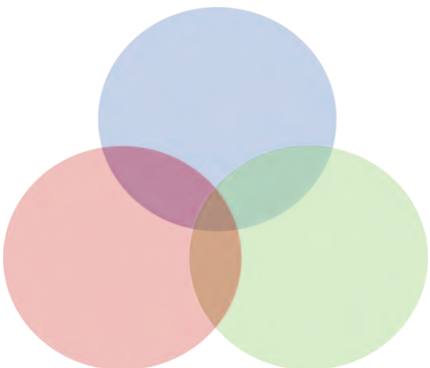
MANUEL URIBE SOTO

Neglected parasites circulating in Neotropical wild- and domestic animals:

A feasible threat to animals and humans under One Health perspective



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Abbreviations

A: Argentina
Ab: antibody prevalence
Af: tropical rainforest climate
Ag: antigen prevalence
AL: apical length
Am: tropical monsoon climate
Aw: tropical wet and dry climate
BC: blade curvature
BFS: Biomedical Research Center Seltersberg
BL: blade length
Bo: Bolivia
Br: Brazil
BSF: Brazilian spotted fever
BSh: semiarid / hot semi-arid climate
C: Colombia
calBP: calibrated years before the present
CEEA: Ethics Committee for Animal Experimentation
CF: centrifugal flotation with zinc sulphate
Cfa: humid subtropical climate
Cfb: Oceanic climate
CFS: fast carbol–fuchsin stained faecal smears
CI: confidence interval
COI: cytochrome c oxidase subunit I
CSA: *Cryptosporidium*-specific antigens
Csb: warm-summer Mediterranean climate
CSF: cerebrospinal fluid
DH: definitive host
ELISA: Enzyme-linked immunosorbent assay
EN: endangered
ET: Tundra climate
EtOH: ethyl alcohol/ethanol
FDA: Food and Drug Administration
gDNA: genomic deoxyribonucleic acid
GL: guard length
GSA: *Giardia*-specific antigens
GW: guard width
HW: handle width
IH: intermediate host
K2P: Kimura2-parameter
L1: first-stage larvae
L2: second-stage larvae
L3: third-stage larvae
L4: fourth-stage larvae
L5: fifth-stage larvae
LC: least concern
LMC: *larva migrans cutanea*
mAb: monoclonal antibodies
Mya: millions of years ago
NE: not evaluated
NGOs: Non-governmental organizations
NSAIDs: non-steroidal anti-inflammatory drugs
NTDs: Neglected tropical diseases
NT: near threatened
NWF: Neotropical wild felids
OD: optical density
OWL: oocyst wall layers
Pa: Panama
PA: Polyamide
PBS: physiological buffered saline solution
Pe: Peru
PED: Polyethylene terephthalate
PH: paratenic host
PRD: poverty-related disease
PRV: Priority Review Voucher
PO: *per os*
SAF: standardized sodium acetate-acetic acid-formalin
SF: combined sedimentation–flotation
SID: sem´el in di´e (once a day)
SQ: subcutaneously
SS: simple sedimentation
TL: total length
TroCCAP: Tropical Council for Companion Animal Parasites
TW: total width
UICN: International Union for Conservation of Nature
V: Venezuela
VU: vulnerable
WA: wild animals
WB: Whole blood
WHO: World Health Organization

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Preface

The focus of herein presented doctoral thesis was to investigate the pivotal role that human-animal interphase (i. e., wildlife-/domestic animals as well as human populations) might have on infectious parasitic diseases dynamics; through a wide multistage parasitological survey in different neotropical wildlife- and domestic animal species.

A brief introduction to the topic, which highlights the current worldwide relevance that emerging and re-emerging parasite diseases have within the One Health concept, is presented as an overview to contextualise the reader. Subsequent chapter subdivision comprehends published original research articles, research letters, communications, literature reviews and manuscripts in preparation as feasible examples of human-animal interphase importance to better understand complex eco-epidemiology of parasitic diseases. All these investigations were conducted in Colombian Neotropical regions where human-wildlife interactions are increasing due to continuous anthropogenic pressure in these mega-biodiverse ecosystems, where ideal social and environmental conditions converge favouring parasitic maintenance, parasite proliferation, and transmission of a plethora of public- and animal-health concerning parasitosis.

Thereafter, a series of chapters in which the results of parasitological approaches in wild- and domestic animals published articles, international conference contributions, a literature review, and manuscripts under preparation, are summarized. Included articles showed unexpected and novel parasitological findings which are discussed both individually in each of the chapters (i. e., research articles, research letter, communication, review, and poster), and analysed toward the end of current doctoral thesis within 'discussion section' entitled "General overview and Discussion - Regarding pleasant and unexpected surprises" to finally finish with a brief conclusion and future work perspectives.

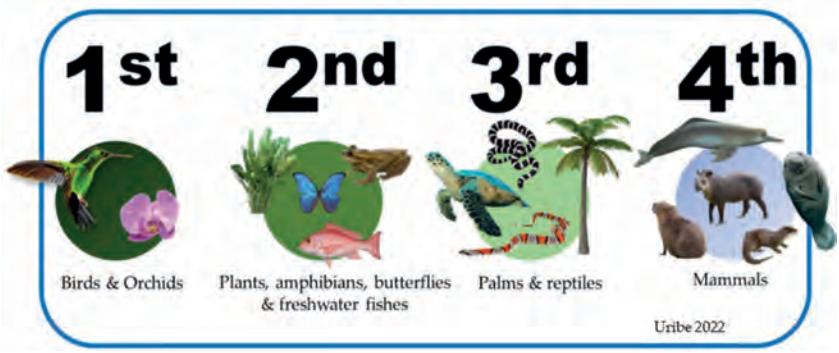
Introduction - Neotropics, biodiversity and parasites

Wildlife biodiversity has an undisputable effect on stable and healthy ecosystems in different biomes in which they inhabit, including the Neotropical realm. As such, wild animals (WA) have shown to be excellent bioindicators of important zoonotic-relevant pathogens in incrementally anthropogenic environments. Thus, WA are indirect indicators of ecosystem health since they are sentinels of some neglected anthropozoonotic ecto- and endoparasitic diseases (e. g. sarcoptosis, tungiasis, amebiasis, baylisascariosis, capillariosis, cryptosporidiosis, dipylidiasis, dirofilariosis, alveolar- and cystic echinococcosis, giardiasis, ancylostomatosis, neobalantidiosis, onchocercosis, sparganosis, strongyloidiosis, taeniasis, thelaziosis, toxocariasis, toxoplasmosis, trichinellosis, leishmaniasis and trypanosomiasis) (Brabec *et al*., 2022; Hermosilla *et al*., 2015; Mackenstedt *et al*., 2015; Odeniran and Ademola, 2016; Otranto and Deplazes, 2019; Thompson *et al*., 2009; Uribe *et al*., 2022b). As a result of continuous increase of anthropogenic pressure on fragile ecosystems, the contact of human populations with WA is constantly increasing. Therefore, it is important to know the parasite fauna occurring in Neotropical wildlife, not only to strengthen conservation plans for threatened species, but also for the generation of valuable public health information to avoid potential human infections. Since Neotropics is an extensive and highly heterogeneous region, here we selected the hinge joining key territory of Colombia because remains as a poorly investigated area for wildlife parasitology. The Colombian national territory is in the extreme north of the South American continent, holder of multiple thermal floors and life zones in which great biological wealth are reported and consequently listed as one of the 17 mega-biodiverse countries of the globe. Consequently, Colombia ranks second after Brazil in the top 5 of worldwide biodiversity index, followed by Indonesia, China and Mexico as shown in Figure 1 (Jenkins *et al*., 2013; Pimm *et al*., 2014).

Figure 1: Top 5 mega-biodiverse countries according to biodiversity index

Furthermore, regarding biodiversity and species richness within the national territory, Colombia is the world's first country in birds and orchids diversity, second in plants, amphibians, butterflies and freshwater fishes, third in palms, reptiles and finally fourth in mammalian abundance (Arbeláez-Cortés, 2013; Avendaño *et al*., 2017; DoNascimiento *et al*., 2017; Mora *et al*., 2011) (Figure 2). Hence Colombia is a territory of privileged geopolitical location and naturally diverse in a plethora of mammalian species, fishes, amphibians, reptiles, arthropods, gastropods, and plants among others.

Figure 2: Colombia's world biodiversity ranking according to different biological groups



Among the vertebrate's abundance distributed within Colombian territories, a total of 543 mammal species are reported to date in the country, thus representing the current known inventory of Colombian mammals (Ramírez-Chaves *et al*., 2022, 2016), nonetheless coupled

with lack of knowledge on ecto- and endoparasite fauna affecting them. Therefore, it seems essential to generate novel data on metazoan and protozoan parasitizing different wildlife species distributed within Colombian Neotropical territories. Thus, the focus of here presented doctoral thesis was to generate a first nationwide approaches on neglected parasite fauna present in Neotropical wild- and domestic animal populations closely related to human populations in different habitats or biomes in which they inhabit, thereby trying to cover different animal species (e. g. apex predators) and biomes along Colombian territories and being as close as possible to the real scenario. Consequently, this approach allowed us to indirectly evaluate ecosystem integrity, establishing general risk factors for public health issues through occurrence of zoonotic parasitosis, and to better understand parasite epizootiology among neglected wildlife species and synanthropic domestic animals as an unattended issue.

In recent years, large previously non- or little explored Colombian territories with low anthropogenic intervention have been opened for economic exploitation purposes by the government. Unfortunately, these political measurements have allowed not only the establishment of legal but also of illicit agro-industrial crop plantations for commercial purposes (Barrera-Ramírez *et al*., 2019), the expansion of agricultural frontiers, livestock development and excessive use of natural resources (e. g. water and soil) through illegal mining, indiscriminate logging (Unda and Etter, 2019), illegal hunting activities (Petriello and Stronza, 2019), and trafficking of wild fauna and flora (Goyes and Sollund, 2016). It is therefore of great relevance, to generate knowledge on baseline health population status of WA species throughout Colombian ecosystems. Among the different infectious agents which can infect WA are viruses, fungi, bacteria and parasites with many of the latter with zoonotic potential, i. e. transmissibility and main causes of morbidity and mortality in wildlife (Borgsteede, 1996; Mackenstedt *et al*., 2015) and humans. As a result of increasing anthropogenic pressure on fragile ecosystems, human-animal contact becomes increasingly close and more frequent, thus generating an evident biological risk of which little is known, due to limited parasitological surveys on Colombian wildlife. Thus, there are scarce and old dated literature reports on various infectious agents that Colombian wildlife may harbour.

Consistently, there are also few reports in scientific literature concerning parasitosis affecting Colombian wildlife and/or tightly linked to animal populations (Jaramillo, 2015; Uribe *et al*., 2022a, 2021c, 2021a, 2021b; Vélez *et al*., 2019, 2018).

Additionally, the harmful or deleterious effects that parasites have on wildlife population and ecosystem health are unknown in this country. Many of these parasitic species are distributed in highly anthropized areas, seeing the ecosystem integrity of the biomes they inhabit and therefore their sanitary status affected considerably (Bossart, 2011). Some of these parasitoses with clinical importance on wildlife populations include ancylostomosis (Lyons *et al*., 2011), ascariosis, neobalantidiosis, infections with various cestode species (e. g. *Diphyllobothrium* sp., *Spirometra* sp., *Adenocephalus* sp.) (Klotz *et al*., 2018; Seguel *et al*., 2018), cryptosporidiosis, dracunculiasis (Cleveland *et al*., 2018), echinococcosis, amoebiasis, sarcoptosis (Silva, 2013), schistosomiasis, giardiasis, filariasis (Keroack *et al*., 2018; Lehnert *et al*., 2016), onchocerciasis, angiostrongylosis (Spratt, 2015), sarcocystosis, piroplasmid infections such as *Babesia* sp., *Theileria* sp., *Cytauxzoon* sp. and *Rangelia* sp. (Alvarado-Rybak *et al*., 2016), toxoplasmosis, trichuriasis or trichocephalosis among others, may alter and even lead to the death of their hosts.

The increase of parasitoses in WA is directly related to the disturbance and loss of their natural habitats (Rendón-Franco *et al*., 2014). Knowledge of parasite species, including life cycles, and associated pathologies in Colombian Neotropical fauna is a subject that has so far been little studied. Therefore, profound knowledge on neglected parasitoses under One Health perspective can significantly contribute to the conservation of threatened WA as well as to prevent transmission to humans. It is noteworthy that the current doctoral thesis did not involve the direct capture or disturbance of free-living WA in the field. WA have been subjected to a variety of conservation means, which could be better monitored and managed if physiological and pathophysiological information, such as parasite infections, could already be gathered from free-ranging WA instead of carcasses (Hermosilla *et al*., 2015). Therefore, environment faecal/stools samples and/or from areas of community defecation like latrines, according to the ethology of the species, were used to indirectly evaluate

ecosystem integrity. Non- or minimally invasive sampling methods should always be implemented in WA-related investigations if possible, thereby preventing individuals from experiencing pain, distress, anguish, and avoiding alterations of health status of involved animals as proposed elsewhere (Ebmer *et al*., 2020; Hermosilla *et al*., 2018, 2015; Kleinertz *et al*., 2014; Uribe *et al*., 2021a). Thus, physical appearance or body weight were not affected in sampled WA, and above all neither sedation nor euthanasia protocols were here used. The national laws that govern the duty and the correct exercise of the Doctor Veterinary Medicine (DVM) profession in the Colombian national territory were as well considered. Furthermore, the Directive *2010/63/EU* of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals used for Scientific Purposes (Unión Europea, 2010), and the Guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes, 2016; Sikes and Gannon, 2011) were here considered. Any procedure or clinical decision arising from this research proposal was conducted under the light and scope of the same DVM profession in Colombian- and German national territories. In fact, for the execution of this proposal research we had already the approval of the Ethics Committee for Animal Experimentation (CEEA) of the Universidad de Antioquia, Colombia, through the minutes of session No. 131 of the 11th of February 2020 and the No. 132 of 14th of April 2020, respectively. Only in fortuitous and sporadic cases the samples were collected from dead, road-killed, euthanized animals and/or necropsies performed by national environmental entities, private veterinary doctors and/or non-governmental organizations (NGOs).

As already stated above, large Colombian geographic areas of little exploration and corresponding low anthropogenic intervention, have so far been opened for national or international timber industry. Thus, contemporary human-related defaunation has reduced food web complexity and therefore undergoing steep regional declines of biodiversity through the loss of food web links after the arrival and expansion of human populations into these areas (Fricke *et al*., 2022). These geographic areas do not have complete inventories of fauna and flora due to difficult access for government, private entities, and independent researchers. In addition, difficult terrain conditions, the ongoing armed conflict and public

order difficulties still present in Colombia, have significantly impeded research activities in these territories. Until now, effective multistage efforts have begun to elucidate the ecological and conservation status of these emblematic areas. All these adverse factors have hindered the generation of new knowledge about the status and parasitological distribution on different wildlife species along these relatively pristine areas. It is therefore of great relevance to know the sanitary status of a multiplicity of wild species associated with both continental and oceanic, coastal, and marine water resources throughout the Colombian national territory (Vélez *et al*., 2019, 2018). Among the different infectious agents that can affect the health status of endemic wildlife are viruses, fungi, bacteria and parasites, and many of them with zoonotic transmissibility (Mackenstedt *et al*., 2015) and being considered as main causes of mortality in WA (Borgsteede, 1996). Colombia has 213 genera and 543 formerly described mammal species, of which 65 are endemic as shown in Table 1 (Ramírez-Chaves *et al*., 2022, 2016).

Table 1: A complete summary of the taxonomic representation and endemic mammal species of Colombia

Order	Families	Genera	Species	Endemic
Didelphimorphia	1	12	39	2
Paucituberculata	1	1	2	0
Cingulata	2	3	6	0
Pilosa	4	5	8	0
Sirenia	1	1	2	0
Eulipotyphla	1	1	8	5
Chiroptera	9	67	217	9
Carnivora	8	24	35	0
Perissodactyla	1	1	3	0
Artiodactyla	7	24	42	0
Primates	4	15	38	10
Rodentia	10	58	137	34
Lagomorpha	1	1	6	5
Total	50	213	543	65

From: (Ramírez-Chaves *et al*., 2022).

In Colombia, the harmful or deleterious impact that parasitoses might have on wildlife health and therefore on ecosystems are still unknown. Hence, not only the understanding

of biological, behavioural and distribution level of WA parasitoses is of importance but also the better understanding of parasite-derived pathogenicity. In particular apex predators are essential players in the biodiversity composition of food web (Brabec *et al*., 2022; Uribe *et al*., 2021c). Consistently, apex predators can be considered as important sentinels of infectious agents of public health concern (Bossart, 2011; Hermosilla *et al*., 2018, 2015; Hunt *et al*., 2008), including zoonotic water-borne infections (Waltzek *et al*., 2012), thereby becoming ideal indirect bioindicators of water pollution, semi-aquatic- and terrestrial ecosystems in which they inhabit (Baskin, 2006; Brabec *et al*., 2022; Nelms *et al*., 2019; Uribe *et al*., 2022a; Vélez *et al*., 2019). Unfortunately, many of these Colombian endemic apex species are currently distributed in highly anthropic areas, with fragile ecosystems. The integrity of these fragile biomes, including biodiversity of endemic flora- and fauna-species, are thus essential for preservation purposes (Bossart, 2011; Hermosilla *et al*., 2015). However, in Colombian wildlife only macroscopic parasitic arthropods and platyhelminths have so far been the most frequently investigated in scarce existing scientific literature (González-Astudillo and Gillespie, 2016) but practically nothing is yet known on protozoan parasites within these fragile ecosystems.

Moreover, free-ranging WA have also been shown to be effective bioindicators of ecosystem health concerning chemical contaminants, toxins and plastic in both terrestrial- and aquatic biomes they inhabit and indirectly tightly linked to human populations in these areas (Bossart, 2011; Hernández-González *et al*., 2018; Jepson *et al*., 2016; Lusher *et al*., 2018; Uribe *et al*., 2021a). Consistently, in other parts of the world microplastic particles and fibres have been detected in free-ranging grey seals (*Halichoerus grypus*) along the British coast line (Nelms *et al*., 2019) and potential bioaccumulation in the food web chain with repercussions for higher marine predators have been found (Nelms *et al*., 2018). Microplastic polymers of polyethylene terephthalate (PED) and polyamide (PA) have also been found in faeces of Eurasian otters (*Lutra lutra*) (Smiroldo *et al*., 2019). An indirect indication of general contamination and animals' health status is the occurrence of certain parasites (both endo- and ectoparasites), since some of them exclusively depend on the presence of secondary metabolites accumulating in host tissues to survive.

