Methods for Ecology. Cambridge University Press.

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., Alexiev, A., Amato, K. R., Metcalf, J. L., Kowalewski, M., Avenant, N. L., Link, A., Di Fiore, A., Seguin-Orlando, A., Feh, C., Orlando, L., Mendelson, J. R., Sanders, J., & Knight, R. (2017). The Effects of Captivity on the Mammalian Gut Microbiome. Integrative and Comparative Biology, 57(4), 690–704. https://doi.org/10.1093/icb/icx090

Miller, S., Zieger, U., Ganser, C., Satterlee, S. A., Bankovich, B., Amadi, V., Hariharan, H., Stone, D., & Wisely, S. M. (2015). INFLUENCE OF LAND USE AND CLIMATE ON SALMONELLA CARRIER STATUS IN THE SMALL INDIAN MONGOOSE (HERPESTES AUROPUNCTATUS) IN GRENADA, WEST INDIES. Journal of Wildlife Diseases, 51(1), 60–68. https://doi.org/10.7589/2014-02-046

Molina Benavides, R. A., Campos Gaona, R., Sánchez Guerrero, H., Giraldo Patiño, L., & Atzori, A. S. (2019). Sustainable Feedbacks of Colombian Paramos Involving Livestock, Agricultural Activities, and Sustainable Development Goals of the Agenda 2030. Systems, 7(4), 52. https://doi.org/10.3390/systems7040052

Monsalve-Buriticá, S. (2019). Enfermedades emergentes y reemergentes de origen viral o bacteriano en Colombia. Fondo Editorial Biogénesis, 49–62.

Montilla, S. O., Mopán-Chilito, A. M., Murcia, L. N. S., Triana, J. D. M., Ruiz, O. M. C., Montoya-Cepeda, J., Gutierrez-Barreto, D. A., Holguín-Vivas, J. A., Agámez, C. J., Pérez-Grisales, L. J., Cruz-Moncada, M., Corredor-Durango, N. J., Díaz, E. A. C., Cardona-Cardona, A. H., Franco-Pérez, E., Rivera-Ospina, A. M., & Link, A. (2021). Activity Patterns, Diet and Home Range of Night Monkeys (*Aotus griseimembra* and *Aotus lemurinus*) in Tropical Lowland and Mountain Forests of Central Colombia. International Journal of Primatology, 42(1), 130–153. https://doi.org/10.1007/s10764-020-00192-1

Muehlenbein, M. P. (2006). Intestinal parasite infections and fecal steroid levels in wild chimpanzees. American Journal of Physical Anthropology, 130(4), 546–550. https://doi.org/10.1002/ajpa.20391

Nourani, L., Zakeri, S., & Dinparast Djadid, N. (2020). Dynamics of prevalence and distribution pattern of avian Plasmodium species and its vectors in diverse zoogeographical areas—A review. Infection, Genetics and Evolution, 81, 104244. https://doi.org/10.1016/j.meegid.2020.104244

Nunn, C., & Altizer, S. M. (2006). Infectious diseases in primates: Behavior, ecology and evolution. Oxford University Press, USA.

O'Callaghan, T. F., Ross, R. P., Stanton, C., & Clarke, G. (2016). The gut microbiome as a virtual endocrine organ with implications for farm and domestic animal endocrinology. Domestic Animal Endocrinology, 56, S44–S55. https://doi.org/10.1016/j.domaniend.2016.05.003

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). Package 'vegan'. Community Ecology Package, Version, 2(9), 1–295.