Many highly pathogenic parasites are among the main morbidity and mortality causes in WA (Borgsteede, 1996). Some of these parasitoses can be zoonotic and therefore acquire constant surveillance from a public health perspective (Mackenstedt *et al*., 2015; Uribe *et al*., 2022a, 2021c). The increase in WA parasitic diseases is directly related to the ecosystem disturbance and the habitat loss (Fricke *et al*., 2022; Rendón-Franco *et al*., 2014). Thus, better comprehension of these circulating parasites in WA actively contributes to wild animals' conservation programs, and additionally to better understand their real impact on public health and livestock industry. That is why wildlife-related parasitological approaches will provide valuable information to design and develop sustainable strategic conservation plans not only in the Colombian neotropics but also in the tropics, subtropics and even with applicability to other ecosystems and latitudes.

Nationwide investigations on neglected gastrointestinal parasite genera (e. g. *Spirometra, Oncicola, Plagiorchis, Neobalantidium*) of various free-living WA [i. e. jaguars (*Panthera onca*), pumas (*Puma concolor*), ocelots (*Leopardus pardalis*), jaguarundis (*Herpailurus yagouaroundi*), capybaras (*Hydrochoerus hydrochaeris*), crab-eating foxes (*Cerdocyon thous*) and bush dogs (*Speothus venaticus*) as well as re-emerging metastrongyloid angio- neurotropic nematodes (i. e. *Angiostrongylus vasorum* and *Gurltia paralysans*) of domestic dogs and cats, are included in the current doctoral thesis. Individual chapters containing respective published articles are summarized below in an organized manner to present herein obtained results. Each one of these chapters are citable original research articles already published on open-access peer-reviewed international journals.

Chapter 1 - Intestinal Parasites of Neotropical Wild Jaguars, Pumas, Ocelots, and Jaguarundis in Colombia: Old Friends Brought Back from Oblivion and New Insights.

This chapter is based on the following published research article:

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

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Project planning: as far as possible

Conceptualization: essential

Methodology: essential

Sample collection: essential

Validation: as far as possible

Formal analysis: essential

Investigation: essential

Writing—original draft preparation: essential

Writing—review and editing: as far as possible

Visualization: essential

pathogens

MDPI

Article

Intestinal Parasites of Neotropical Wild Jaguars, Pumas, Ocelots, and Jaguarundis in Colombia: Old Friends Brought Back from Oblivion and New Insights

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Abstract: Neotropical wild felids (NWF) are obligate carnivore species present in Central and South America, and some are considered endangered due to constantly decreasing populations. NWF can become infected by a wide range of protozoan and metazoan parasites, some of them affecting their health conditions and others having anthropozoonotic relevance. Parasitological studies on NWF are still very scarce, and most data originated from dead or captive animals. On this account, the current study aimed to characterize gastrointestinal parasites of free-ranging jaguars (*Panthera onca*), pumas (*Puma concolor*), ocelots (*Leopardus pardalis*), and jaguarundis (*Herpailurus yagouaroundi*), i.e., four out of six NWF species endemic to Colombia. Fecal samples from jaguars ($n = 10$) and ocelots ($n = 4$) were collected between 2012 and 2017 as part of the Jaguar Corridor Initiative from six geographic locations in Colombia. In addition, cestode specimens were obtained during puma and jaguarundi necropsies. Scat samples were processed by standardized sodium acetate-acetic acid-formalin (SAF), sedimentation, and flotation techniques and by carbol fuchsin-stained fecal smears. Morphological evaluation of feces showed the presence of one cestode (*Spirometra* sp.), a nematode (*Toxocara cati*), an acanthocephalan (*Oncicola* sp.), and one cyst-forming coccidian (*Cystoisospora*-like oocysts). Feces oocysts were submitted to a *Toxoplasma gondii*-specific PCR for species identification, but no product was amplified. The cestodes isolated from a puma and jaguarundi were molecularly characterized by sequencing cytochrome c oxidase subunit I, identifying them as *Taenia omissa* and as a *T. omissa* sister lineage, respectively. These results collectively demonstrate the potential role of NWF as natural reservoir hosts for neglected zoonotic parasites (e.g., *Spirometra* sp., *T. cati*) and highlight their possible role in parasite transmission to human communities. Due to public health concerns, the occurrence of these parasites should be monitored in the future for appropriate zoonotic management practices in conservation strategies and wild felid health management programs.

Keywords: jaguar; puma; ocelot; jaguarundi; *Spirometra* sp.; *Toxocara cati*; *Oncicola* sp.; *Cystoisospora* sp.; *Taenia omissa*

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1. Introduction

The family Felidae (order: Carnivora) is currently composed of 45 recognized non-hybrid extant wild species with a worldwide distribution throughout all biomes except the Antarctic polar ice caps and insular Oceania [1,2]. All members are obligate carnivores acting as apex predators or mesocarnivores in many terrestrial ecosystems. Large wild felids serve as effective umbrella and keystone species, contributing to maintaining and

regulating associated biodiversity and ecosystems where they occur [3]. Neotropical wild felids (NWF) are well-known hosts of important zoonotic protozoan parasites, such as *Toxoplasma gondii* [4,5], *Cryptosporidium* sp., and *Giardia* sp. [6,7] and are often reservoirs of hemoparasites such as *Trypanosoma cruzi* [8] and tick-borne piroplasmids such as *Babesia* sp., *Cytauxzoon felis* [9], and *Anaplasma* sp. [10]. Moreover, the presence of metazoan parasites has also been reported in non-domestic NWF, showing them as feasible hosts of gastropod-borne metastrongyloid lungworms [11,12] or *Dirofilaria immitis*, the causative agent of heartworm disease [13]. Other helminths, for instance, hookworms [14], trematodes [15], and cestodes, [13,16–18] have also been reported in non-domestic wild felids as well as ectoparasites like ticks, mites, and fleas [19]. The sophisticated ways in which parasite life cycles have evolved to ensure transmission involve complex interactions with vertebrate and invertebrate hosts, and parasite assemblage reflects the host's trophic position within the food web [20]. Thus, parasite populations and communities are useful indicators of environmental stress, food web structure, and biodiversity [20,21]. The neotropics are the most diverse region with the largest number of animal species in the world [22,23], felids not being the exception. Colombia is home to six species of NWF (Table 1). Several of these species co-occur or are wholly sympatric; for example, puma, jaguarundi, and ocelot are sympatric to jaguar ranges in Colombia, but not necessarily the other way round [24–26].

Table 1. Neotropical wild felid (NWF) species of Colombia.

			Risk Classification		
Genus	**Species**	**Common Name**	**CITES** [a]	**UICN** [b]	**National** [b]
Herpailurus	*yagouaroundi* *	Jaguarundi, Eyra cat	Appx II	LC	NE
Leopardus	*pardalis* *	Ocelot	Appx I	LC	NE
Leopardus	*wiedii*	Margay, Tree ocelot	Appx I	NT	NE
Leopardus	*tigrinus*	Northern tiger cat	Appx I	VU	NE
Panthera	*onca* *	Jaguar	Appx I	NT	VU
Puma	*concolor* *	Puma, Cougar	Appx I	LC	NE

* Species included in the current study. [a] All appendix I species are threatened with extinction. [b] LC: least concern; NT: near threatened; VU: vulnerable; NE: not evaluated.

The potential multiplicity of NWF parasite species has never been evaluated in the unique Central and South American hinge-joining key territory of Colombia. Despite numerous data on the ecology and biology of non-domestic felids in Colombia [25,27,28], little is known about free-ranging NWF-associated infectious diseases (e.g., virus, bacteria, fungi) and their parasite fauna. Additionally, parasite surveillance in natural ecological systems is an important tool to understand wildlife health, parasite biodiversity, ecology, and conservation [29]. Hence, the current study aims to present the first description of gastrointestinal parasite fauna from free-ranging jaguars, pumas, ocelots, and jaguarundis at eight sampling locations in Colombia through copromicroscopic and necropsy-based approaches on detailed morphology and further molecular identification.

2. Results

2.1. Copromicroscopical Evaluation

Parasitological evaluation of jaguar (*P. onca*) and ocelot (*L. pardalis*) faeces through basic coprological standard techniques simultaneously evidenced three metazoan parasite taxa belonging to Platyhelminthes and Acanthocephala, plus a protozoan of the phylum Alveolata (Subphylum: Apicomplexa). A high infection rate (~36%; 5/14) of cestode eggs belonging to *Spirometra* sp. was found (Figure 1a). The oval-shaped diphyllobothriid eggs corresponded to *Spirometra* sp. These asymmetric yellowish eggs showed a slightly distinct operculum at the cone-shaped pole (Figure 1b). Furthermore, we also identified golden, slightly pear-shaped ascarid-type eggs with characteristic thick-pitted eggshells (Figure 1c). Therefore, the traits of ascarid-type eggs depicted above corresponded well to the zoonotic nematode *Toxocara cati*. Additionally, pale and slightly oval eggs of *Oncicola* sp., with a delicate external membrane, were detected. Finally, un-

sporulated *Cystoisospora*-like oocysts (Sarcocystidae) were also identified in jaguar and ocelot scat samples (Figure 1d,e, respectively).

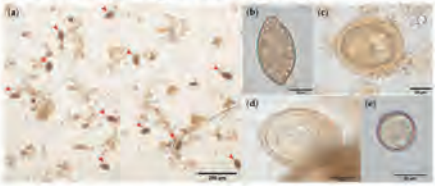

Figure 1. Illustrations of parasite eggs detected in faecal samples of free-ranging jaguars and ocelots: **(a)** High number of *Spirometra* sp. eggs; **(b)** Single *Spirometra* sp. egg (60.72 µm × 33.38 µm); **(c)** Non-embryonated *Toxocara cati* egg (63.86 µm × 53.43 µm) carrying a zygote; **(d)** *Oncicola* sp. egg (64.30 µm × 46.68 µm); **(e)** Un-sporulated *Cystoisospora*-like oocyst (12 µm × 12 µm; Sarcocystidae). Scale-bars: **(a)** 200 µm; **(b–d)** 20 µm; **(e)** 10 µm.

2.2. *Cestode Identification and Characterization of Rostellar Hooks*

The macroscopical analyses of helminth specimens collected from the small intestine of a puma (*P. concolor*) and a jaguarundi (*H. yagouaroundi*) evidenced the presence of taeniid cestodes in both felids during necropsy procedures. Both cestodes presented a ribbon-like strobila with many proglottids. Immature and mature proglottids were wider than longer, increasing in length towards the posterior part. Additionally, two rows of hooks in a well-developed rostellum were noticed. All rostellar hooks of anterior row were larger and alternated with those of second row, which were consistently smaller (please refer to Videos S1 and S2). Armed rostellum evidences a total of 48 hooks. The basic morphological measures of large and small hooks (n = 24, n = 21, respectively) were: 282.64 µm and 205.31 µm total length (TL), 135.26 µm and 99.57 µm total width (TW), 202.89 µm and 164.62 µm blade length (BL), 135.59 µm and 97.69 µm apical length (AL), 59.28 µm and 45.34 µm guard length (GL), 47.49 µm and 38.86 µm guard width (GW), 39.32 µm and 27.46 µm blade curvature (BC), and 45.03 µm and 22.07 µm handle (HW). Along the strobila, each proglottid showed marginal alternating irregular genital pores, demonstrating the presence of a single set of reproductive organs (Figure 2). These morphological traits correspond well to the cyclophyllidean genus *Taenia*.

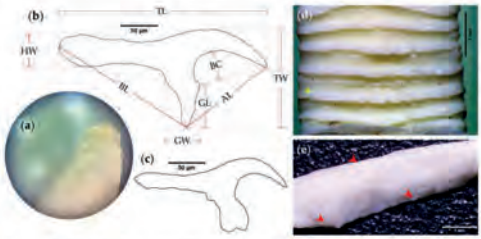

Figure 2. Morphological traits of putative *Taenia omissa* specimens. **(a)** Scolex photograph of *T. omissa* obtained from puma; **(b)** Large and **(c)** small rostellar hooks outline drawings of adult *Taenia* sp. specimen from jaguarundi gastrointestinal tract (ileum); **(d)** jaguarundi, and **(e)** puma cestodes ribbon-like strobila (red arrows indicate genital pores). TL: total length, TW: total width, BL: blade length, AL: apical length, GL: guard length, GW: guard width, BC: blade curvature, HW: handle width. Scale-bars: **(b,c)** 50 µm; **(d,e)** 1 mm.

In order to identify the species of adult cestodes found in puma and jaguarundi, 858 bp-long fragments of cytochrome c oxidase subunit I (COI) gene of both specimens were characterized and subjected to phylogenetic analysis. Representative COI sequences of relevant *Taenia* species including all specimens reported from felids were included. The specimen from puma clustered within the lineage composed of representatives of *Taenia omissa*, while the jaguarundi isolate formed a sister lineage to them (Figure 3).

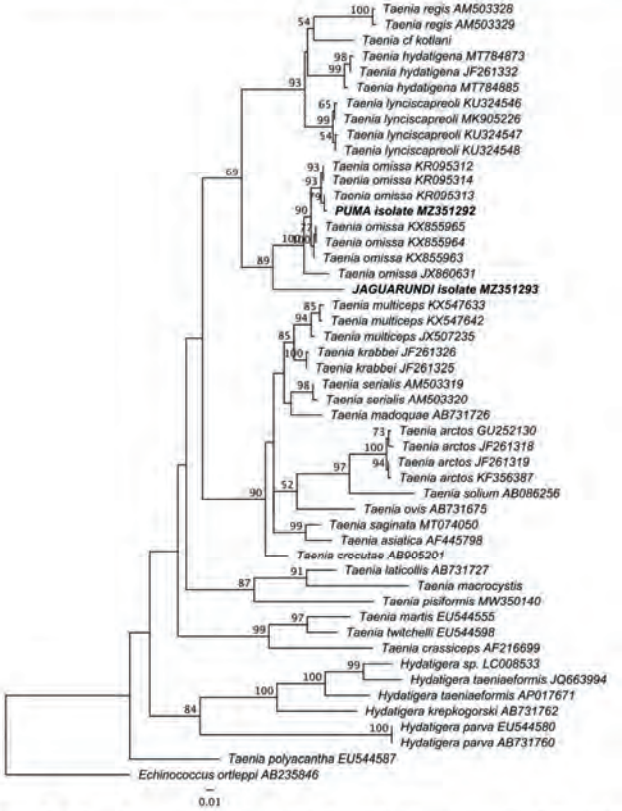

Figure 3. Phylogenetic position of *Taenia* sp. isolates obtained from puma and jaguarundi. Maximum likelihood tree from IQ-Tree based on cytochrome c oxidase subunit I gene sequences analyzed as single partition using GTR + F + I + G4 model selected according to corrected Akaike information criterion. Nodal values show standard bootstrap supports above 50 (100 replicates). Specimens collected from puma and jaguarundi are shown in bold. GenBank accessions are given after taxa names. The branch length scale bar indicates number of substitutions per site.

2.3. *Toxoplasma gondii* PCR

None of the jaguar nor ocelot faecal oocyst samples analyzed showed effective amplification of the *T. gondii*-specific 529 bp DNA repetitive fragment. Nevertheless, both the internal and positive controls of each test amplified normally, and negative controls never showed amplification.

3. Discussion

Since the vast majority of available data on wild felid parasite fauna come from captive, deceased, or highly anthropized individuals, and data on free-ranging NWF are scarce [30], the findings presented here constitute an important contribution to baseline understanding of the parasite fauna harbored by free-ranging wild felids (~67% of species) of Colombia. Agricultural expansion negatively impacts the occupancy of wild felid communities across human-modified landscapes [31,32], and these adverse anthropogenic factors may in turn influence their respective parasite communities. Thus, more frequent domestic animal–human–wildlife interface favors a plethora of infectious pathogens to emerge, spread, cross species barriers, and eventually evolve [14,33]. Here, we describe free-ranging wild felid parasites, including zoonotic parasites, heightening the importance of NWF living in human-modified landscapes and highlighting the need for appropriate zoonotic management practices in wild felid health management programs, due to public health concern and conservation.

Sparganosis is a globally distributed neglected water- and food-borne disease caused by larval stages of *Spirometra* sp. located in various human body tissues [34]. The occurrence of *Spirometra* infections across South America has been reported since the beginning of the 20th century [35]. This cestode has been previously recorded in Geoffroy's cat (*Leopardus geoffroyi*), puma, and jaguarundi in western Paraguay [36]; the guiña (*Leopardus guigna*) in Chile [37]; and jaguar, puma, and margay (*Leopardus wiedii*) in Brazil [16,38]. Additionally, ocelots from Peru [39] and Brazil [38] have been shown to represent feasible definitive hosts of *Spirometra* sp. To the best of our knowledge, we report *Spirometra* sp. here for the first time in Colombian free-ranging jaguars and ocelots. Human cases of sparganosis have been previously reported in South America [40–42], but to date there is only a single six-decades-old case report from Colombia [43]. In addition, ascarid nematodes were shown to be broadly prevalent in some wild felids, and *T. cati* is the dominant parasite in some of them due to its complex life cycle, including lactogenic transmission and a wide array of paratenic hosts (e.g., rodents) [44]. We report *T. cati* in jaguars and ocelots, highlighting again the potential role of NWF in parasite transmission to local human communities. Despite the worldwide distribution of anthropozoonotic *T. cati* and its endemicity in most American countries [45], feline as well as human toxocarosis is still poorly understood in Colombian rural areas, since most studies have been conducted in urban areas, including large cities of the country [46].

Despite *T. gondii* negative molecular assays, here we describe un-sporulated *Cystoisospora*-like oocysts in wild jaguars and ocelots. Since there are at least eight *Eimeria* species described previously in felids as spurious findings, meaning that identified *Eimeria* oocysts belonged to prey animals and passed through the felid's gut into faeces [47], it would be recommended to posteriorly identify if *Cystoisospora*-type oocysts reported here belonged to *Cystoisospora rivolta*, *Hammondia hammondi*, or *Besnoitia* spp., frequently reported as cyst-forming coccidians in domestic and wild felids [48]. Furthermore, the cyclophyllidean cestode *T. omissa*, which was firstly reported in 1910 [49], was also evidenced during necropsy procedures of the deceased puma. To date, *T. omissa* molecular data information is restricted to reports in natural intermediate hosts such as domesticated alpacas (*Vicugna pacos*) [50] and free-living red brockets (*Mazama americana*). Meanwhile, puma [51] and Eurasian lynx (*Lynx lynx*) [18] have also been reported as *T. omissa*-definitive hosts. Therefore, the present study enlarges the sequence data for this tapeworm of felids, expands the geographical distribution range of *T. omissa* to Colombia, and adds jaguarundi as a new definitive host for an uncharacterised sister linage of *T. omissa*. In comparison to

faeces, carcass evaluation increases the chance of parasite detection. The copromicroscopic detection of *Taenia* sp. eggs tends to be of lower sensitivity when compared to carcass evaluation, since taeniid eggs are usually passed within mature proglottids, and show intermittent shedding analogously to other cestodes. [52]. The acanthocephalan genus *Oncicola*, consisting of twenty-four recognized species, has been circulating in South American felines for almost 9000 years [53,54]. Some scattered reports of *O. canis*, *O. oncicola*, and *O. venezualensis* have been reported in jaguars, ocelots, pumas, and margays across the American continent [13,55–60]. We report this parasite genus in free-ranging jaguars and ocelots in Colombia for the first time since 1968 [61].