Ostfeld, R., Glass, G., & Keesing, F. (2005). Spatial epidemiology: An emerging (or re-emerging) discipline. Trends in Ecology & Evolution, 20(6), 328–336. https://doi.org/10.1016/j.tree.2005.03.009

Ottman, N., Smidt, H., De Vos, W. M., & Belzer, C. (2012). The function of our microbiota: Who is out there and what do they do? Frontiers in Cellular and Infection Microbiology, 2. https://doi.org/10.3389/fcimb.2012.00104

Palma, A. C., Vélez, A., Gómez-Posada, C., López, H., Zárate, D. A., & Stevenson, P. R. (2011). Use of space, activity patterns, and foraging behavior of red howler monkeys (*Alouatta seniculus*) in an Andean forest fragment in Colombia. American Journal of Primatology, 73(10), 1062–1071. https://doi.org/10.1002/ajp.20973

Pang, K.-L., Hassett, B. T., Shaumi, A., Guo, S.-Y., Sakayaroj, J., Chiang, M. W.-L., Yang, C.-H., & Jones, E. B. G. (2021). Pathogenic fungi of marine animals: A taxonomic perspective. Fungal Biology Reviews, 38, 92–106. https://doi.org/10.1016/j.fbr.2021.03.008

Parajuli, A., Hui, N., Puhakka, R., Oikarinen, S., Grönroos, M., Selonen, V. A. O., Siter, N., Kramna, L., Roslund, M. I., Vari, H. K., Nurminen, N., Honkanen, H., Hintikka, J., Sarkkinen, H., Romantschuk, M., Kauppi, M., Valve, R., Cinek, O., Laitinen, O. H., … Sinkkonen, A. (2020). Yard vegetation is associated with gut microbiota composition. Science of The Total Environment, 713, 136707. https://doi.org/10.1016/j.scitotenv.2020.136707

Park, H., Yeo, S., Arellano, K., Kim, H. R., & Holzapfel, W. (2018). Role of the gut microbiota in health and disease. Probiotics and Prebiotics in Animal Health and Food Safety, 35–62.

Peterson, B. G., Carl, P., Boudt, K., Bennett, R., Ulrich, J., Zivot, E., Cornilly, D., Hung, E., Lestel, M., & Balkissoon, K. (2018). Package 'performanceanalytics'. R Team Cooperation, 3, 13–14.

Pfeiffer, D. U., Robinson, T. P., Stevenson, M., Stevens, K. B., Rogers, D. J., & Clements, A. C. A. (2008). Identifying factors associated with the spatial distribution of disease. Spatial Analysis in Epidemiology, 81–109. https://doi.org/10.1093/acprof:oso/9780198509882.003.0007

Phillips, J. N., Berlow, M., & Derryberry, E. P. (2018). The Effects of Landscape Urbanization on the Gut Microbiome: An Exploration Into the Gut of Urban and Rural White-Crowned Sparrows. Frontiers in Ecology and Evolution, 6, 148. https://doi.org/10.3389/fevo.2018.00148

Qin, W., Song, P., Lin, G., Huang, Y., Wang, L., Zhou, X., Li, S., & Zhang, T. (2020). Gut Microbiota Plasticity Influences the Adaptability of Wild and Domestic Animals in Co-inhabited Areas. Frontiers in Microbiology, 11, 125. https://doi.org/10.3389/fmicb.2020.00125

Rashid, M., Rashid, M. I., Akbar, H., Ahmad, L., Hassan, M. A., Ashraf, K., Saeed, K., & Gharbi, M. (2019). A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. Parasitology, 146(2), 129–141. https://doi.org/10.1017/S0031182018001282

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G., Gasbarrini, A., & Mele, M. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms, 7(1), 14. https://doi.org/10.3390/microorganisms7010014

Rizzetto, L., De Filippo, C., & Cavalieri, D. (2014). Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease. European Journal of Immunology, 44(11), 3166–3181. https://doi.org/10.1002/eji.201344403

Rodríguez-Vivas, R. I., Grisi, L., Pérez de León, A. A., Silva Villela, H., Torres-Acosta, J. F. de J., Fragoso Sánchez, H., Romero Salas, D., Rosario Cruz, R., Saldierna, F., & García Carrasco, D. (2017). Potential economic impact assessment for cattle parasites in Mexico. Review. Revista Mexicana de Ciencias Pecuarias, 8(1), 61. https://doi.org/10.22319/rmcp.v8i1.4305

Roncancio Duque, N. J. (2021). Effect of landscape modification on primate assemblages of the Magdalena River Valley, Colombia. Caldasia, 43(2), 261–273. https://doi.org/10.15446/caldasia.v43n2.84845

Roncancio Duque, N. J., Rojas Días, V., Ríos Franco, C. A., Gómez-Posada, C., Gutiérrez-Chacón, C., Giraldo, P., Velasco, J. A., & Franco, P. (2012). Plan de conservación y manejo del tití gris (Saguinus leucopus). 45.