Based on the fact that parasites are associated with retarded growth, reproductive disorders, tissue damage, inflammation, and mortality in wildlife [14], constant parasitological investigations of Colombian NWF are needed. This should be considered not only for conservation strategies and wild felid health management programs but also for public health concerns. For a better comprehension of parasite fauna infecting free-ranging NWF in Colombia, we also encourage further studies of the highly arboreal margay and the rare Northern tiger cat (*L. tigrinus*) to complement baseline data for the complete set of six endemic species reported to date in Colombian territories. Likewise, for comparative purposes, further parasitological surveys of the species included in the present study (i.e., jaguar, puma, ocelot, and jaguarundi) should be performed at a larger sample size in different biomes and seasons throughout the American continent. Finally, since the indirect life cycles of *Spirometra* sp. and *T. cati* require two to three hosts, including humans as aberrant hosts, it is desirable to analyze potential intermediate hosts (e.g., tetrapods, invertebrates, and copepods) in agricultural and semi-aquatic landscapes for a better understanding of these neglected parasites in the tropics and to delineate appropriate zoonotic health management practices to avoid human infections. Intriguing felid metastrongyloid cardiopulmonary nematodes have become spotlighted in the parasitology of wild felids [12,62]. Thus, we encourage future studies on epizootiological drivers of feline , aelurostrongylosis, angiostrongylosis, crenosomosis, gurltiosis, and troglostrongylosis in NWF [12,37,63], as these parasitoses have been discredited in populations of wild felids [11,37].

4. Materials and Methods

4.1. Study Area

The current study was conducted across the highly heterogeneous Colombian biomes of the Andean, Amazonian, and Orinoquía regions. Based on the Köppen–Geiger climate classification [64], the eight sampling geographic locations included in the present study belong to tropical monsoon (Am), tropical rainforest (Af), and tropical wet and dry climate (Aw) (see Table 2). The jaguar and ocelot faecal samples were collected from three locations in Santander, and one from Antioquia, Casanare, and Córdoba departments, respectively. Furthermore, cestode specimens (i.e., scolex, strobila, and proglottids) were collected from a deceased wild puma in Caquetá and a road-killed jaguarundi in Cundinamarca (refer to Figure 4).

Table 2. Detailed sampling areas and climate classification.

Department	Municipality	Sampling Location	Climate [a]	Sample Type
Antioquia	Yondó	Ciénaga de Barbacoas	Am Af	Feces [◇]
Caquetá	San José del Fragua	Puerto bello	Af	Metazoan [Δ]
Casanare	Hato Corozal	La Chapa	Am	Feces [◇]
Córdoba	Puerto Libertador	La Esmeralda	Af	Feces [◇]
Cundinamarca	-	-	Aw	Metazoan [◊]
Santander	El Hato	Las Pampas	Af	Feces [□]
Santander	Puerto Wilches	Las Palmas	Am	Feces [□]
Santander	Puerto Wilches	Caño Limón	Am	Feces [□]

[a] Köppen–Geiger Am: Tropical monsoon; Af: Tropical rainforest; Aw: Tropical wet and dry. [◇] Jaguar (*Panthera onca*) faeces. [□] Ocelot (*Leopardus pardalis*) faeces. [Δ] Puma (*Puma concolor*) collected helminths. [◊] Jaguarundi (*Herpailurus yagouaroundi*) collected helminths.

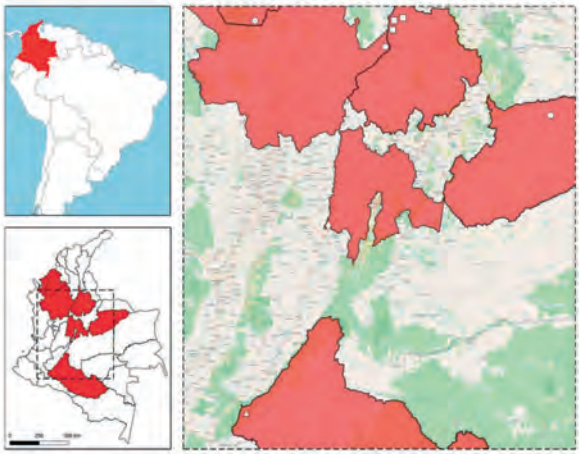

Figure 4. Geographical locality of sampled free-ranging neotropical wild felids (NWF).

4.2. Sample Collection and Laboratory Procedures

The current study includes free-ranging wild jaguar (n = 10) and ocelot (n = 4) faecal samples collected between 2012 and 2017 as part of the Jaguar Corridor Initiative, a conservation and monitoring program carried out by the Panthera organization across the jaguar's range [65]. Collected faeces came from direct sampling sites of trails that were regularly monitored by trap cameras (refer to Figure 5). Faeces were identified by associated tracks and followed the general features and morphometric characteristics of wild felid depositions [66]. The traditional ecological knowledge of locals was also very helpful for monitoring and sampling these reclusive individuals [67]. Once identified in the field, well-formed faeces were dry preserved and fixed in 70% EtOH until subsequent copromicroscopic evaluation, as recommended for challenging tropical environments [68]. Furthermore, we collected a cestode sample serendipitously during the necropsy of a young road-killed wild jaguarundi male in Cundinamarca. The cestode specimen was carefully extracted from the ileum. Additionally, free cestode proglottids and whole tapeworms firmly attached by their scolex to the jejunum mucosa of an adult deceased female puma at Caquetá were collected. Cestode specimens were gently rinsed in physiological buffered saline solution (PBS) and thereafter preserved in 96% EtOH until further molecular evaluation. All sampling procedures were performed in agreement with the Guidelines of the American Society of Mammologists for the Use of Wild Mammals in Research and Education [69,70], the EU Directive 2010/63/EU, and the final approval of the Ethics Committee for Animal Experimentation of the Universidad de Antioquia (AS No. 132) under collection permit No. 0524 of 2014 (IDB0321), Colombia.

4.2.1. Basic Copromicroscopic Analyses

Since there is no single copromicroscopic method to diagnose all parasitic stages concomitantly, the jaguar and ocelot faeces examination was performed by means of the following qualitative techniques for cysts, oocysts, eggs, and parasite larvae detection to optimize data collection: modified sodium-acetate aceticacid formaldehyde (SAF) technique [71], simple sedimentation technique [72], zinc sulfate centrifugal flotation technique,

and fast carbol-fuchsin stained faecal smears [73]. The parasitic specimens were identified through morphometry under an Olympus BX53 (Olympus Corporation, Tokyo, Japan) semi-motorized direct light microscope (100×, 400×, and 1000×) equipped with an Olympus DP74 (Olympus Corporation, Tokyo, Japan) digital camera using the *cellSens* standard imaging software (Olympus Corporation, Tokyo, Japan). The parasites' identification was based on general morphology, shape, size, and color, according to Deplazes et al. (2016) [74].

Figure 5. Camera trap-images of monitored free-ranging neotropical wild felids (NWF) of the present study: (**a**) jaguar female (*Panthera onca*) with two cubs; (**b**) adult ocelot (*Leopardus pardalis*); (**c**) puma (*Puma concolor*); (**d**) jaguarundi (*Herpailurus yagouaroundi*).

4.2.2. Molecular Phylogenetics

Adult cestodes obtained from the jejunum of the female puma and the ileum of the male jaguarundi were carefully removed from the epithelium and/or lumen of the small intestine, trying to preserve the scolex, strobila, and proglottids integrity. The obtained helminths were first photographed using a stereomicroscope (Nikon SMZ25R, Tokyo, Japan). Amplification of partial cytochrome c oxidase subunit I (COI) was achieved with primers JB3 [75] and Cox1R [76] using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Inc., Ipswich, USA) and the following cycling conditions: 35 cycles of 10 s at 98 °C, 15 s at 60 °C, and 50 s at 72 °C. PCR products were gel-checked, purified with Exonuclease I and FastAP alkaline phosphatase (Thermo Fisher Scientific, Waltham, USA), and directly Sanger-sequenced at SeqMe (Dobříš, Czech Republic). Contiguous gene sequences were assembled and inspected for errors in Geneious 7.1.9 (http://www.geneious.com, accessed on 3 June 2021 [77]). COI coding sequences were aligned using MAFFT's L-INS-i [78] translational align of Geneious. The phylogenetic tree was estimated by maximum likelihood in IQ-TREE 1.6.5 [79]. The best-fitting model of nucleotide evolution was chosen according to the corrected Akaike information criterion in IQ-TREE ([80,81]) and nodal supports estimated through running 100 standard nonparametric bootstrap replicates.

4.2.3. Faecal DNA Isolation and *Toxoplasma gondii* Molecular Evaluation

Total DNA isolation was performed using the Class II type B2 BSC and the DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands) following manufacturer's instructions. A

200- to 300-fold repetitive 529 bp DNA fragment conserved among 60 strains and more sensitive than B1 gene was used for *T. gondii* molecular detection. Amplification of repeated fragments was performed using Toxo4 and Toxo5 primers set under previously described conditions [82]. Tachyzoites of the *T. gondii* RH- and ME49 strain were used as positive DNA controls.

Supplementary Materials: The following material is available online at https://www.mdpi.com/article/10.3390/pathogens10070822/s1, Video S1: 3D model of *T. omissa* rostellar large hook, Video S2: 3D model of *T. omissa* rostellar small hook.

Author Contributions: Conceptualization, M.U., E.P., C.H. and J.J.C.-G.; methodology, M.U., E.P. and J.B.; sample collection, E.P. and M.U.; validation, M.U. and J.B.; formal analysis, M.U. and J.B.; investigation, M.U.; resources, E.P., J.B. and J.J.C.-G.; writing—original draft preparation, M.U.; writing—review and editing, C.H., J.B., E.P., J.V. and J.J.C.-G.; visualization, M.U.; supervision, J.J.C.-G. and C.H.; funding acquisition, J.J.C.-G., J.B. and A.T. All authors have read and agreed to the final published version of the manuscript and authorship is limited to those who have contributed substantially to the work reported.

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Institutional Review Board Statement: The current study was conducted according to the Guidelines of the American Society of Mammologists for the use of wild mammals in research and education, the EU Directive 2010/63/EU, and approved by the Ethics Committee for Animal Experimentation of Universidad de Antioquia (AS No. 132) under collection permit No. 0524 of 2014 (IDB0321).

Informed Consent Statement: Not applicable.

Data Availability Statement: The putative *Taenia omissa* sequences obtained from puma (*Puma concolor*) [PUM_CPB_Fem_20] and jaguarundi (*Herpailurus yagouaroundi*) [YAG_CG_Male19] were deposited in GenBank database (National Center for Biotechnology Information, NIH, Bethesda, USA) and are available at ENA (European Nucleotide Archive) in Europe and the DDBJ (DNA Data Bank of Japan) under accession numbers MZ351292 and MZ351293, respectively.

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Conflicts of Interest: The authors declare no conflict of interest. No funder had any role in the design of the study, in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Chapter 2 - Parasites Circulating in Wild Synanthropic Capybaras (*Hydrochoerus hydrochaeris*): A One Health Approach.

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Article

Parasites Circulating in Wild Synanthropic Capybaras (*Hydrochoerus hydrochaeris*): A One Health Approach

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Abstract: Capybaras (*Hydrochoerus hydrochaeris*) are affected by a wide range of protozoan and metazoan-derived parasitic diseases. Among parasites of free-ranging capybaras are soil-, water-, food- and gastropod-borne parasitosis, today considered as opportunistic infections in semiaquatic ecosystems. The overlapping of the capybara's natural ecological habitats with human and domestic animal activities has unfortunately increased in recent decades, thereby enhancing possible cross- or spillover events of zoonotic parasites. Due to this, three synanthropic wild capybara populations in the Orinoco Basin were studied for the occurrence of gastrointestinal parasite infections. A total of forty-six fecal samples were collected from free-ranging capybaras in close proximity to livestock farms. Macroscopical analyses, standard copromicroscopical techniques, coproELISA, PCR, and phylogenetic analysis revealed thirteen parasite taxa. In detail, the study indicates stages of five protozoans, four nematodes, one cestode, and three trematodes. Two zoonotic parasites were identified (i.e., *Plagorchis muris*, and *Neobalantidium coli*). The trematode *P. muris* represents the first report within South America. In addition, this report expands the geographical distribution range of echinocoelosis (*Echinocoleus hydrochoeri*). Overall, parasitological findings include two new host records (i.e., *P. muris*, and *Entamoeba*). The present findings collectively constitute baseline data for future monitoring of wildlife-derived anthropozoonotic parasites and call for future research on the health and the ecological impact of this largest semiaquatic rodent closely linked to humans, domestic and wild animals.

Keywords: capybara; *Echinocoleus hydrochoeri*; *Plagiorchis muris*; *Neobalantidium coli*; *Cryptosporidium*; zoonoses; rodents

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1. Introduction

The Rodentia order is the most numerous and diverse within the Mammalia class composing up to 42% of worldwide mammalian biodiversity with at least 2277 species reported so far [1]. Rodents are distributed all over the continents except for Antarctica [2]. Capybaras (*Hydrochoerus*, Rodentia: Caviidae) are New World semiaquatic and herbivorous hystricomorphs, which represent the world's largest and heaviest species among extant rodents [3]. The genus *Hydrochoerus* includes two species, namely *H. hydrochaeris* and *H. isthmius*. The first one is conspicuously larger and broadly distributed across South America, and the second one is an endemic species of Colombia, Panama, and Venezuela [4]. The biology of this giant rodent is closely linked to water sources such as rivers, swamps, natural lakes, and manmade water cumulus for domestic animal usage [5].

Moreover, capybaras are synanthropic species distributed in riparian habitats with strong anthropogenic impact and representing an important bushmeat source for traditional communities [6]. Accordingly, the Orinoco River constitutes a huge tropical watershed with extensive wetlands, marshes, and lakes nourished by the fourth largest river in the world. Unfortunately, this diverse and unique ecosystem has suffered dramatic anthropogenic changes during the last decades because of legal and illegal agroindustrial crop development [7], the expansion of the agricultural frontier due to livestock, illegal mining, logging [8], hunting [6], and wildlife trafficking [9]. Thus, free-ranging capybaras distributed in the human-altered Orinoco Basin are constantly interacting with a plethora of endemic wildlife species, humans, and domestic animals such as horses (*Equus caballus*), cattle (*Bos taurus indicus*), chickens (*Gallus gallus domesticus*), dogs (*Canis familiaris*), and cats (*Felis catus*). Thus, this demands One Health concept-oriented attention on parasitic infections. In this sense, a particular interplay between humans, animals, and the environment may lead to the emergence and transmission of zoonotic diseases as is highlighted by the increased transmission of wildlife-origin pathogenic agents [10]. Consistently, it seems essential to monitor parasitic infections in synanthropic species such as capybaras within their natural habitats, to identify potential zoonotic parasites and their impact not only on human health but also on domestic animals and endemic wildlife populations.

Currently, both endogenous proto- and metazoan parasites have been described in capybaras (Table 1) but little attention has been paid to zoonotic infections, particularly soil-, water-, food- and gasteropod-borne parasites. Therefore, the present study aims to describe the gastrointestinal parasite fauna of wild synanthropic capybara populations in the Orinoco Basin.

Table 1. Reported endoparasite species occurring in capybaras (*Hydrochoerus* spp.), including the findings of the present study.

Parasite	Localization [a]	Tissue	Feces	Blood	Literature
Metazoa					
Nematoda					
Dipetalonema hydrochoerus	C	x			[11]
Cruorifilaria tuberocauda	C, Br	x			[12–15]
Protozoophaga obesa ‡	A, Br, Bo, C, V		x		[15–22]
Strongyloides ‡	A, Br, C		x		[16,17,19,22]
Strongyloides chapini	Br		x		[15,21]
Capillaria spp.	Br		x		[23]
Echinocoleus hydrochoeri [b,‡]	A, Br, C		x		[15,17,21,22, 24]
Viannella spp.	Br		x		[19]
Vianella hydrochoeri	Br, Bo, V		x		[15,17,18,20, 21]
Hydrochoerisnema anomalobursata	Br		x		[17,21]
Trichuris spp.	Br		x		[17]
Trichuris cutillasae n. sp.	A		x		[25]
Trichostrongylus axei	Br		x		[15,21]
Habronema clarki	Br, Pa		x		[20,26]
Yatesia hydrochoerus	Br		x		[15]
Fam: Trichostrongyloidea ‡	A, C		x		[22]
Ord: Ascaridida	A		x		[22]
Cestoda					
Monoecocestus ‡	C				Present study
Monoecocestus hagmanni	Br, Bo, V		x		[18,20,21]
Monoecocestus hydrochoeri	A, Br, Bo		x		[15–17,20–22]

Table 1. *Cont.*

Parasite	Localization [a]	Tissue	Feces	Blood	Literature
Monoecocestus jacobi	Br		x		[17]
Monoecocestus macrobursatus	Br, Bo				[16,20,21]
Fam: Anoplocephalidae	Br		x		[16]
Trematoda					
Fasciola hepatica	A, Br		x		[23,27,28]
Hippocrepis fuelleborni	Br		x		[16]
Hippocrepis hippocrepis ‡	Br, C, V		x		[15–18,21,29]
Hydrochoeristrema cabrali	Br		x		[17]
Neocotyle neocotyle	Br		x		[21]
Nudacotyle tertius	Br		x		[15,21]
Nudacotyle valdevaginatus	Br		x		[21]
Plagiorchis muris ‡	C		x		Present study
Philophthalmus lachrymosus	Br	x	x		[16,30]
Taxorchis schistocotyle ‡	A, Br, C, V		x		[15,16,18,21, 22]
Protozoa					
Neobalantidium coli ‡	A, C		x		[22]
Blastocystis sp.	A		x		[22]
Cryptosporidium ‡ /*C. parvum*	B		x		[31]
Entamoeba ‡	C		x		Present study
Eimeria sp./spp.	A, C		x		[22,32]
Eimeria araside	Br		x		[33]
Eimeria boliviensis	Bo, Br, V		x		[33,34]
Eimeria ichiloensis	Bo, Br, V		x		[33,34]
Eimeria trinidadensis ‡	Bo, Br, C, V		x		[33,34]
Trypanosoma evansi	A, Br, Pe, V			x	[35–38]
Toxoplasma gondii	Br			x	[39]
Giardia spp.	C		x		[32]
Sarcocystis spp.	C		x		[32]
Fam: *Cyclopostiidae* ‡	C		x		Present study

[a] A: Argentina, Bo: Bolivia, Br: Brazil, C: Colombia, Pa: Panama, Pe: Peru, V: Venezuela; [b] *Echinocoleus hydrochoeri* syn. *Capillaria hydrochoeri*. ‡ Parasites found in this study. x: Parasite location.