Rondón, S., Cavallero, S., Renzi, E., Link, A., González, C., & D'Amelio, S. (2021). Parasites of Free-Ranging and Captive American Primates: A Systematic Review. Microorganisms, 9(12), 2546. https://doi.org/10.3390/microorganisms9122546

Rondón, S., León, C., Link, A., & González, C. (2019). Prevalence of Plasmodium parasites in non-human primates and mosquitoes in areas with different degrees of fragmentation in Colombia. Malaria Journal, 18(1), 1–10.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: Metabolism of nutrients and other food components. European Journal of Nutrition, 57(1), 1–24. https://doi.org/10.1007/s00394-017-1445-8

Ryan, U., Zahedi, A., & Paparini, A. (2016). Cryptosporidium in humans and animals—A one health approach to prophylaxis. Parasite Immunology, 38(9), 535–547. https://doi.org/10.1111/pim.12350

Sauvaitre, T., Etienne-Mesmin, L., Sivignon, A., Mosoni, P., Courtin, C. M., Van de Wiele, T., & Blanquet-Diot, S. (2021). Tripartite relationship between gut microbiota, intestinal mucus and dietary fibers: Towards preventive strategies against enteric infections. FEMS Microbiology Reviews, 45(2), fuaa052. https://doi.org/10.1093/femsre/fuaa052

Schurer, J., Mosites, E., Li, C., Meschke, S., & Rabinowitz, P. (2016). Community-based surveillance of zoonotic parasites in a 'One Health' world: A systematic review. One Health, 2, 166–174. https://doi.org/10.1016/j.onehlt.2016.11.002

Schwab, C., Cristescu, B., Northrup, J. M., Stenhouse, G. B., & Gänzle, M. (2011). Diet and environment shape fecal bacterial microbiota composition and enteric pathogen load of grizzly bears. PLoS One, 6(12), e27905.

Seabolt, M. H., Konstantinidis, K. T., & Roellig, D. M. (2021). Hidden Diversity within Common Protozoan Parasites as Revealed by a Novel Genomotyping Scheme. Applied and Environmental Microbiology, 87(6), e02275-20. https://doi.org/10.1128/AEM.02275-20

Simon, A., Rousseau, A. N., Savary, S., Bigras-Poulin, M., & Ogden, N. H. (2013). Hydrological modelling of Toxoplasma gondii oocysts transport to investigate contaminated snowmelt runoff as a potential source of infection for marine mammals in the Canadian Arctic. Journal of Environmental Management, 127, 150–161. https://doi.org/10.1016/j.jenvman.2013.04.031

Smith, K. F., Behrens, M., Schloegel, L. M., Marano, N., Burgiel, S., & Daszak, P. (2009). Reducing the risks of the wildlife trade. Science, 324(5927), 594–595. https://doi.org/10.1126/science.1174460

Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology, 20(5), 1349–1357.

Sokal, R. R., Rohlf, F. J., & Rohlf, J. F. (1995). Biometry. Macmillan.

Solórzano-García, B., Gasca-Pineda, J., Poulin, R., & Pérez-Ponce de León, G. (2017). Lack of genetic structure in pinworm populations from New World primates in forest fragments. International Journal for Parasitology, 47(14), 941–950. https://doi.org/10.1016/j.ijpara.2017.06.008

Solórzano-García, B., & Pérez-Ponce de León, G. (2018). Parasites of Neotropical Primates: A Review. International Journal of Primatology, 39(2), 155–182. https://doi.org/10.1007/s10764-018-0031-0

Solórzano-García, B., White, J. M., & Shedden, A. (2023). Parasitism in heterogeneous landscapes: Association between conserved habitats and gastrointestinal parasites in populations of wild mammals. Acta Tropica, 237, 106751. https://doi.org/10.1016/j.actatropica.2022.106751

Song, Q., Wang, Y., Huang, L., Shen, M., Yu, Y., Yu, Q., Chen, Y., & Xie, J. (2021). Review of the relationships among polysaccharides, gut microbiota, and human health. Food Research International, 140, 109858. https://doi.org/10.1016/j.foodres.2020.109858

Spiegelhalter, D., Thomas, A., Best, N., & Lunn, D. (2018). OpenBUGS version 3.0. 2.

Stensvold, C. R., Jirků-Pomajbíková, K., Tams, K. W., Jokelainen, P., Berg, R. P. K. D., Marving, E., Petersen, R. F., Andersen, L. O., Angen, Ø., & Nielsen, H. V. (2021). Parasitic Intestinal Protists of Zoonotic Relevance Detected in Pigs by Metabarcoding and Real-Time PCR. Microorganisms, 9(6), 1189. https://doi.org/10.3390/microorganisms9061189

Thompson, R. C. A. (2013). Parasite zoonoses and wildlife: One health, spillover and human activity. International Journal for Parasitology, 43(12–13), 1079–1088. https://doi.org/10.1016/j.ijpara.2013.06.007

Ujvari, B., & Belov, K. (2011). Major histocompatibility complex (MHC) markers in conservation biology. International Journal of Molecular Sciences, 12(8), 5168–5186.

Uribe, M., Payán, E., Brabec, J., Vélez, J., Taubert, A., Chaparro-Gutiérrez, J. J., & Hermosilla, C. (2021). Intestinal Parasites of Neotropical Wild Jaguars, Pumas, Ocelots, and Jaguarundis in Colombia: Old Friends Brought Back from Oblivion and New Insights. Pathogens, 10(7), 822. https://doi.org/10.3390/pathogens10070822

van der Linden, A., de Olde, E. M., Mostert, P. F., & de Boer, I. J. M. (2020). A review of European models to assess the sustainability performance of livestock production systems. Agricultural Systems, 182, 102842. https://doi.org/10.1016/j.agsy.2020.102842

Van Voorhis, W. C., Hulverson, M. A., Choi, R., Huang, W., Arnold, S. L. M., Schaefer, D. A., Betzer, D. P., Vidadala, R. S. R., Lee, S., Whitman, G. R., Barrett, L. K., Maly, D. J., Riggs, M. W., Fan, E., Kennedy, T. J., Tzipori, S., Doggett, J. S., Winzer, P., Anghel, N., … Ojo, K. K. (2021). One health therapeutics: Target-Based drug development for cryptosporidiosis and other apicomplexa diseases. Veterinary Parasitology, 289, 109336. https://doi.org/10.1016/j.vetpar.2020.109336

VanWormer, E., Miller, M. A., Conrad, P. A., Grigg, M. E., Rejmanek, D., Carpenter, T. E., & Mazet, J. A. K. (2014). Using Molecular Epidemiology to Track Toxoplasma gondii from

Terrestrial Carnivores to Marine Hosts: Implications for Public Health and Conservation. PLoS Neglected Tropical Diseases, 8(5), e2852. https://doi.org/10.1371/journal.pntd.0002852

Vijay, A., & Valdes, A. M. (2022). Role of the gut microbiome in chronic diseases: A narrative review. European Journal of Clinical Nutrition, 76(4), 489–501. https://doi.org/10.1038/s41430-021-00991-6

Williams, A. R., Myhill, L. J., Stolzenbach, S., Nejsum, P., Mejer, H., Nielsen, D. S., & Thamsborg, S. M. (2021). Emerging interactions between diet, gastrointestinal helminth infection, and the gut microbiota in livestock. BMC Veterinary Research, 17(1), 62. https://doi.org/10.1186/s12917-021-02752-w

Wisely, S. M., Howard, J., Williams, S. A., Bain, O., Santymire, R. M., Bardsley, K. D., & Williams, E. S. (2008). An unidentified filarial species and its impact on fitness in wild populations of the black-footed ferret (Mustela nigripes). Journal of Wildlife Diseases, 44(1), 53–64.