2. Results

Overall, thirteen gastrointestinal parasite taxa were found comprising five protozoans of the phyla Apicomplexa, Amoebozoa, and Ciliophora, and eight metazoans of the phyla Nematoda and Platyhelminthes (Class: Cestoda and Trematoda), thereby covering a rather wide range of parasites when compared to previous studies (see Table 1).

2.1. Fecal Macroscopical Examination

The feces' gross examination revealed three helminths (i.e., one nematode and two trematodes). Nematodes found in Cinaruco were identified as *Protozoophaga obesa* female pinworms based on taxonomic traits (see Figure 1). The collected specimens had straight caudal extremity and an evident thin tail uncoiled posterior end. The mean morphometric measurements ($n = 6$) obtained were total body length 3.21 mm, body width 227.69 µm, and a total tail length of 815.72 µm. The mouth opening had four oxyurid-characteristic lips and four corresponding amphids. A straight thick short cylindrical oesophagus (118.25 µm in length) was observed with a wide cavity and a poorly chitinized pyriformous oesophagus bulb (49.85 µm × 48.89 µm). A wide straight intestine in the anterior portion of the body, which ended slightly contorted posteriorly was noticed.

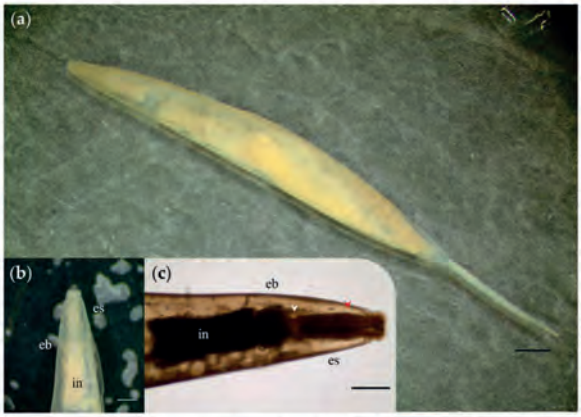

Figure 1. Female adult specimen of *Protozoophaga obesa*. (**a**) Whole specimen´s body on a dark-ground stereomicroscope image. (**b**,**c**) Anterior end; notice oesophagus (es), subtle bulge of the nerve ring (red arrowhead), isthmus of oesophagus (white arrowhead), oesophagus bulb (eb), and intestine (in). Scale bars: (**a**,**b**) 200 µm; (**c**) 50 µm.

Furthermore, an elongated reddish trematode was found in La Maporita collected feces (Figure S1). Studies of wet-mounted specimen and dark-ground stereomicroscopy evidenced asymmetric rows of spines along the integument decreasing in size towards the posterior region. The subterminal position of the oral sucker presented two notorious bilateral papillae. The morphometric data obtained from body measurements were 9.34 mm of total body length and 1.64 mm in width. The oral sucker was slightly ellipsoidal (74.87 µm × 80.76 µm). A narrow excretory terminal pore was also evidenced. Therefore, the digenean was identified such as *Hippocrepis hippocrepis* as previously described elsewhere [29]. Additionally, a dehydrated digenean specimen was found inside a fecal pellet in Bocas del Arauca. Due to poor conservation the specimen was thereafter subjected to subsequent phylogenic analysis.

2.2. *Microscopical, Coproantigen, and Molecular Parasite Identification*

The copromicroscopical analysis (n = 46) revealed nine parasite taxa comprising three protozoans and nine metazoans. Unsporulated *Eimeria*-like oocysts were detected and bilayered oocyst walls. Both oocyst wall layers (OWL) were smooth, with the outer OWL slightly yellowish and the inner OWL darker. The above depicted traits of the identified oocysts corresponded well with *Eimeria trinidadensis* (Figure 2a). Likewise, *Echinocoleus hydrochoeri* brownish barrel-shape plugged eggs were identified (Figure 2b and Video S1). In addition, *P. obesa*-eggs were also detected (Figure 2c). Cestode eggs of *Monoecocestus* were found in a low number (Table 2); the eggs showed typical embryphore with a pyriformous apparatus containing a developed hexacanth embryo (oncosphere; Figure 2d). Furthermore, *Neobalantidium coli* cysts were identified (Figure 2e). In addition, two trematode species were found, i.e., *Hippocrepis hippocrepis* (Figure 2f and Video S2), eggs with typical long and bilateral filaments in the poles of a capsulated miracidium (Figure 2g), as well as *Taxorchis schistocotyle* eggs (Figure 2h). Rhabditiform larvae of *Strongyloides* (Figure 2i,j) were also identified. Moreover, ascarid eggs were found (Figure 2k). Additionally, *Entamoeba* immature cysts were detected (Figure 2l).

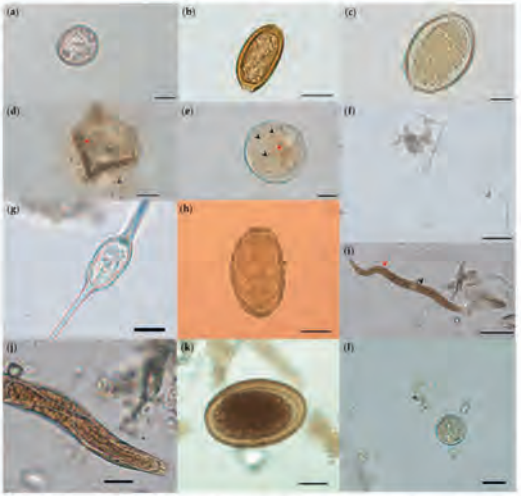

Figure 2. Illustrations of identified parasites stages in fecal samples of free-ranging capybaras (*Hydrochoerus hydrochaeris*) in the Orinoco Basin: (**a**) *Eimeria trinidadensis* oocyst (20 µm × 21.18 µm). (**b**) *Echinocoleus hydrochoeri* (44.72 µm × 24.65 µm) egg. (**c**) *Protozoophaga obesa* egg (70.58 µm × 44.85 µm). (**d**) *Monoecocestus* egg (55.67 µm × 61.84 µm), notice the rounded hexacanth embryo (red arrowhead). (**e**) A *Neobalantidium coli* cyst (46.32 µm × 47.82 µm), notice large macronucleus (red arrowhead) and cytoplasmic contractile vacuoles (black arrowheads). (**f**) *Hippocrepis hippocrepis* egg, notice long filaments (131.92–170.79 µm) and (**g**) miracidium capsule (20.42 µm × 11.27 µm). (**h**) *Taxorchis schistocotyle* egg (157.44 µm × 90.51 µm). (**i**) *Strongyloides*-like larvae (full length 349 µm), notice the rhabditiform oesophagus (87.09 µm; white dotted line), the buccal canal (white arrowhead), oesophageal bulb (black arrowhead) and ventral genital primordium (red arrowhead). (**j**) The strongyloid larvae intestinal esophagus junction width was 19 µm and intestinal undifferentiated cells were noticed. (**k**) Ascarid egg (72.73 µm × 48.89 µm), notice the embryo in advanced cleavage stage. (**l**) *Entamoeba* immature cyst (13.28 µm × 13.44 µm). Scale bars: (**a**,**g**,**l**) 10 µm; (**b**–**e**,**k**) 20 µm; (**f**,**h**,**j**) 50 µm; (**i**) 200 µm.

Coproantigen ELISA showed a *Cryptosporidium* occurrence of 34.8% (16/46). Cinaruco had the highest *Cryptosporidium* occurrence (6/8), followed by La Maportia and Bocas del Arauca [(8/23) and (2/15), respectively]. In contrast, no *Giardia*-derived antigen was here detected. The complete list of parasitic stages, detection technique, occurrence, and geographic locale are summarized in Table 2. Moreover, though outside the scope of the current study, it is worth mentioning that a wide variety of commensal ciliated protozoans belonging to the Cycloposthiidae family were found (refer to Figure S2). Additionally, different shapes and colors of microplastic fibers in most analyzed samples were evidenced.

In order to identify the dehydrated digenean specimen found in capybara feces, the ~1300 bp-long fragments of 28S rDNA gene subjected to phylogenetic analysis showed that the analyzed specimen clustered within the lineage composed of the representative of *Plagiorchis muris* (Figure 3).

Table 2. Occurrence of endoparasites detected in capybaras (*Hydrochoerus hydrochaeris*).

Phylum	Parasite	Stage [a]	Technique [b]	Bocas del Arauca $n = 15$	Cinaruco $n = 8$	La Maporita $n = 23$	Total (%)
Apicomplexa	*Cryptosporidium*	O	coproELISA	2	6	8	34.8 (16/46)
	Eimeria trinidadensis	O	SAF	2	2	6	21.7 (10/46)
Amoebozoa	*Entamoeba*	C	SAF	4	2	2	17.4 (8/46)
Ciliophora	*Neobalantidium coli*		SAF	1		1	4.3 (2/46)
Platyhelminthes	Cycloposthiidae	C	SAF	6		4	21.7 (10/46)
Nematoda	Ascarididae	E	SAF	3	2	8	28.3 (13/46)
	Echinocoleus hydrochoeri	E	CF/SAF	7	7	13	58.7 (27/46)
	Protozoophaga obesa	E/L/A	SS/CF/SAF	3	1	4	17.4 (8/46)
	Strongyloides-like	L	SAF	7	2	10	41.3 (19/46)
Cestoda	*Monoecocestus*	E	CF/SAF	1		2	6.5 (3/46)
Trematoda	*Hippocrepis hippocrepis*	E/A	SF/SS/SAF	4	1	3	17.4 (8/46)
	Plagiorchis muris	A	Sequencing	1			2.2 (1/46)
	Taxorchis schistocotyle	E	SS/SAF	6	6	8	43.5 (20/46)

[a] O: oocysts, C: cysts, E: eggs, L: larvae, A: adult; [b] SF: sedimentation–flotation, SAF: modified sodium acetate–acetic acid–formalin, SS: simple sedimentation, CF: centrifugal flotation, CFS: fast carbol–fuchsin stained fecal smear.

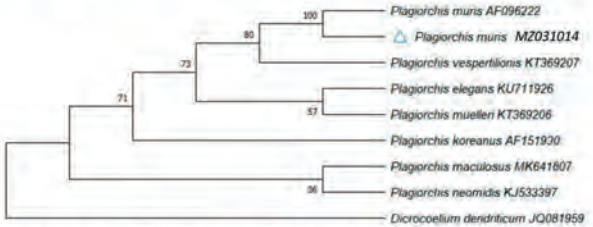

Figure 3. Phylogenetic position of *Plagiorchis muris* isolate obtained from capybara feces. Neighbor-Joining 28S rDNA phylogenic tree. The analysis involved nine nucleotide sequences and all ambiguous positions were removed for each sequence pair (pairwise deletion). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The specimen collected from capybara feces is indicated by a green triangle.

3. Discussion

Synanthropic populations of free-ranging capybaras are associated not only with cattle farming but also with human settlements. Additionally, this giant rodent is an important meat source for countryside populations [6]. It is estimated that approximately 61% of human diseases are zoonotic, and wildlife reservoirs are the source of most human emerging infectious diseases [40,41]. Consistently, capybaras have been reported as natural reservoirs of various zoonotic pathogens such as the tick-borne Brazilian spotted fever (BSF) [42], the liver fluke *Fasciola hepatica* [27], and the enteropathogen *Cryptosporidium parvum* [31]. This highlights the role of capybaras in the ecoepidemiology of infectious diseases. Thus, free-

ranging capybaras should be considered as reservoir hosts for various zoonotic-relevant water-, food-, soil- and gastropod-borne parasites of public health concern.

Literature reporting on Colombian capybara parasitic infections is scarce and restricted to solely two four decades old reports, which describe microfilariae [11,12]. Thus, neither morphological data nor molecular descriptions have been reported for gastrointestinal parasites occurring in Colombian capybara populations. To the best of our knowledge, we present for the first time a wide gastrointestinal parasite study of free-ranging capybaras. Overall, the current study revealed thirteen parasite taxa, five protozoans (i.e., *Cryptosporidium*, *E. trinidadensis*, *Entamoeba*, *N. coli*, and *Cyclophosthium* sp.), four nematodes [i.e., *E. hydrochoerid* (Trichuridae), *P. obesa* (Oxyuridae), strongyloid-like (Strongyloididae), and Ascarididae], one cestode [*Monoecocestus* sp. (Anoplocephalidae)], and three digenean trematodes [i.e., *H. hippocrepis* (Notocotylidae), *P. muris* (Plagiorchiida), and *T. schistocotyle* (Cladorchiidae)].

Here we identify *P. muris* infecting capybaras for the first time. Within the Plagiorchiidae family, this is the only species capable of infecting humans and it has been reported across continents [43]. This neglected trematode infects mainly humans from Korea, Japan, and North America proving an effective transmission route from rodents to humans [44–48]. Often reported as a rodent-borne disease with public health concern in mainland southeast Asia [49], Iran [50] and the Netherlands [51], this trematode has never been reported in South America before. Despite its well characterized occurrence and zoonotic potential across Asian countries, detailed information on epidemiology, life cycle, and reports on human plagiorchiosis remain still scarce in Africa, the Americas, and Europe [51–53]. Thus, the present description expands the previously known geographic distribution range of this parasite and constitutes the first host record of capybaras. Meanwhile, *P. muris* has been reported to infect cats, dogs, and chickens [54–56]. Therefore, we recommend future activities on this euryxenous zoonotic trematode in South American domestic animals and humans for a better life cycle comprehension, potential obligate first gastropod intermediate host spectrum, second intermediate host, other final hosts, and possible public health impact of plagiorchiosis in rural populations.

Moreover, surveillance of zoonotic parasitic agents by local public health authorities should be recommended as capybaras are still frequently consumed [6]. Notably, bushmeat-related activities have been linked to parasitic emerging diseases outbreaks to human populations such as neobalantidiosis, amoebiasis, strongyloidiosis, and giardiasis [57]; it is worth mentioning that the present study has shown a general coinfection occurrence of 17.4% (8/46) for *H. hippocrepis*, *P. obesa*, and *Entamoeba* in capybaras. Since *E. histolytica* is a cosmopolitan extracellular enteric parasite causing amoebiasis with an average of 50 million cases and 55,000 to 100,000 human deaths each year globally [58], further monitoring of this zoonosis in capybara populations should be addressed. Interestingly, the water/food-borne parasite *N. coli* was detected with low occurrence in Bocas del Arauca and La Maporita. This enteropathogenic ciliated protozoa can be found throughout the world infecting mainly pigs, wild boars (*Sus scrofa*), rodents, equines, ruminants, nonhuman primates, and humans [59,60]. It is possible that *N. coli* cysts have a multidirectional interchange and transmission among humans, capybaras, and domestic animals. Additionally, a general occurrence of 41.3% (19/46) for *Strongyloides*-like larvae were reported. The strongyloidiosis is a parasitic disease caused by nematodes in the genus *Strongyloides* that remain largely neglected [61]. Thus, infected capybara herds may contribute to the dissemination of related soil-borne nematodes.

Overall, the highest parasite occurrence found in capybaras were recorded for *E. hydrochoeri* [(58.7% (27/46)] and the trematode *T. schistocotyle* [43.5% (20/46)]. The capillariid genus *Echinocoleus* has been reported in natural populations of capybaras in the northeast Argentinian Iberá Wetlands [22]. Despite the difficulty of studying this nematode group, the paucity of good morphological characteristics, and complex systematics [24], *E. hydrochoeri* is widely distributed in Brazil from the Pantanal, Mato Grosso do Sul, to Rio Grande do Sul where it has been identified in capybaras linked to cattle breeding areas [15,17]. Irrespectively, the present study constitutes the first report of this parasite in Colombia, thus expanding the distribution range of echinocoelosis to the Orinoco Basin. Capybara echinocoelosis may result in gastrointestinal disturbances due to enteric and intestinal location of pre- and adult stages [24]. Additionally, *T. schistocotyle* has been associated with multifocal necrotizing colitis in capybaras [62], and it is worth considering as a major concomitant lethal cause in heavily infected rodents, as well as fasciolosis [27].

Furthermore, *Cryptosporidium*-specific antigens (CSA) were found in studied areas, thus indicating a potentially wide occurrence along the Orinoco Basin. While anthropozoonotic *Cryptosporidium* species have already been described in nine rodent families [63–68], there is only one report of *C. parvum* in capybaras [31]. Interestingly, the highest occurrence of CSA was found in Cinaruco, furthest away from populated centers whose closest human settlement is located at 20.598 Km. In contrast, areas with greater anthropogenic effect show lower occurrence. Since the hypertransmissible IIaA15G2R1 *Cryptosporidium* subtype naturally infect Brazilian wild capybaras [31], and the oocysts could easily spread in aquatic/semiaquatic ecosystems [69], it is necessary to identify the species/genotype and the related zoonotic potential of *Cryptosporidium* detected in capybaras in this study.

The present findings collectively reflect the parasitological status of wild synanthropic capybara populations in the Orinoco Basin, a well-known neotropical grassland region for extensive cattle production. Therefore, this region faces a special risk of parasites transmission among capybaras, wild/domestic animals, and humans. Based on these results, we encourage further parasite monitoring studies on wild capybara populations. For comparative reasons, parasitological surveys of the species *H. isthmius* should be considered in the near future. Further investigations are required to reveal the importance of described parasites not only for public health concern but also for neotropical wildlife conservation issues. Since detected trematodes (i.e., *P. muris*, *H. hippocrepis*, and *T. schistocotyle*) require either terrestrial/amphibious or aquatic obligate gastropod intermediate hosts, it would be appropriate to analyze in depth the gastropod fauna (e.g., snails and slugs) inhabiting flooded areas, rivers, natural lakes, and ponds shared by humans, domestic animals, and wildlife to prevent zooanthroponotic parasite spillovers.

4. Materials and Methods

4.1. Study Areas and Sample Collection

Three wild populations of capybaras were investigated in west-side lowlands of the Orinoco Basin (Figure 4). The geographic study areas have an average annual precipitation of 1477 mm, 90% relative humidity, 120 m above sea level, and a temperature range between 27.8 and 30.9 °C. In accordance with the Köppen–Geiger climate classification sampling areas were located in tropical savannah [70]. Individual fecal samples were collected during dry season from March to June 2020 in La Maporita (06°55′37.77″ N; 070°27′46.54″ W), Cinaruco (06°40′46.88″ N; 070°7′9.36″ W), and Bocas del Arauca (07°01′10.31″ N; 070°35′28.97″ W), Arauca, Colombia.

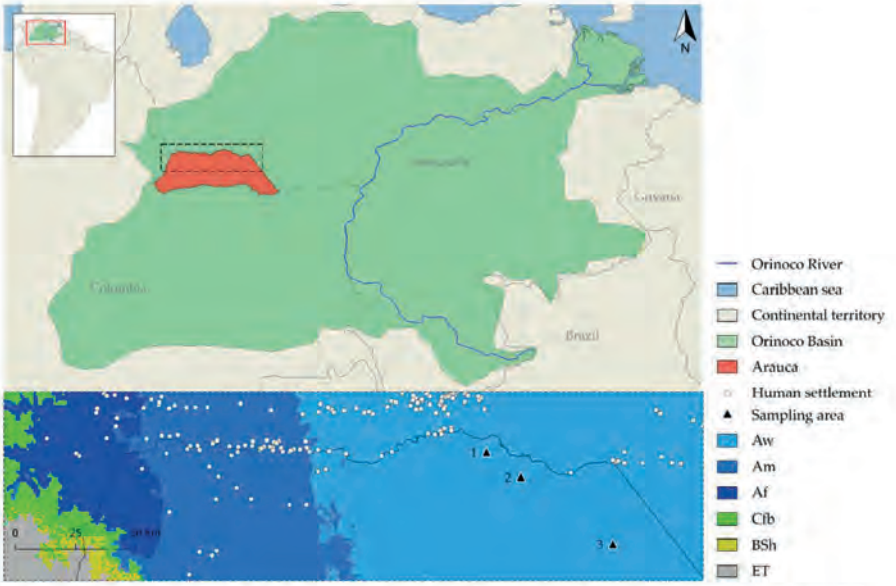

Figure 4. Precise geographic location of sampling areas; Aw: Tropical savanna, Am: Tropical monsoon, Af: Tropical Rainforest, Cfb: Oceanic, BSh: Semiarid, and ET: Tundra. (1) La Maporita, (2) Cinaruco, and (3) Bocas del Arauca.

The noninvasive methodology allows the collection of feces after spontaneous defecation without disturbing the social dynamics and natural behavior of capybara herds as has been successfully performed for other wild mammals [71]. Free-ranging capybaras feces (n = 46) were randomly collected from manure pellet piles regardless of the gender and age (Figure 5). Fecal samples were immediately placed in 50 mL conical tubes (Sarstedt, Nümbrecht, Germany) containing 80% EtOH for fixation until further analyses. Helminth stages spontaneously shed within feces were gently rinsed in sterile 0.9% PSS and afterwards fixed in 96% EtOH for subsequent taxonomic and molecular identification. Sampling procedures were conducted in agreement with the international guidelines for the use of wild mammal species in research [72], the EU Directive 2010/63/EU, and the approvement of Ethics Committee for Animal Experimentation of the Universidad de Antioquia (AS N° 131), Colombia.

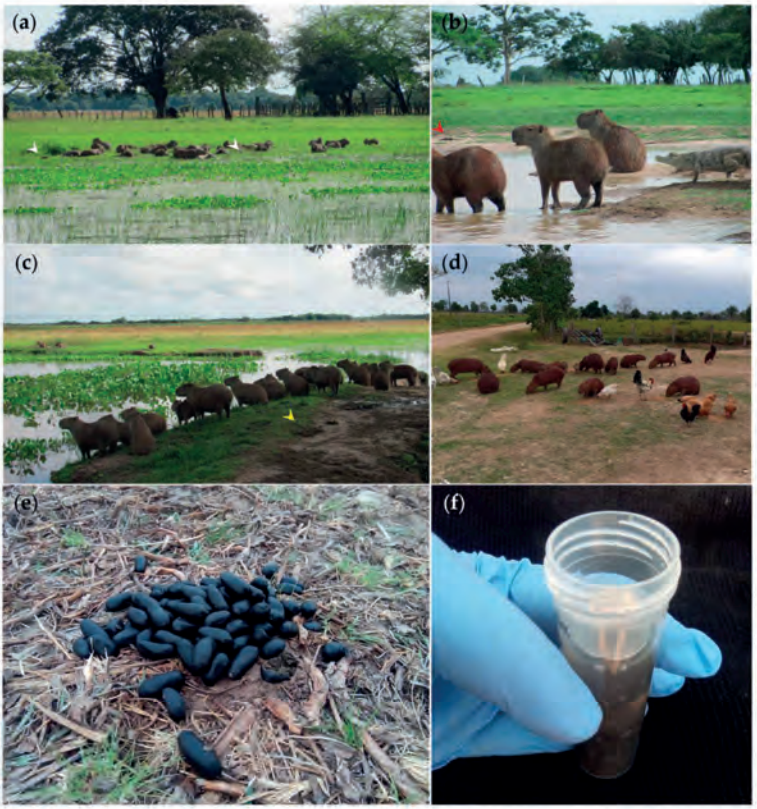

Figure 5. Illustration of free-ranging capybaras (*Hydrochoerus hydrochaeris*) in floodable savannas of the Orinoco Basin. (**a**) Twenty-seven capybara hare resting in Bocas del Arauca mudflats, notice the tight interaction with Charadiiform wader birds (*Vanellus chilensis*) (white arrowheads); (**b**) Small riverside group of capybaras sharing their habitat with spectacled caiman (*Caiman crocodilus*), red arrowhead indicates feces; (**c**) Twenty-three capybara hare in La Maportia, yellow arrowhead indicates feces; (**d**) Peridomestic chickens (*Gallus gallus domesticus*) and synanthropic capybaras feeding in close proximity demonstrating domestic animal–wildlife–human interface; (**e**) Freshly defecated capybara manure pellets piles; and (**f**) EtOH preserved capybara feces.

4.2. Parasitological and Immunological Analysis

Fecal samples were processed following standardized parasitological techniques: combined sedimentation–flotation (SF), modified sodium acetate–acetic acid–formalin (SAF) [73], simple sedimentation (SS) [74], centrifugal flotation with zinc sulfate (CF), and fast carbol–fuchsin stained fecal smears (CFS) for *Cryptosporidium*-oocyst detection [75].

Copromicroscopical findings of parasitological stages, such as cysts, oocysts, sporocysts, eggs, and larvae were identified through corresponding morphology and morphometry analyses under an Olympus BX53 (Olympus Corporation, Tokyo, Japan) semimotorized light microscope equipped with an Olympus DP74 digital camera and the *cellSens* standard imaging software. Macroscopic parasites were identified using an Olympus SZX7 (Olympus Corporation, Tokyo, Japan) stereomicroscope system with Olympus DP27 digital camera and the above-described software. The parasite stages' identification was based on general morphology, shape, size, and color, according to [24,29,33,62,76–78].

Furthermore, commercially available coproantigen ELISA for detection of *Cryptosporidium*- and *Giardia*-specific antigens (CSA and GSA) were performed by the membrane-bounds solid phase immunoassays ProSpecT *Cryptosporidium* Microplate Assay and *Giardia* Microplate Assay (Thermo Scientific, Waltham, MA, USA; Oxoid, Basingstoke, UK), following the manufacturer's instructions. The interpretation of the results considered as negative all colorless reactions via visual reading and/or when OD values were < 0.05 after the blank of negative controls, indicating none or undetectable levels of GSA/CSA in analyzed samples. On the other hand, a variable intensity of yellow was considered as positive when OD values were > 0.05.

4.3. Molecular Analyses

To characterize helminths found in feces a small sample of the midsection body tissue (≈10 mg) was dissected to extract gDNA using the DNeasy Blood and Tissue kit (Qiagen, Dusseldorf, Germany) following the manufacturer´s instructions. A ~1300 bp fragment of 28S rDNA gen was PCR-amplified using the primers: for-5′-aagcatatcactaagcgg-3′, and rev-5′-gctatcctgagggaaacttcg-3′, following the thermocycle profiles previously described [79]. The 28S rDNA gene was selected considering it is a more conserved gene compared to the mtCOI region among the Plagiorchiidae family [80]. All PCR products were bidirectionally sequenced by LGC Biosearch Technologies (Berlin, Germany). Representative 28S rDNA sequences of the recognized extant species were included in order to reveal the phylogenetic relationships. *Dicrocoelium dendriticum* [JQ081959; (Dicrocoeliidae)] was here used as the outgroup digenean species. SeqManPro 7.1.0 (DNASTAR Inc., Madison, WI, USA) was used to in silico edit, and finally assembled the sequence. The 28S rDNA alignment were conducted using the online version of MAFFTv. 7 (available at https://mafft.cbrc.jp/alignment/server/ accessed on 21 February 2020) [81]. A Neighbor-Joining algorithm analysis under 1000 bootstrap replicates was conducted in MEGAX software. Nucleotide sequence divergences were calculated using the Kimura2-parameter (K2P) model for multiple substitution distance correction and were in the units of the number of base substitutions per site [82]. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the analyzed helminth [83]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed.

5. Conclusions

Capybaras are effective indirect indicators of ecosystem health and therefore helpful to better understand potential parasites transmission routes to humans and domestic animal populations. Here we report the presence of thirteen parasite taxa in capybaras, some of them baring zoonotic potential. Thus, this rodent should be constantly monitored as public health concern issue. The noninvasive sampling methodology allows detection of large number of protozoan and metazoan parasites without altering wildlife populations. It is very important to broaden these epidemiological studies to other wildlife species inhabiting the Orinoco Basin and more frequent parasitological survey of this rodents within South America, since accurate identification of soil-, water-, food-, and gastropod-borne parasites will contribute to early detect and prevent parasitic spillovers events within the One Health concept.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pathogens10091152/s1, Figure S1: Adult specimen of *Hippocrepis hippocrepis* (Trematoda: Notocotylidae) found in capybara manure pellet piles collected in a flooded area of La Maporita. Scale bar: 2 mm, Figure S2: Cycloposthiidae cyst (47.28 µm × 33.03 µm), notice adoral ciliary zone (white arrowhead), vestibulum (red arrowhead), and cytoproct (black arrowhead). Scale bar: 10 µm, Video S1: Biopercular plugged *Echinocoleus hydrochoeri* egg, Video S2: Biflagellate egg of *Hippocrepis hippocrepis*.

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Institutional Review Board Statement: The current study was conducted according to the Guidelines of the American Society of Mammologists for the use of wild mammals in research and education, the EU Directive 2010/63/EU, and approved by the Ethics Committee for Animal Experimentation of Universidad de Antioquia (AS No. 131) under collection permit No. 0524 of 2014 (IDB0321).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the Supplementary Material files (Figures S1, S2 and Videos S1, S2). The putative *Plagiorchis muris* sequence obtained from capybara (*Hydrochoerus hydrochaeris*) feces was deposited in the GenBank database (National Center for Biotechnology Information, NIH, Bethesda, USA), and are simultaneously available at ENA (European Nucleotide Archive) in Europe and the DDBJ (DNA Data Bank of Japan) under accession number MZ031014.

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Chapter 3 - The Neglected Angio-Neurotrophic Parasite *Gurltia paralysans* (Nematoda: Angiostrongylidae): Northernmost South American Distribution, Current Knowledge, and Future Perspectives.

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Review

The Neglected Angio-Neurotrophic Parasite *Gurltia paralysans* (Nematoda: Angiostrongylidae): Northernmost South American Distribution, Current Knowledge, and Future Perspectives

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Abstract: *Gurltia paralysans* is a rare metastrongyloid nematode in South America that has begun to gain relevance in feline internal medicine as a differential diagnosis of progressive degenerative myelopathy disorders. The parasite life cycle has not been fully elucidated but probably involves invertebrate gastropod fauna as obligate intermediate hosts; thus, *G. paralysans* remaining an extremely neglected parasitosis. Feline gurltiosis *intra vitam* diagnosis is highly challenging due to lack of evidence in the excretion of *G. paralysans* eggs and larvae, neither in feces nor in other body secretions because environmental stages and the transmission route of the parasite remain unknown. Unfortunately, no experimental trials for the treatment of feline gurltiosis have been conducted to date. However, there are some reports of the successfully antiparasitic drugs used with different effectiveness and clinical improvement results in diagnosed cats. Further studies are needed to evaluate the parasite occurrence among domestic cats and the neotropical wild felid species distributed within Colombia in addition to the gastropod fauna that may harbor the developing larvae (L1–L3) stages of this underestimated parasite.

Keywords: *Gurltia paralysans*; gurltiosis; paralysis worm; metastrongyloid; feline; South America

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1. Introduction, Brief History, and the Enigmatic Life Cycle

Tropical and subtropical geographic areas offer appropriate ecological characteristics for re-emergence, maintenance, and dissemination of multiple parasitic infectious diseases, with some of them extremely neglected [1–3]. Rare and underestimated felid parasites such as the nematodes *Dracunculus insignis* (Dracunculidae) [4], *Brugia malayi* (Onchocercidae) [5,6], *Thelazia callipaeda* (Thelaziidae) [7,8], *Ollanulus tricuspis* (Molineidae) [9,10], *Physaloptera praeputialis* (Physalopteridae) [11], *Oslerus rostratus* (Filariodidae), *Troglostrongylus subcrenatus* (Crenosomatidae), and *Angiostrongylus chabaudi* (Angiostrongylidae) [12]; the trematode *Amphimerus sp. (Opisthorchiidae)* [13]; and the cestode *Mesocestoides corti* (Mesocestoididae) [14] have been documented in domestic cats (*Felis catus*) in the Americas, Asia, and Europe. Additionally, *Gurltia paralysans* is a rare metastrongyloid nematode in South America that has begun to gain relevance in feline internal medicine as a differential diagnosis of progressive degenerative myelopathy disorders. However, it is still neglected and underestimated [15]. This angio-neurotrophic nematode is infrequently described, often unnoticed, and out of the diagnostic scope of even specialist veterinary clinicians, similarly to other neglected parasitoses [16,17]. The poorly understood felid angio-neurotrophic parasite was first classified as *Hemostrongylus*, then as *Angiostrongylus*, and finally located as the only species of the genus *Gurltia* (Nematoda: Angiostrongylidae). The first description of *G. paralysans* took place in the temperate forest ecoregion of Valdivia in southern Chile by Wolffhügel in 1933 [18]. The nematode genus was named in honor

of both Dr. Ernst Friedrich Gurlt (1794–1882) and the species *paralysans*, due to observed paralysis being the main clinical sign in infected felids [16,18]. This metastrongyloid nematode was reported parasitizing domestic cats (*F. catus*) in southern Chile, and the small wild kodkod (*Leopardus guigna*) has been proposed as the natural host [16,19]. The parasite has been reported in other wild felids such as the margay (*Leopardus wiedii*) [20]. Moreover, the Goeffroy's cat (*Leopardus geoffroyi*) and the northern tiger cat (*Leopardus tigrinus*) have also been proposed as potential native final hosts [15,21].

The nematode life cycle has not been fully elucidated but probably involves hypothesized paratenic hosts such as amphibians, birds, insects, lizards, and rodents, and invertebrate gastropod fauna as obligate intermediate hosts in which the larvae developmental process occurs from first-stage larvae (L1), second-stage larvae (L2), and final infective third-stage larvae (L3) (Figure 1), as is the case for several metastrongyloid parasites in the Angiostrongylidae family.

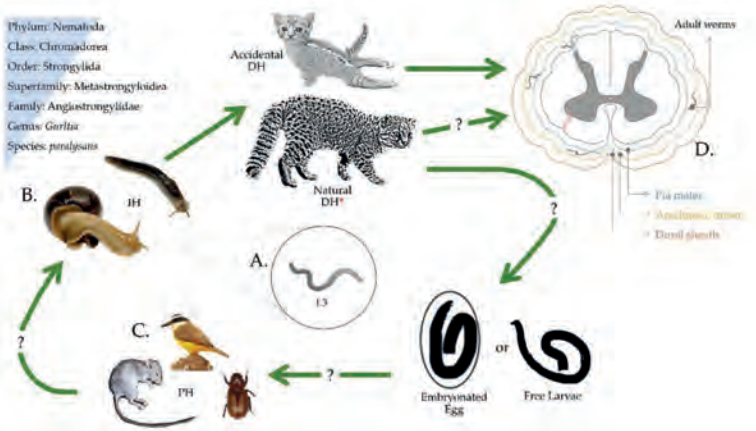


Figure 1. Proposed life cycle of *Gurltia paralysans*. Felids become infected by consuming the L3 larvae (**A**) from an infected intermediate host (**B**) or paratenic host (**C**). L3 migrates from the intestinal tract to the central nervous system and invades the veins of the subarachnoid space (**D**) of the spinal cord. There, larvae mature to adult worms and produce eggs and reproduce through eggs. The elimination route of the eggs or first larval stage (L1) or how the intermediate host becomes infected with L1 is still unknown. * Definitive host.

An extensive survey to identify *G. paralysans* larval stages was conducted in terrestrial gastropods (n = 835) collected from a previous feline hotspot located in southern Chile (Valdivia) showed that neither semi-nested PCR, enzymatic digestion nor histopathological examination could identify the presence of *G. paralysans* larvae in mollusks of the families Arionidae, Limacidae, Helicidae, and Milacidae [22]. Therefore, the obligate intermediate hosts of this parasitic disease are still unknown, and the morphological traits of larvae have not been identified so far. Notwithstanding the lack of epidemiological and life cycle knowledge of *G. paralysans*, it has been known since 1930s that the kodkod is the natural definitive host, allowing the pulmonary development of the parasite, while domestic cats are accidental hosts [19,20]. Adult male specimens of *G. paralysans* have a total body length of 12–18 mm and a width that varies from 72–103 µm to 26–32 µm anterior to the bursa and the cephalic region, respectively. Consistently larger female adults show a total body length ranging from 27 to 28 mm [21]. The parasite adult stages are located in the definitive host's

meningeal veins and spinal cord subarachnoid space [23], where undeveloped eggs passed by the females, and 16-cell embryos eggs, have been found in the felid bloodstream [16]. The morphometrical measurements of known parasitic stages are summarized in Table 1. To the best of our knowledge, to date, neither eggs nor larvae have been found in any felid host gastrointestinal tract, nor in feces [16].

Table 1. Comparative morphometrical traits of *Gurltia paralysans* adult stages among related Angiostrongylidae nematodes.

	G. paralysans [a]	*Ael. Abstrusus* [b]	*Ang. Vasorum* [c]
Male			
Body length	12,000–18,000 µm	5440–7080 µm	14,000–16,000 µm
Body width	72–103 µm	42.5–92.7 µm	–
Esophagus length	360–432 µm	219.4–360.9 µm	219 µm
Spicule length	650–816 µm	103.7–138.9 µm	360–490 µm
Gubernaculum length	37–39 µm	18.8–31 µm	34–45 µm
Female			
Body length	20,500–30,000 µm	7950–10,587 µm	15,000–21,000 µm
Vulva to tail tip	102–150 µm	223.6 µm	–
Tail length	30–50 µm	27–29 µm	27–29 µm
Eggs	40–72 × 26–54 µm	37.8–48.4 × 94.5–99.6 µm	70–80 × 40–50 µm

References [a] [21–23], [b] [24,25], [c] [25–29].

The migration pathways of *G. paralysans* in vertebrate hosts begin when domestic cats or wild felids acquire the infective third-stage larvae (L3) through ingestion of intermediate/paratenic hosts. Infective larvae penetrate the stomach into the vascular bed, migrating to the portal vein toward the inferior vena cava and/or the thoracic venous system to finally reach the spinal cord via the vertebral venous plexus and the intervertebral veins [30]. Understanding the current lifecycle brings up many questions, and therefore *G. paralysans* remains an extremely neglected parasite.