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 257. https://doi.org/10.1186/s13059-019-1891-0

Xiao, L., & Fayer, R. (2008). Molecular characterisation of species and genotypes of Cryptosporidium and Giardia and assessment of zoonotic transmission. International Journal for Parasitology, 38(11), 1239–1255. https://doi.org/10.1016/j.ijpara.2008.03.006

Yoo, J., Groer, M., Dutra, S., Sarkar, A., & McSkimming, D. (2020). Gut Microbiota and Immune System Interactions. Microorganisms, 8(10), 1587. https://doi.org/10.3390/microorganisms8101587

Synthesis

The risk to wildlife species is influenced by environmental, climatic, and behavioral factors. Changes in ecosystems, increased urbanization, and interaction with domestic animals may elevate wildlife exposure to these parasites. Furthermore, immunosuppression due to factors such as stress, malnutrition, or disease can increase individual susceptibility. Generally, livestock is not sustainable socially, economically, and environmentally (Molina-B, 2019). In this sense, based on the protozoa found in livestock that could be or have been detected in wildlife mammals, cattle become an important zoonotic reservoir (Buret et al., 2019; Echeverría et al., 2019; Ryan et al., 2016). The positive relationship observed between the forest area and number of the forest parches in focal landscapes and the richness of intestinal microbiota and gastrointestinal protozoa in bovine and equine livestock can be attributed to various ecological mechanisms. Native forests have a more diverse community including the level of microorganisms and prebiotics that favor their proliferation and development (Blum et al., 2019). Higher biodiversity in native forests may dilute the prevalence of specific pathogenic protozoa. A more diverse ecosystem could reduce the concentration of parasites that specifically target livestock, leading to a lower risk of infection (Williams et al., 2021). This complexity may stimulate a more diverse microbial community in the intestines of livestock, promoting overall gut health and resilience to gastrointestinal parasites (Berrilli et al., 2012; Stensvold & van der Giezen, 2018).

The diversity of gut microbiota among wild and domestic species in the central Andes revealed greater similarity between domestic canines and equines. Closest to these, the puma exhibited a 19% similarity in intestinal microbiota with dogs and a 29% similarity with horses and mules. *T. pinchaque* primarily shared its intestinal microbiota with bears at a 29% similarity. The most distant organism in this landscape, in terms of intestinal microbiota, was the domestic bovine, with a maximum similarity of only 13% with the puma. Regarding protozoa, a similar pattern emerged, with a 78% similarity between domestic canines and equines, while similarities decreased among other species. About the primate-associated sampling, the highest similarity, both in intestinal microbiota and protozoa, was observed between domestic equines and bovines, with a greater

resemblance in protozoa compared to the overall microbiota. Conversely, equines and bovines exhibited greater similarities with the red howler monkey than with other primates. In this regard, the howler monkey tends to cluster more closely with bovines than with other species, both in terms of microbiota and protozoa. The two nocturnal monkeys are proximate to each other in terms of microbiota. *S. leucopus* appears to be the species furthest away, both in terms of intestinal microbiota and protozoa.

A negative relationship was identified in the Weighted Cover Index over soon the dissimilarity of intestinal microbiota between wildlife and domestic mammals. Equally, in a multiscale analysis from 50 to 64000 ha done to *Alouatta seniculus* (Red howler monkey) the Weighted Cover Index that reflects the structure and composition of the plant cover in each focal landscape was the variable with the most weight in the modeling. In this study, probably, several first-time records of protozoan species were documented in different hosts at both the species and, in some cases, genus levels. Despite the limited sample size, the discovery of these findings reflects the broad information gap in this aspect for many species and their landscapes (Da Silveira et al., 2017; Rondón et al., 2021; Solórzano-García & Pérez-Ponce de León, 2018; Uribe et al., 2021). This underscores the need for further research to enhance our understanding of protozoan diversity and distribution in various wildlife species and their ecosystems. However, many of these results need to be reviewed with more in-depth studies. The use of metabarcoding and the analytical approach of Kraken2 may introduce uncertainty in taxonomic determination (Lu et al., 2022; Wood et al., 2019).