2. Worldwide Distribution Range of Gurltiosis

Until recently, gurltiosis was a parasitic disease found only in South America, distributing from Aysén in southern Chile to the northernmost report located in Antioquia, Colombia [31,32]. One confirmed case of gurltiosis has been reported in South America in a domestic cat from the island of Tenerife, and this is also the first report of ophthalmic *G. paralysans* parasitism [33]. However, a necropsy performed at Cornell University on a cat with severe neuropathy revealed an extensive hemorrhagic lesion between the third and sixth lumbar vertebrae, which contained a metastrongyloid female consistent with *G. paralysans* morphologic traits [16]. Those reports possibly constitute imported cases to Spain and the USA, respectively. Feline gurltiosis is often reported as sporadic single case reports and limited sample size studies across South America. The vast majority of reports had a small sample size, ($x \approx 9.4$), and were located in rural and suburban areas (Table 2). In South America, the mean age of feline gurltiosis reported cases was 2.5 years old.

Table 2. Chronologic confirmed case report of the angio-neurotrophic *Gurltia paralysans* parasite.

Year	Location	Area	Host	(*n*)	Diagnosis Method	Reference
1933	Chile	Rural	*Felis catus*	-	M	[18]
1933	Chile	Rural	*Leopardus guigna*	-	-	[19]
2010	Chile	Rural	*Felis catus*	4	Ct, Hp, M	[34]
2011	Uruguay	Rural	*Felis catus*	2	Hp, M	[35]
2011	Colombia	Rural	*Felis catus*	6	Ct, Hp	[32]
2011	Argentina	Suburban	*Felis catus*	1	Hp	[36]
2012	Chile	Rural	*Felis catus*	3	Hp, M	[23]
2013	Chile	Rural	*Felis catus*	9	Ct	[37]
2013	Brazil	Rural	*Felis catus*	4	Hp	[38]
2016	Chile	Rural	*Felis catus*	1	Hp	[39]
2016	Argentina	Rural	*Felis catus*	3	Hp, M	[40]
2017	Chile	Suburban/Rural	*Felis catus*	-	SEM, phylogeny	[21]
2018	Spain	Suburban	*Felis catus*	1	M, phylogeny	[33]
2019	Brazil	Rural	*Felis catus*	7	Hp	[41]
2020	Chile	Rural	*Felis catus*	4	Serology, M	[42]
2020	Chile	Suburban	*Felis catus*	1	PCR	[31]
2020	Chile	Rural	*Felis catus*	7	PCR	[43]
2020	Brazil	Rural	*Leopardus wiedii*	1	M	[20]
2021	Chile	Urban	*Felis catus*	93	PCR	[15]

M: morphology, Ct: Computed tomographic-myelography, Hp: histopathology, SEM: Scanning electron microscopy. PCR: Polymerase chain reaction.

A descriptive molecular epidemiology study from a total of 93 domestic cats located in urban areas of Southern Chile, where 54.4% of the studied animals were *G. paralysans*-positive, showed the feasible transmission of parasite among domestic cat populations in urban environments and proposed that predictors such as age, sex, lifestyle (indoor/outdoor), anthelmintic use, and cat hunting behavior should be considered as potential risk factors associated with this angio-neurotrophic parasite infection [15]. Thus, those risk factors should be considered in rural, suburban, and urban areas from Argentina, Brazil, Chile, Colombia, Spain, and Uruguay, where *G. paralysans*-positive domestic cat cases have been confirmed.

Parasite biological characteristics such as host specificity, life cycle complexity, and climatic tolerance may render parasite species particularly vulnerable or allow them to proliferate [44]. As previously stated, gurltiosis in South America ranges from Colombia to Chile, covering a wide range of environmental conditions. As a result, a climate analysis could provide epidemiological insights into the ecoepidemiology of *G. paralysans*. In accordance with the Köppen–Geiger climate classification, the parasite shows great adaptation to different thermal floors and climates because the nematode has been identified in domestic and wild felids located in tropical rainforest (Af), savanna (Aw), hot semi-arid (BSh), humid subtropical (Cfa), oceanic (Cfb), warm-summer Mediterranean (Csb), and tundra (ET) climates [15,32,33,44,45] (Figure 2). Thus, confirmed gurltiosis cases have been reported in tropical, arid (dry), and temperate climates, showing a considerable climatic tolerance. Despite the natural gurltiosis distribution throughout South America, it is essential to note that southern Chile is a well-known gurltiosis hotspot where the main geographic distribution of gurltiosis cases in domestic cats occurs [22]. Meanwhile, the only reports of the disease in wild felids are parasitic myelopathy in a Brazilian margay [21] and the first Wolffhügel report of the disease's native host, the kodkod [20].

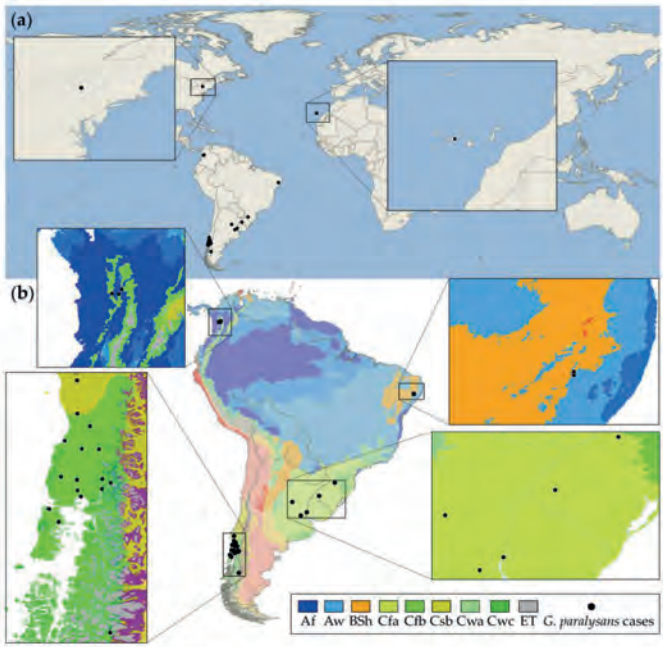


Figure 2. Worldwide reported cases of feline gurltiosis in domestic cats and wild felids. (**a**) Enlargement of the areas outside South America (i.e., Tenerife Island, Spain, and New York, NY, USA) where imported cases have been recorded. (**b**) Köppen–Geiger climate classification from South American case reports of the angio-neurotrophic parasite *G. paralysans*. Close-up location of the parasite reports from Colombia, Brazil, and the triple border area between Brazil, Uruguay, and Argentina. Additionally, feline gurltiosis hotspot area in Southern Chile where the most significant number of occurrence disease cases have been reported. Af: Tropical rainforest, Aw: Savanna, Bsh: Hot semi-arid, Cfa: Humid subtropical, Cfb: Oceanic, Csb: Warm-summer Mediterranean, Cwc: Monsoon-influenced subpolar oceanic, Cwa: Monsoon-influenced humid subtropical, ET: Tundra.

3. Clinical Signs and Diagnostic

Feline gurltiosis should always be considered as a differential diagnosis in cats with neurological signs related to thoracolumbar/lumbosacral spinal cord damage [39]. Demonstration and morphological identification of the nematodes in the spinal cord vasculature is the definitive diagnosis. This approach, however, may only be used postmortem [34,37]. Necropsy findings include diffuse sub-meningeal congestion of spinal cord segments (i.e., lumbar, sacral, and coccygeal). The intravascular presence of adult nematodes and larvae stages cause the thickening and congestion of subarachnoid vessels [24,36,43]. Microscopic findings include vascular myelitis and intralesional adult parasites, which principally locate at subarachnoid space in some segments of the spinal cord (third thoracic vertebra to third lumbar vertebrae and fourth lumbar vertebrae to third sacral vertebrae) [38,39]. The gurltiosis *intra vitam* diagnosis is highly challenging due to the lack of evidence in the excretion of *G. paralysans* eggs and larvae stages, neither in feces nor in other body

secretions [42], because the environmental stages and parasite transmission route remain unknown. Veterinary clinicians should include exhaustive clinical examination in cats with typical hind limbs neurological signs and complementary tests to approach the diagnosis of this parasitosis. Cats with a clinical history of progressing paraparesis or paraplegia between 2 weeks and 48 months [32,34,37,39], pelvic limb ataxia, tail paralysis, and fecal and urine incontinency in endemic areas should be considered for the diagnosis of feline gurltiosis.

The neurological signs are related to the neuroanatomical lesions caused by the parasite and include bowel incontinence, urinary dysfunction, tail trembling/atony, pelvic limbs ataxia and tights muscle atrophy and tremor, pelvic limbs tremors, and proprioceptive deficit [37]. The classical chronic myelopathy signs are caused by the vascular proliferation produced by the parasite and result from compression of the white matter in the thoracolumbar and lumbosacral dorsal cord [38]. Some paraclinical findings include non-regenerative anemia, hypochromia, and high blood urea nitrogen (BUN) levels with consequently azotemia [37,46]. Neither eosinophilia nor coagulopathy are common findings in cats with gurltiosis [37]. Ocular lesions and the parasite presence in the fluid-filled space between the cornea and iris (i.e., anterior chamber of the eye) in a domestic cat have also been reported [33]. Paraclinical analysis includes complete blood examination (haemogram), fecal examination, analysis of cerebrospinal fluid (CSF), and imaging (computed tomography, myelography, and magnetic resonance imaging) [37]. Recently, Gomez et al. evaluated the commercial serological Angio Detect™ test (IDEXX™ Laboratories, ME, USA) as a suitable *intra vitam* diagnostic method for feline gurltiosis. They suggested a cross-reaction between *Angiostrongylus vasorum* and *G. paralysans*-specific antigens, which could be used as a new diagnostic tool for feline gurltiosis. Nevertheless, it is necessary to analyze the sensibility and specificity compared with the specific antigen for this parasite to validate the serological test result [42].

Additionally, semi-nested PCR analysis has been proposed as a routinary test for early diagnosis of the parasite in serum. The PCR amplifies a 450 bp fragment of a common metastrongyloid sequence using universal oligonucleotides (please see Table 3). The semi-nested PCR differentiates between *G. paralysans* DNA and *Aelurostrongylus abstrusus* with bands of 356 and 300 bp, respectively [43]. It has been proposed that serum samples are more effective than CSF in detecting the parasite by molecular analysis; nevertheless, the result was not statistically significant, and further research is needed [43]. Furthermore, phylogenetic analysis of 28S rRNA (D2-D3 region), ITS1 and ITS2 of the 5.8S rRNA, and partial 18S rRNA sequences demonstrated that *Gurltia* spp. belongs to the family Angiostrongylidae and is therefore morphologically similar to related genera (i.e., *Aelurostrongylus* sp., *Angiostrongylus* sp., *Didelphostrongylus* sp., and *Heterostrongylus* sp.) [21,23]. Additionally, the molecular analysis concluded that *G. paralysans* is most closely related to *A. vasorum* [30]. The parasite D2-D3 region is considered an adequate molecular marker [21,45]. Nevertheless, to date, it has not been possible to identify *G. paralysans*-DNA in fecal samples with any coproparasitological tests, so it is alternatively recommended to use serum or CSF samples [43]. Preliminary analysis also indicates the presence of *G. paralysans* DNA in bronchoalveolar lavage, but it has not yet been validated [15]. Some primers used to determine the presence of *G. paralysans* DNA are listed in Table 2. It is worth mentioning that the accurate *intra vitam* diagnosis of the disease remains highly challenging and more feasible via necropsy in clinically ill felids [18,19,21,30].

Table 3. Reported PCR analysis for *Gurltia paralysans* proposed by López-Contreras et al., 2020, and Barrios et al., 2021.

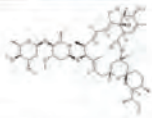
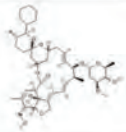
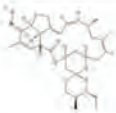
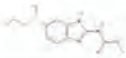
PCR Type & Gene	Primer Set (5′→3′)	Amplicon Size (bp)	Identification
semi-nested PCR 28S rDNA	AaGp28Sa1-R: AGGCATAGTTCACCATCT AaGp28Ss1-F: CGATRATATGTATGCCATT	450	Common sequence Metastrongyloidea
	E1:Aa28Ss2-F: CGTTGATGTTGATGAGTATC E2:Gp28Sa3-R: TCTTGCCGCCATTATAGTA	300 356	*A. abstrusus* *G. paralysans*
Endpoint-PCR rDNA Feline	F: AGCAGGAGGTGTTGGAAGAG R: AGGGAGAGAGCCTAATTCAAAGG	100	Internal control

4. Treatment

Even though the Tropical Council for Companion Animal Parasites (TroCCAP) has affirmed neither a pharmacological treatment schedule nor a therapy that has been proven effective against *G. paralysans* infection in cats [47], some empirical treatments have demonstrated variable degrees of effectiveness against this angio-neurotrophic parasite in felids. Unfortunately, no experimental trial for treating gurltiosis has been conducted to date. However, there are some reports of the successfully antiparasitic drugs used with different effectiveness and clinical improvement results in diagnosed cats (Table 4). For example, the oral (PO) administration of ivermectin resulted in clinical recovery in mild or moderate neurodegenerative feline gurltiosis cases [48]. Moreover, the prophylactic use of other macrocyclic lactones, such as selamectin and milbemycin, may prevent *G. paralysans* infection in cats located in endemic areas [30], together with the control of paratenic/intermediate hosts and responsible pet ownership practices such as keeping the cats indoor and avoiding hunting behavior in gurltiosis hotspot areas could reduce the possibility of acquiring this not yet wholly understand parasitosis [15]. Furthermore, broad-spectrum fenbendazole (benzimidazole) and macrocyclic lactone moxidectin concomitantly administrated with the neonicotinoid imidacloprid may reduce the risk of *G. paralysans* infection, as they do for other related nematode species, such as *A. vasorum* [30].

The PO or subcutaneously (SQ) ivermectin administration in cats is well-tolerated at ranges between 0.2 and 1.3 mg/kg [51,52], the no-effect level is approximately 0.5 to 0.75 mg/kg of body weight, and toxicosis has been reported in a limited number of cats. Routinely, a monthly deworming PO dose of moxidectin of 0.003 mg/kg and a sustained release injectable formulation at a dose of 0.17 mg/kg could be preventively administrated every six months in cats [50]. Additionally, a PO milbemycin monthly dose of 2 mg/kg is available for cats, but adverse effects such as hypersalivation, ataxia, mydriasis, and central nervous system depression should be constantly monitored [53]. Furthermore, the highest safe level of selamectin, which has been proven up to ten times its recommended dose in kittens [49,50], is also a suitable and safe option to treat gurltiosis in domestic cats. However, the PO selamectin administration at the recommended topical dose (6 mg/kg) may cause salivation and vomiting in malnourished or underweight cats [49]. As in other metastrongyloid parasitoses, it is well known that an efficient treatment involves repeated check-ups and repeated treatments when necessary [54]. The use of anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, neuroprotective vitamins (i.e., Vitamins B, C, E, and K), and other medicaments is left to clinician discretion. The animal's recovery depends on the gravity of the vascular damage and the grade of limb cord compression. The longer it takes to establish treatment, the lower is the recovery percentage [30].

Table 4. Suitable pharmacological drugs for treating the metastrongyloid *Gurltia paralysans* in domestic cats.

Drug	Structure [§]	Dose [†]	Route and Frequency of Administration	Reference
Ivermectin [a,*]		0.2–0.4	PO, SID, 1× weekly, 4 doses	[30,32,48]
Selamectin [b]		6	PO, SID	[30,49]
Milbemycin oxime [c]		2	PO, SID, 1× monthly	[30,50]
Ricobendazole [d,*]		20	PO, SID, 2× days	[30]
Fenbendazole [e]		50	PO, SID, 1× day, 3–5 doses	[30]

[†] The doses are indicated in mg (milligrams) per Kg (kilogram) of body weight. [§] Chemical structure depiction adapted from PubChem™. [a] 22,23-Dihydroavermectin B1a. [b] 25-cyclohexyl-25-de(1-methylpropyl)-5-deoxy-22 23-dihydro-5-(hydroxyimino)-avermectin B1 monosaccharide. [c] Milbemycin a4 5-oxime. [d] Methyl [5-(propane-1-sulfinyl)-1H-benzoimidazol-2-yl]-carbamate. [e] 2-(Methoxycarbonylamino)-5-(phenylthio)benzimidazole. * Drugs successfully administered to *G. paralysans* diagnosed cats.

5. The Northernmost *G. paralysans* Case Report in South America

In Colombia, a total of six feline gurltiosis case reports have been documented. Five Siamese cats that lived in the same household (i.e., two kittens, one adult male, and two adult females) from Tarso and one domestic mixed-breed cat from Amagá, Antioquia, exhibited moderate to severe paraplegia with general ataxia, decreasing of superficial sensitivity, and deep sensitivity loss. Additionally, the felids anamnesis evidence progressive paralysis more severe in adults due to the chronic clinical manifestation of the disease. Moreover, the cats showed hind limb atrophy and bladder and bowel dysfunction [32]. All described clinical signs correspond well with the chronic clinical evolution recorded for this angio-neurotrophic parasitic disease [17]. Subsequently, the cats´ necropsy and histopathology analysis showed the presence of *G. paralysans* specimens in the meningeal veins from the tenth thoracic vertebrae to the fourth lumbar vertebrae with medullar compression concomitantly myelomalacia [32]. Locals have frequently reported chronic long-lasting degenerative myelopathy in rural cats in the southwest and eastern Atrato subregion in Antioquia and Chocó, respectively, thereby suggesting *G. paralysans* infection in other Colombian areas not yet studied.

The presence of metastrongyloid parasites such as *Aelurostrongylus abstrusus (Angiostrongylidae)*, *Troglostrongylus brevior (Crenosomatidae)*, *Crenosoma vulpis (Crenosomatidae)*, and the zoonotic *Angiostrongylus vasorum (Angiostrongylidae)* have been reported in gas-

tropods from the *Achatinidae* family in several Colombian regions [55], including Antioquia, where *G. paralysans* cases have been identified [32,55], and the traditional ecological knowledge of peasant communities describing clinical signs in cats compatible with feline gurltiosis (see Figure 3). Additionally, an epidemiological approach showed the patent occurrence of *A. abstrusus* infections in domestic cats from Antioquia [56]. This region has also recently shown the presence of neglected zoonotic parasites such as *Spirometra* sp. *(Diphyllobothriidae)* and *Toxocara cati (Toxocaridae)* in neotropical wild felids [57]. It is essential to highlight that the Gastropoda class has been proposed as an obligate intermediate host for *G. paralysans* and is a highly biodiverse taxon in Colombia with at least 56 families and 120 species spread across various biomes (Table S1). Terrestrial gastropod species do not only inhabit humid and cool environments; they can also cope and prevail in hot and dry environments, therefore successfully adapting to insolation, heat, and drought [58]. Thus, those findings collectively demonstrate and highlight the epidemiological feasibility of a wider distribution of metastrongyloid nematodes such as *G. paralysans* in Colombia, given that the country has all the climates in which the parasite has been reported. Furthermore, studies on concomitant infections have not been consistently conducted across reported areas, despite their importance in understanding and identifying the epidemiological risk factors associated with feline gurltiosis [15] in domestic cats and wild felid populations.