In summary, in this study, we found that landscape alteration due to the loss of natural vegetation cover leads to changes in animal assemblage composition, associated diets, and exposure to pathogens. These factors contribute to the observed negative relationship in the dissimilarity of the intestinal microbiota between domestic and wild animals in specific environments (Biek & Real, 2010; Williams et al., 2021). The positive effect of the weighted index of coverages, including more natural vegetation such as forests, and the number of forest patches on the diversity of microbiota and intestinal protozoa in red howler monkeys may be attributed to these coverages providing a wide variety of plants, fruits, and insects, offering a more diverse diet to the primates (Giraldo et al., 2007; McKenzie et al., 2017; Palma et al., 2011). Regarding the relative abundance of *G. intestinalis* and *C. parvum*, the results are inconclusive, and it is necessary to increase the sample size. In these types of organisms and variables, many zero values are common, making it challenging to obtain robust models (Solórzano-García et al., 2017). The impact on wildlife mammals of infectious disease can act synergistically with other threats like isolation and low

population size, reducing even further their numbers and increasing their vulnerability to habitat reduction and degradation, dietary stress, hunting, and pathogens (Ishibashi et al., 2017; Schwab et al., 2011). The interaction between wildlife and domestic life cycles plays an important role in the transmission of different infectious agents.

These explanations are based on general ecological principles, and the specific relationships may vary based on the characteristics of the landscape, climate, and management practices. Continued research, including longitudinal studies and molecular analyses, is essential to unravel the intricate dynamics between landscape features, microbial diversity, and gastrointestinal protozoa abundance in livestock. However, to improve the sustainability standards in the environmental, economic, and social dimensions based on the animal health component, the livestock landscape configuration in Colombia must be changed from a matrix of grassland with forest patches to a matrix of native forest with grassland patches.

4.8 References

Berrilli, F., Di Cave, D., Cavallero, S., & D'Amelio, S. (2012). Interactions between parasites and microbial communities in the human gut. Frontiers in Cellular and Infection Microbiology, 2. https://doi.org/10.3389/fcimb.2012.00141

Biek, R., & Real, L. A. (2010). The landscape genetics of infectious disease emergence and spread. Molecular Ecology, 19(17), 3515–3531. https://doi.org/10.1111/j.1365-294X.2010.04679.x

Blum, W. E. H., Zechmeister-Boltenstern, S., & Keiblinger, K. M. (2019). Does Soil Contribute to the Human Gut Microbiome? Microorganisms, 7(9), 287. https://doi.org/10.3390/microorganisms7090287

Buret, A. G., Motta, J.-P., Allain, T., Ferraz, J., & Wallace, J. L. (2019). Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: A role for iron? Journal of Biomedical Science, 26(1), 1. https://doi.org/10.1186/s12929-018-0495-4

Da Silveira, A. W., De Oliveira, G. G., Menezes Santos, L., da Silva Azuaga, L. B., Macedo Coutinho, C. R., Echeverria, J. T., Antunes, T. R., do Nascimento Ramos, C. A., & Izabel de Souza, A. (2017). Natural Infection of the South American Tapir (Tapirus terrestris) by Theileria equi. Journal of Wildlife Diseases, 53(2), 411–413. https://doi.org/10.7589/2016-06-149

Echeverría, G., Reyna-Bello, A., Minda-Aluisa, E., Celi-Erazo, M., Olmedo, L., García, H. A., Garcia-Bereguiain, M. A., & de Waard, J. H. (2019). Serological evidence of Coxiella burnetii infection in cattle and farm workers: Is Q fever an underreported zoonotic disease in Ecuador? Infection and Drug Resistance, Volume 12, 701–706. https://doi.org/10.2147/IDR.S195940

Giraldo, P., Gómez-Posada, C., Martínez, J., & Kattan, G. (2007). Resource Use and Seed Dispersal by Red Howler Monkeys (*Alouatta seniculus*) in a Colombian Andean Forest. Neotropical Primates, 14(2), 55–64. https://doi.org/10.1896/044.014.0202

Ishibashi, Y., Oi, T., Arimoto, I., Fujii, T., Mamiya, K., Nishi, N., Sawada, S., Tado, H., & Yamada, T. (2017). Loss of allelic diversity in the MHC class II DQB gene in western populations of the Japanese black bear Ursus thibetanus japonicus. Conservation Genetics, 18(2), 247–260.