Figure 3. Ecoepidemiological factors that allow gurltiosis occurrence in the Colombian Andean region. Images of *G. paralysans* gastropod intermediate hosts (IH) such as (**a**) smooth land slugs of the genus *Deroceras* sp. found in the Southwest subregion of Antioquia, Colombia and (**b**) Giant African snail (*Lissachatina fulica*) in an urban environment (Medellín city). (**c**) The natural domestic cat's curious behavior on a Giant African land snail favors close interaction with IH, demonstrating the feasibility of parasite transmission in urban areas. (**d**) Clinically ill mixed-breed domestic cat in an Andean rural area with long-lasting degenerative myelopathy and severe paraplegia.

As mentioned above, the wild felid of the species *Leopardus guigna*, commonly known as kodkod, is the natural definitive host, and domestic cats are considered accidental hosts for gurltiosis [19,20]. The parasitic myelopathy due to *G. paralysans* adult nematodes

and eggs inside the veins of subarachnoid space in the spinal cord of a margay has been described in Santa Catarina, Brazil [20]. Both the Goeffroy's cat (*Leopardus geoffroyi*) and the northern tiger cat (*Leopardus tigrinus*) have been proposed as definitive hosts [15,21]. Neither the kodkod nor Goeffroy's cat are distributed within Colombia; therefore, the margay and the northern tiger cat should be considered potential gurltiosis hosts in this geographical location. Other neotropical wild felid species found in Colombia such as jaguarundi (*Herpailurus yagouaroundi*), ocelot (*Leopardus pardalis*), jaguar (*Panthera onca*), and puma (*Puma concolor*) should not be ruled out as the natural definitive host in the Northernmost South American distribution area, where the parasite has so far been documented (Table 5).

Table 5. Potential *Gurltia paralysans* definitive wild felid host species in South America.

Genus	Species	Common Name	Conservation Status [§]
Herpailurus	*yagouaroundi* [1]	Jaguarundi	LC
Leopardus	*colocolo*	Pampas cat	NT
Leopardus	*geoffroyi* [2]	Geoffroy's cat	LC
Leopardus	*guigna* [2]	Kodkod	VU
Leopardus	*guttulus*	Southern tiger cat	VU
Leopardus	*jacobita*	Andean cat	EN
Leopardus	*pardalis* [1]	Ocelot	LC
Leopardus	*wiedii* [1,2]	Margay	NT
Leopardus	*tigrinus* [1]	Northern tiger cat	VU
Panthera	*onca* [1]	Jaguar	NT
Puma	*concolor* [1]	Puma	LC

[1] Felid species distributed within Colombia. [2] Species in which the parasite has been reported. [§] Based on the IUCN threat levels of classifications for endangered species. LC: Least-concern, NT: Near threatened, VU: Vulnerable, EN: Endangered.

6. Conclusions

In parallel with other neglected felid diseases frequently underestimated by veterinary clinicians, gurltiosis should be included in the differential diagnoses of feline spinal cord disorders. The prompt and accurate diagnosis of *G. paralysans* will contribute to improved health of infected definitive hosts and result in proper anthelminthic treatments impeding further gurltiosis spreading among felid populations. The poor understanding of this neglected angio-neurotrophic parasite's life cycle demands further research to identify the potential gastropod intermediate host species and paratenic hosts such as amphibians, birds, insects, lizards, and rodents. Additionally, comprehensive sampling efforts should be developed throughout the Americas, where the majority of feline gurltiosis cases are reported. Particular attention should be taken in North America, Africa, and Europe because cases outside of South America were recently reported in the west Africa palearctic realm and the north Nearctic realm, on the island of Tenerife, Spain, and NY, USA, respectively [16,33]. In conclusion, further studies are needed to evaluate the parasite occurrence among domestic cats and neotropical wild felid species distributed within Colombia as well as the plethora of gastropod/paratenic fauna that may harbor the developing larvae (L1–L3) stages of this underestimated parasite.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pathogens10121601/s1, Table S1: Gastropod species as potential intermediate hosts for *G. paralysans* in Colombia.

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Chapter 4 - Nationwide Seroprevalence Survey of *Angiostrongylus vasorum*-Derived Antigens and Specific Antibodies in Dogs from Colombia**.**

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Personal contribution to published paper

Initiative: essential

Project planning: as far as possible

Software: essential

Investigation: as far as possible

Writing—original draft preparation: essential

Visualization: essential

microorganisms

MDPI

Communication

Nationwide Seroprevalence Survey of *Angiostrongylus vasorum*-Derived Antigens and Specific Antibodies in Dogs from Colombia

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Abstract: *Angiostrongylus vasorum* is a cardiopulmonary nematode, causing several clinical manifestations in dogs, e.g., severe respiratory signs, coagulopathy, and gastrointestinal or neurological signs. In the last decades, this parasite has been described to spread and emerge in Europe and North America. Scant studies on *A. vasorum* occurrence in South America exist. Recently, *A. vasorum* was detected in gastropod intermediate hosts in Colombia, where data on definitive host prevalence, e.g., dogs and wild canids, are still limited. Therefore, the sera of 955 dogs, varying in age and breed from seven different departments all over Colombia, were collected and analysed for *A. vasorum* antigens and parasite-specific antibodies by ELISA. In total, 1.05 % ($n = 10$; 95 % CI 0.40–1.69) of the samples were antigen-positive and 2.62 % ($n = 25$; 95 % CI 1.61–3.63) were antibody-positive. These results confirm the presence of *A. vasorum* in Colombia, although positive results in antigen and antibody reactions in the same dog were not detected. This study is the first large-scale survey on *A. vasorum* seroprevalences in dogs from Colombia.

Keywords: *Angiostrongylus vasorum*; ELISA; canine angiostrongylosis; epidemiology; South America

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1. Introduction

The canid heartworm *Angiostrongylus vasorum* is a metastrongyloid lungworm species belonging to the most pathogenic cardiorespiratory parasites in dogs, and also infects a broad range of wild carnivore families, e.g., Canidae, Mephitidae, Mustelidae, Procyonidae, and Ailuridae [1,2]. This nematode has a heteroxenous lifecycle, where terrestrial gastropods act as natural obligate intermediate hosts [3]. Clinical manifestations of angiostrongylosis in dogs vary broadly from non-specific signs like lethargy, anorexia, diarrhoea, and weight loss to more specific signs such as coughing, dyspnoea, and haemorrhages caused by verminous pneumonia and disseminated coagulopathies [4–6]. Furthermore, canine angiostrongylosis can be associated with neurological signs, ocular manifestations, or even pneumothorax due to alveolar rupture [7–11]. Domestic dogs may be clinically unapparent for months to years before manifesting clinical signs, or may become chronically ill, or even die acutely [12]. Because subclinical *A. vasorum* infections can occur, detecting infected dogs can be difficult. Thus, veterinarians' awareness of this parasitic disease is important for the early detection of the infection, thereby performing an appropriate diagnostic interpretation, anthelmintic treatments, and preventive measures. Rapid serological assays, ultrasonography, nonclassical coprological tests such as Mini-FLOTAC, and molecular detection, but also classical and low-cost Baermann funnel techniques, have

all shown usefulness in *A. vasorum* diagnosis [13]. A rapid and precise diagnosis is essential, not only for proper anthelminthic treatments, but also for disease control among host populations [14–16]. The advantages of serological assays like ELISAs principally are the possibility to detect non-patent infections and to test efficiently large groups of animals, and to therefore better understand epizootiology of canine angiostrongylosis [17].

In the last decades, *A. vasorum* was reported to spread and emerge in previously supposed non-endemic regions [18–20]. The worldwide geographical distribution reveals the parasite's occurrence in Africa, America, and Europe, which is shown in Figure 1 [2,19,21–25]. To the best of our knowledge, to date there have been no autochthonous occurrence reports of this cardio–pneumotropic parasite in Asia, Oceania, or the Middle East, either in carnivore definitive hosts or in obligate gastropod intermediate hosts (e.g., snails, semi-slugs, and slugs). Scientific attention on this parasite was raised and many epidemiological studies, especially in Europe, were performed [2]. A wide distribution of the parasite is reported in this continent, despite the absence of reports for some European countries such as Andorra, Bosnia and Herzegovina, Kosovo, Liechtenstein, Luxembourg, and Slovenia. Notwithstanding, given the intermediate host density, spatial variations, and environmental factors that influence parasite transmission and local movement [26], it is very likely that the parasite occurs in these territories, but remains unreported for domestic dogs until now.

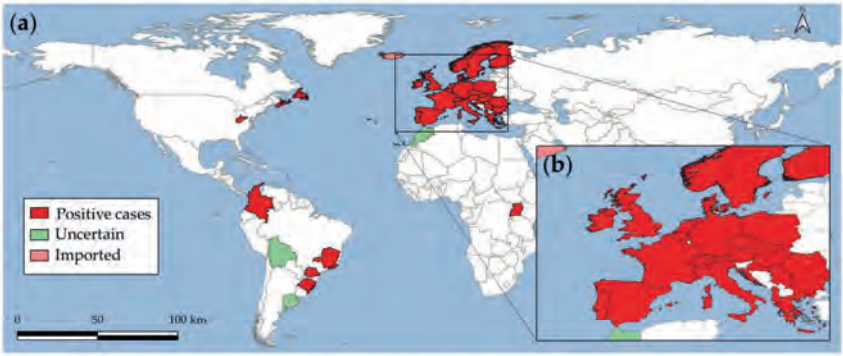

Figure 1. Worldwide geographical distribution range of *Angiostrongylus vasorum*. **(a)** Map shows the global location of parasite case reports in definitive and intermediate hosts. **(b)** Europe close-up.

In Africa, the parasite was observed during the 1960s in Ugandan domestic dogs [22]. Additionally, in Morocco, a potential autochthonous *A. vasorum* case in a 6-month-old asymptomatic dog was reported [21]. For the North American subcontinent, there are *A. vasorum* reports in canids from West Virginia (USA), and Nova Scotia and Prince Edward Island in Canada [12,27]. The historically endemic focus for *A. vasorum* in North America occurs in the south-eastern part of Newfoundland island [12]. In South America, data on *A. vasorum* prevalence are still scarce and ambiguous 'lungworm larvae' descriptions make it difficult to establish the real parasite distribution in the Bolivian Chaco and Buenos Aires province in Argentina [19,28]. Throughout southern Brazil, natural *A. vasorum* infections were reported both in wild canids (i.e., *Cerdocyion thous* and *Lycalopex vetulus*) and domestic dogs in the state of Minas Gerais, Paraná, Rio de Janeiro, and Rio Grande do Sul [19,29]. Recently, *A. vasorum* larvae were found in the invasive giant African snail species *Lissachatina fulica* as well as other metastrongyloid lungworm species (i.e., *Troglostrongylus brevior*, *Crenosoma vulpis*, *Aelurostrongylus abstrusus*) in Colombia for the first

time [30]. In definitive hosts, *A. vasorum* was identified in Colombia initially in a domestic dog sixty years ago by Gonçalves et al. (1961) in accordance with [31], and in 2014, another patent *A. vasorum* infection was reported in a crab-eating fox (*C. thous*), thus constituting the only two nationally published records of this metastrongyloid parasite [32]. Consistently, data on the actual prevalence or the spread of canine angiostrongylosis to new Colombian areas are still lacking, despite the overlapping spectrum of natural intermediate hosts and definitive hosts carrying *A. vasorum* infections [28,29]. Moreover, classical diagnostic methods such as the Baermann funnel technique, by which first-stage larvae (L1) can be microscopically identified in the faeces of definitive hosts, are rarely used in veterinary clinics around the country. Naturally *A. vasorum*-infected dogs may show highly variable clinical manifestations, thus the accurate, prompt, and effective diagnosis of this parasite is not a straightforward task [6,33]. As canine angiostrongylosis is a neglected disease and the knowledge of *A. vasorum* epidemiology in South America is still poorly understood, it can be easily overlooked by veterinary clinicians [19].

In previously published large-scale epidemiological studies on *A. vasorum*-infected dogs and foxes within Europe [34], novel diagnostic tools such as serology have frequently been used to reveal occurrences. Thus, the aim of the current epidemiological survey was to perform a nationwide prevalence survey for *A. vasorum* in Colombian domestic dogs using the combined detection of circulating antigens and specific antibodies through a serological approach.

2. Materials and Methods

Briefly, from March 2017 to May 2017, a total of 1024 domestic dog blood samples were collected from across Colombia through collaboration with veterinary clinicians, universities, veterinary diagnostic laboratories, and animal hospitals. Based on the Köppen-Geiger classification, the serological survey was carried out in Amazonian, Andean, Caribbean, Orinoco, and Pacific eco-epidemiological regions of Colombia, and in seven departments (i.e., Antioquia, Atlántico, Córdoba, Cundinamarca, Risaralda, Sucre, and Meta) throughout tropical rainforest (Af), tropical monsoon (Am), tropical wet savannah (Aw), and temperate oceanic (Cfb) climates [35].

The study inclusion criteria for dogs were being older than one month and not having received prophylactic anti-parasitic treatments. All sampled animals were owned pets or dogs from animal shelters; no stray or feral dogs were included. Whole blood (WB) samples were collected mainly from the venipuncture of the cephalic or jugular veins and placed into 3 mL vacutainer tubes without anticoagulant. The WB samples were stored at 4 °C for a maximum of 24 h before sera isolation, avoiding thawing and freezing the sample several times. Thereafter, the samples were centrifuged at 2200 rpm for 20 min to separate the sera and blood cells. The serum samples were stored at −20 °C until further use. From the total of the collected WB samples (n = 1024), a total of 979 serum samples were successfully obtained, and 963 samples were finally suitable for serological analyses, discarding haemolytic or lipaemic samples. Finally, a overall of 955 sera samples were analysed, due to insufficient sample quantity and haemolysis of some samples. The sera (n = 955) were tested for the presence of circulating *A. vasorum* antigens using monoclonal and polyclonal antibodies in a sandwich ELISA, with a sensitivity of 95.7% and a specificity of 94.0%, as previously described [36], and for antibodies against *A. vasorum* using adult *A. vasorum* somatic antigen purified with monoclonal antibodies (mAb 5/5), with a sensitivity of 81% and a specificity of 98.8% [17]. Both tests were performed at the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland. Test thresholds were determined with 300 randomly selected samples based on the mean value of optical density (A_{405} nm) plus three standard deviations. All test runs included a background control, a conjugate control, three positive control sera from three experimentally infected dogs, and two control sera from negative dogs. For a complete summary list of each sample in which the sex, age, breed, origin, and serological results (Ag/Ab) are described, please refer to Table S1.

3. Results

The *A. vasorum* antigen- and antibody-positive tested samples originated from different areas of Colombia, distributed in five out of seven departments (i.e., departments of Antioquia, Atlántico, Córdoba, Meta, and Sucre). The location and seropositivity of all tested samples is shown in Figure 2.

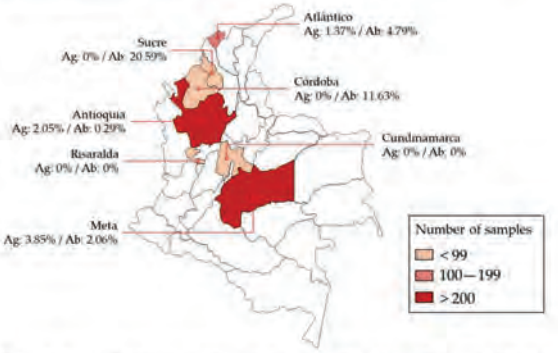

Figure 2. Geographical origins of domestic dogs (n = 955) serologically examined for *Angiostrongylus vasorum* occurrence. Different red shades represent the sampling density by area. Ag: antigen prevalence; Ab: antibody prevalence.

Overall, 1.05% (n = 10; 95% CI 0.56–1.92) of the evaluated dog sera tested *A. vasorum* antigen-positive. Furthermore, 2.62% (n = 25; 95% CI 1.77–3.83) of the sampled dogs tested *A. vasorum* antibody-seropositive. Table 1 summarizes the seropositivity status of all examined animals. None of the analysed sera tested positive for both, i.e., *A. vasorum* antigen and antibody detection. Antigen-positive dogs originated from the municipalities of Medellín, Barranquilla, and Villavicencio. Antibody-positive dogs came, likewise, from the same areas with additional seropositive dogs coming from Montería, Sincelejo, and Cumaral. The origin of 43 samples was unknown. The prevalence of both antibodies and antigens was only reported in two municipalities (i.e., Barranquilla and Medellín). The highest regional antigen serological prevalence of *A. vasorum* was detected in Villavicencio at 3.85% (1 out of 26; 95% CI 0.68–18.89), followed by Medellín at 2.05% (7 out of 341; 95% CI 0.997–4.17), and Barranquilla at 1.37% (2 out of 146; 95% CI 0.37–4.85). On the other hand, major antibody reactivity was evidenced in Sincelejo at 20.59% (7 out of 34; 95% CI 10.34–36.79) and in Montería at 11.63% (5 out of 43; 95 % CI 5.07–24.47), both located in the north-western Caribbean region, characterized by a tropical wet savannah (Aw) climate.

Table 1. Serological results of dog sera samples from Colombia, tested for the presence of *A. vasorum* antigens and for specific antibodies against *A. vasorum*.

Departments	Municipalities	*n*	Antigen +	%	CI [1]	Antibody +	%	CI [1]
Antioquia	Bello	6	0	0.00%		0	0.00%	
	Caldas	1	0	0.00%		0	0.00%	
	Copacabana	5	0	0.00%		0	0.00%	
	El Retiro	1	0	0.00%		0	0.00%	
	Itagüi	5	0	0.00%		0	0.00%	
	La Ceja	3	0	0.00%		0	0.00%	
	Medellin	341	7	2.05%	0.99–4.17	1	0.29%	0.05–1.64
	Rionegro	3	0	0.00%		0	0.00%	
	Sabaneta	4	0	0.00%		0	0.00%	
Atlántico	Barranquilla	146	2	1.37%	0.37–4.85	7	4.79%	2.34–9.56
	Puerto Colombia	20	0	0.00%		0	0.00%	
Córdoba	Montería	43	0	0.00%		5	11.63%	5.07–24.47
Cundinamarca	NR [2]	39	0	0.00%		0	0.00%	
Risaralda	Pereira	31	0	0.00%		0	0.00%	
Sucre	Sincelejo	34	0	0.00%		7	20.59%	10.34–36.79
Meta	Cumaral	243	0	0.00%		5	2.06%	0.88–4.72
	Villavicencio	26	1	3.85%	0.68–18.89	0	0.00%	
	NR [2]	4	0	0		0	0	
	Total	955	10	1.05%	0.569–1.916	25	2.62%	1.77–3.83

[1] CI = 95% confidence interval; [2] Not recorded. + positive serological reactivity.