Lu, J., Rincon, N., Wood, D. E., Breitwieser, F. P., Pockrandt, C., Langmead, B., Salzberg, S. L., & Steinegger, M. (2022). Metagenome analysis using the Kraken software suite. Nature Protocols, 17(12), 2815–2839. https://doi.org/10.1038/s41596-022-00738-y

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., Alexiev, A., Amato, K. R., Metcalf, J. L., Kowalewski, M., Avenant, N. L., Link, A., Di Fiore, A., Seguin-Orlando, A., Feh, C., Orlando, L., Mendelson, J. R., Sanders, J., & Knight, R. (2017). The Effects of Captivity on the Mammalian Gut Microbiome. Integrative and Comparative Biology, 57(4), 690–704. https://doi.org/10.1093/icb/icx090

Palma, A. C., Vélez, A., Gómez-Posada, C., López, H., Zárate, D. A., & Stevenson, P. R. (2011). Use of space, activity patterns, and foraging behavior of red howler monkeys (*Alouatta seniculus*) in an Andean forest fragment in Colombia. American Journal of Primatology, 73(10), 1062–1071. https://doi.org/10.1002/ajp.20973

Rondón, S., Cavallero, S., Renzi, E., Link, A., González, C., & D'Amelio, S. (2021). Parasites of Free-Ranging and Captive American Primates: A Systematic Review. Microorganisms, 9(12), 2546. https://doi.org/10.3390/microorganisms9122546

Ryan, U., Zahedi, A., & Paparini, A. (2016). Cryptosporidium in humans and animals—A one health approach to prophylaxis. Parasite Immunology, 38(9), 535–547. https://doi.org/10.1111/pim.12350

Schwab, C., Cristescu, B., Northrup, J. M., Stenhouse, G. B., & Gänzle, M. (2011). Diet and environment shape fecal bacterial microbiota composition and enteric pathogen load of grizzly bears. PLoS One, 6(12), e27905.

Solórzano-García, B., Gasca-Pineda, J., Poulin, R., & Pérez-Ponce de León, G. (2017). Lack of genetic structure in pinworm populations from New World primates in forest fragments.

International Journal for Parasitology, 47(14), 941–950. https://doi.org/10.1016/j.ijpara.2017.06.008

Solórzano-García, B., & Pérez-Ponce de León, G. (2018). Parasites of Neotropical Primates: A Review. International Journal of Primatology, 39(2), 155–182. https://doi.org/10.1007/s10764-018-0031-0

Stensvold, C. R., & van der Giezen, M. (2018). Associations between Gut Microbiota and Common Luminal Intestinal Parasites. Trends in Parasitology, 34(5), 369–377. https://doi.org/10.1016/j.pt.2018.02.004

Uribe, M., Payán, E., Brabec, J., Vélez, J., Taubert, A., Chaparro-Gutiérrez, J. J., & Hermosilla, C. (2021). Intestinal Parasites of Neotropical Wild Jaguars, Pumas, Ocelots, and Jaguarundis in Colombia: Old Friends Brought Back from Oblivion and New Insights. Pathogens, 10(7), 822. https://doi.org/10.3390/pathogens10070822

Williams, A. R., Myhill, L. J., Stolzenbach, S., Nejsum, P., Mejer, H., Nielsen, D. S., & Thamsborg, S. M. (2021). Emerging interactions between diet, gastrointestinal helminth infection, and the gut microbiota in livestock. BMC Veterinary Research, 17(1), 62. https://doi.org/10.1186/s12917-021-02752-w

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 257. https://doi.org/10.1186/s13059-019-1891-0

Supplement information