4. Discussion

Canine angiostrongylosis is an emerging and underestimated cardiopulmonary disease reported only sporadically in South America [37]. In contrast, serological studies carried out in European canids have shown an endemic occurrence and a widespread distribution of *A. vasorum* [34,38–42]. The current study presents first-time evidence of *A. vasorum* occurrence among a representative dog population (n = 955) in different Colombian regions, and thereby contributes to a deeper understanding of the actual prevalence in South American dogs. On this continent, the presence of "lungworm larvae" has been previously reported both in wild and domestic canids [28,43–47]. However, the identification of these larvae was not properly confirmed due to confusing morphological traits or non-specific serological reactivity. Notwithstanding, even new approaches to studying the morphological details of intra-molluscan stages of *A. vasorum* have been developed [48–50]. A prevalence of 3.9% *A. vasorum*-infected African giant snails (*Lissachatina fulica*; n = 609) was found in a preliminary gastropod survey in Colombia, which also reported the presence of the *A. vasorum* European genotype in South America [37]. The current study indicates that dogs act as definitive hosts in several regions of Colombia.

With an overall sera prevalence of 1.05%/2.62% (antigen/antibody), these results show similarities to European serological studies for *A. vasorum* infections, but the prevalence remains lower than the reported occurrence in Colombian gastropods [34,39–42]. While most of the *A. vasorum* prevalence data in definitive host populations is higher than the one of intermediate host populations, this discrepancy seems to be unexpected. As *A. vasorum* occurs in hyperendemic foci, the variable sampling locations selected in these different studies might be a possible explanation for the differences [51,52].

Here, we did not find any correlation between the detection of seropositivity for *A. vasorum* and the dog's sex. Regarding geographical origins, in Sincelejo and Barranquilla, seven dogs were antibody-positive, respectively, followed by Montería and Cumaral, both with five positive animals, and one positive sample for Medellín. Additionally, 7 out of 10 antigen-positive samples came from the municipality of Medellín, Antioquia.

It should be highlighted that wild definitive hosts, e.g., red foxes (*Vulpes vulpes*) in Europe, are reported to act as pivotal wild reservoir hosts, which additionally show higher

prevalence than domestic dogs, and therefore play an important role in the spread of *A. vasorum* [53]. Hence, the current data might indicate that other wild canid hosts for *A. vasorum* in Colombia might also represent the natural reservoirs, having higher prevalence, as reported for red foxes in Europe. It is thus important to evaluate the *A. vasorum* infections among wild carnivores in non-endemic and unreported areas, as potential reservoir hosts for domestic animal infections [54]. Consequently, wild carnivores, distributed in the Neotropical realm, should be sampled in the future for a better understanding of *A. vasorum* epidemiology in this highly biodiverse region. Within the little existing data, *A. vasorum*-infected crab-eating foxes (*Cerdocyon thous*) have been reported and seem to be a potential definitive host. However, since the short-eared dog (*Atelocynus microtis*), maned wolf (*Chrysocyon brachyurus*), bush dog (*Speothos venaticus*), hoary fox (*Lycalopex vetulus*), sechuran fox (*Lycalopex sechura*), pampa's fox (*Lycalopex gymnocercus*), Darwin's fox (*Lycalopex fulvipes*), culpeo (*Lycalopex culpaeus*), chilla (*Lycalopex griseus*), and members of the superfamily Musteloidea, like tyra (*Eira barbara*), neotropical otter (*Lontra longicaudis*), ring-tailed coati (*Nasua nasua*), kinkajou (*Potos flavus*), and olingo (*Bassaricyon* sp.) could also be potential hosts for *A. vasorum*, further investigations are required to understand and establish the role of these species in the lifecycle, distribution, and epidemiology of the parasite in South and Central America.

The highest antibody-seroprevalence in the current study was calculated for Sincelejo/Sucre at 20.59% (95% CI: 10.35–36.80) and may indicate a hyperendemic focus area. Surprisingly, the antigen testing resulted negative in samples originating from this region. However, no sampling location was found to be positive for both antibody and antigen tests. A similar unexpected observation was found in other serological studies from dogs in Romania and Bulgaria [42,55]. Seroconversion is directly associated with the sampling time point. Antigen detection is possible 7–11 weeks after infection, while antibody seroconversion may occur as early as 3–6 weeks after infection. In contrast, after parasite elimination, antibodies may persist up to 9 weeks, while circulating antigens were not anymore detectable after 6 weeks. This may explain the generally observed higher number of antibody positive dogs compared to antigen positive dogs in this study [13].

To conclude, the current study confirmed the endemic occurrence of the cardiopulmonary nematode *A. vasorum* in Colombia. It also aims to raise the awareness of veterinary health staff of this neglected parasite in South America, with the goal of early diagnosing and performing treatment of affected dogs. Moreover, it calls for further research activities to evaluate the parasite occurrence among domestic dogs, other endemic wild canids (mainly foxes, bush dogs, and mane wolves), obligate gastropod intermediate hosts, and potential paratenic host populations within non-endemic regions in the Americas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms10081565/s1, Table S1: Dog serum database.

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Informed Consent Statement: The dog owners signed informed consent forms, and the laboratories or clinics sent permission to use the dog serum samples in their serum banks.

Data Availability Statement: Not applicable.

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Chapter 5 - Presence of *Spirometra mansoni*, Causative Agent of Sparganosis, in South America.

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Personal contribution to accepted paper

Initiative: essential	Data curation: as far as possible
Project planning: as far as possible	Writing—original draft preparation: essential
Conceptualization: essential	Writing—review and editing: as far as possible
Methodology: essential	Visualization: essential
Investigation: essential	

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Presence of *Spirometra mansoni*, Causative Agent of Sparganosis, in South America

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We report molecular identification of an adult *Spirometra mansoni* tapeworm retrieved from a crab-eating fox (*Cerdocyon thous*) in Colombia, confirming presence of this parasite in South America. This tapeworm is the causative agent of human sparganosis, commonly reported from Southeast Asia, and represents the second congeneric species with known zoonotic potential in the Americas.

Sparganosis is a neglected human zoonosis caused by migrating larval stages of the broad tapeworm genus *Spirometra* (Diphyllobothriidea), whose natural definitive hosts include wild and domestic canids and felids. The life cycle of this tapeworm involves 2 intermediate hosts: a freshwater copepod crustacean as the first and various vertebrates, mostly amphibians, as the second. Human infections are commonly reported from Southeast Asia and propagate most often in the form of subcutaneous sparganosis; however, the larvae can enter other organs or parts of central nervous system and cause damage.

Taxonomy of *Spirometra* remains highly complicated. Numerous species of *Spirometra* have been described, often poorly (*1*), and representatives of just 6 species-level lineages have been characterized molecularly so far, a key prerequisite to achieve a convincing tapeworm identification when only strobila fragments or larval stages are available. Limitations of morphologic characters of *Spirometra* are numerous and include characters' great intraspecific and even intra-individual variability (overview of problematic traits in *2*). Molecular sequence data thus represent the only unequivocal method of species identification.

Previous phylogenetic analysis of *Spirometra* has shown that the geographic distribution of the 6 lineages respects continental borders (*2*). North

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and South America were shown to share 2 lineages found exclusively on those continents (3), provisionally termed *Spirometra decipiens* complex 1 and 2 because of the lack of essential morphologic data precluding conclusive species determination (2). *S. decipiens* complex 1 was shown to house, among parasites of canids and felids, causative agents of cutaneous and proliferative sparganosis. Representatives of *S. decipiens* complex 2, on the other hand, have not yet been shown to cause the zoonosis. The frequently reported human cases of sparganosis from Southeast Asia, as well as numerous specimens from wildlife from the region, corresponded to *S. mansoni* (2).

We report molecular identification of a tapeworm specimen retrieved from a dead crab-eating fox (*Cerdocyon thous*) from the vicinity of Ciudad Bolívar, Antioquia, Colombia. We characterized the specimen through Sanger-sequencing of 3 genetic loci (Appendix, https://wwwnc.cdc.gov/EID/article/28/11/22-0529-App1.pdf), including the complete mitochondrial cytochrome c oxidase subunit gene (*cox1*) as the most densely sampled and phylogenetically informative gene of broad tapeworms

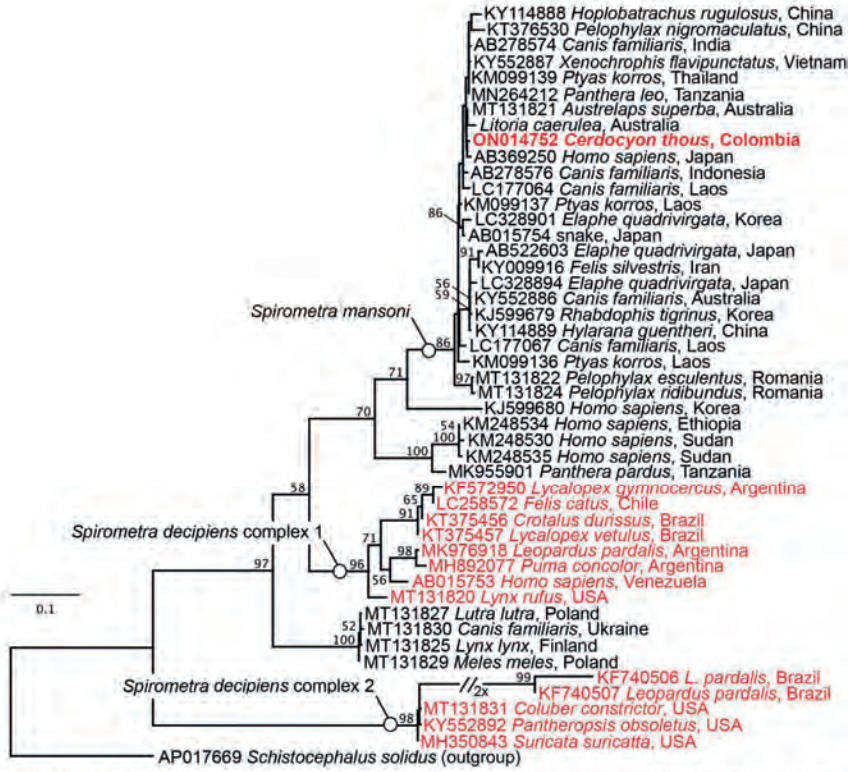

Figure. Maximum-likelihood estimate of the phylogenetic position of a *Spirometra mansoni* tapeworm collected from a crab-eating fox (*Cerdocyon thous*) in Colombia. Red indicates specimens from South America; bold indicates newly characterized *S. mansoni* from this report. Names of the 3 species-level lineages of *Spirometra* in South America are indicated; GenBank numbers are provided. Nodal support values show standard bootstrap supports >50. Scale bar indicates number of substitutions per site.

Phylogenetic analysis under maximum-likelihood criterion resolved the position of the tapeworm nested deep within the clade of *S. mansoni* (Figure), proving the presence of this causative agent of human sparganosis on the American continents.

S. mansoni represents by far the most frequently reported causative agent of sparganosis, previously misidentified as *S. erinaceieuropaei* (2). This species is responsible for virtually all human cases in Asia but has been also shown to infect wildlife in Africa, Australia, and Eastern Europe (2). Our finding of *S. mansoni* in Colombia in a crab-eating fox, a definitive host endemic and widely distributed across South America, from Panama to the Entre Ríos province of Argentina (4), expands the known distribution of *S. mansoni* into broader range than previously thought. This finding contrasts with the distribution of the remaining 5 lineages of *Spirometra*, which seem limited to continental regions (2). *S. mansoni* has been sporadically reported from the Americas in the past; however, morphology-altering fixation techniques and lack of critical molecular evidence did not support species identification. Reported hosts mostly included domestic cats (Appendix) and a single report from a crab-eating fox in Brazil (5).

The crab-eating fox inhabits savannah and woodland areas of various Neotropical habitats from coastal plains to montane forests and is considered omnivorous, opportunistically feeding on fruits, insects, and small vertebrates including amphibians and reptiles, with seasonal shifts to its diet (6,7). A broad range of Neotropical amphibians and reptiles has been found to serve as intermediate hosts of *Spirometra*; however, the record remains skewed toward herpetofauna of the more intensively surveyed coastal regions (8), and species identification of the parasite larvae has been, thanks to the lack of accompanying molecular data, either absent or ungrounded. As a result, the real range and the relevance of different intermediate hosts for the transmission of the sympatric South America species of *Spirometra* remain unknown. The situation in North America is even more obscure because of the virtually missing intermediate host record (1,9). Given the wide spectrum of suitable intermediate hosts of *S. mansoni*, which includes omnivores such as wild boar in Europe (10), the natural pools and the importance of different host species in the etiology of the zoonosis remain dubious. The concurrent presence of the second congeneric species with zoonotic potential urges deeper investigations into the parasite's life cycles and the epizootiology of a disease that could affect public health in the Americas.

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Chapter 6 - New insights into zoonotic helminthiases of neglected Neotropical wild canids.

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New insights into zoonotic helminthiases of neglected Neotropical wild canids.

Personal contribution to published paper

Initiative: essential

Project planning: as far as possible

Conceptualization: essential

Methodology: essential

Sample collection: essential

Validation: as far as possible

Formal analysis: as far as possible

Investigation: essential

Writing—original draft preparation: essential

Visualization: essential

New insights into zoonotic helminthiases of neglected Neotropical wild canids

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Abstract

Wild canids have a pivotal role in the lifecycle, and epidemiology of public health concern diseases, thus are well-known reservoirs of parasites shared with humans and domestic animals. Developing countries have a higher concentration of zoonotic emerging pathogens, which favour diseases transmission into the human-domestic animal-wildlife interface. Rarely clinically reported and underestimated neglected parasitosis have been fallen in obscurity as public health issues. Through coproparasitological, morphological and phylogenetic examination here we evaluate the occurrence of gastrointestinal parasites in the bush dog (*Speothos venaticus*) and crab-eating fox (*Cerdocyon thous*) from Amazonian and Andean region. The bush dog cestodes were molecularly characterized by sequencing COI (cytochrome c oxidase subunit I) and thereafter identified as a canine-specific lineage of *Dipylidium caninum*. Besides, the crab-eating fox faecal examination showed the presence of non-embryonated *Lagochilascaris minor* eggs, both eggs and gravid proglottids of *Spirometra mansoni*. In addition to previously molecular identifed *Spirometra mansoni*, herein we report the occurrence of neglected zoonotic helminthiases in South American wild canids. Current study constitutes the first so-called "*D. caninum* canine genotype" linage identification in wild canids and the first non-human *L. minor* report in Colombia.

Author summary

The global threat of Neglected Tropical Diseases (NTD) constitutes a public health issue endangering the socioeconomic development and well-being specially in low-latitude developing countries. A significant percentage of emerging human infectious diseases are wildlife-derived pathogens and over 75% of global disability adjusted life years lost is attributed to helminth parasites. Wild canids have a pivotal role on public health concern pathogens as well-known reservoirs of zoonotic parasites. The knowledge of zoonotic helminth diseases is often limited and underestimated even by specialists. In this study, we used the COI (cytochrome c oxidase subunit I) gene for the reconstruction of the interrelationship of *Dipylidium caninum* proglottids in bush dog (*Speothos venaticus*). Additionally, herein we identify *Lagochilascaris minor* and *Spirometra mansoni* eggs in crab-eating fox (*Cerdocyon thous*) faeces and morphologically characterized adult stages of *S. mansoni* found during necropsy procedure. Thus, the occurrence of potentially zoonotic helminthiases constitutes a strong call to constantly monitor parasites in wild carnivore populations to better understand the epidemiology and transmission dynamics of parasitic NTD such as dipylidiosis,

lagochilascariosis, and sparganosis among the human-domestic animal-wildlife interface.

Introduction

The old-dated human-wildlife interface favours a plethora of infectious diseases to circulate between human and animal populations. Approximately 60% of the emerging human infectious diseases are zoonoses and among these up to 70% are wildlife-derived pathogens [1,2]. It is important to draw attention that amongst the wide variety of wildlife-originated infectious diseases the worldwide distributed zoonotic helminthiases accounting for over 75% of global disability-adjusted years lost and the fact that many of them have been fallen in obscurity as neglected diseases [3,4]. Around the globe wild canids are well-known zoonotic parasite reservoirs [5–10], and thus have a pivotal role in the lifecycle, epidemiology and transmission chain of public health concern parasites [11–14]. The forested tropical regions where changing land-use take place and present a high mammal species richness are emergence zoonotic infectious diseases hotspots and thus present an elevated disease transmission risk into the human-domestic animal-wildlife interface [15,16]. The lower-latitude developing countries (e. g., Neotropical territories) concentrate zoonotic emerging pathogens from wildlife although the scientific and surveillance effort focused on this issue is scarce [17].

The knowledge of zoonotic cestode human infections is often limited to genera *Dibothriocephalus* (diphyllobothriosis), *Hymenolepis* and *Taenia*, leading uncommon neglected cestodiases such as bertielliosis, dipylidiosis, echinococcosis, inermicapsiferosis, raillietinosis, mesocestoidiosis and sparganosis, rarely clinically reported and underestimated even by specialists [4,18,19]. Likewise, wild canids are a large group of dog-like related facultative carnivore taxa found all over the world and often closely related to human populations [20–22]. From the mammals arrival to South America about 66 Mya, thenceforth species diversification has been shaped by climate change, human-induced population declines, and topography [23]. Nowadays a total of five genera and ten species of wild endemic canids with varied behaviours, diverse habitats, disparate forms, and distribution ranges are reported across the Neotropical region (Table 1).

Table 1. Wild canid species distributed in the Neotropical realm.

Genus	Species	Common name	Classification Risk [§]
Atelocynus	*microtis*	Short-eared dog	NT
Cerdocyon	*thous* [‡]	Crab-eating fox	LC
Chrysocyon	*brachyurus*	Maned wolf	NT
Speothos	*venaticus* [‡]	Bush dog	NT
Lycalopex	*vetulus*	Hoary fox	NT
Lycalopex	*sechura*	Sechuran fox	NT
Lycalopex	*gymnocercus*	Pampa's fox	LC
Lycalopex	*fulvipes*	Darwin's fox	EN
Lycalopex	*culpaeus*	Culpeo	LC
Lycalopex	*griseus*	Chilla	LC

[‡] Wild canid species included in present study.
[§] Based on the International Union for

Conservation of Nature (IUCN) threat levels of classifications for endangered species.

As a result, the current study aims to present the findings on the gastrointestinal helminth parasite occurrence in two highly divergent free-ranging neotropical wild canid species (i. e., the semiaquatic diurnal/crepuscular elusive bush dog and the nocturnal ground-dwelling crab-eating fox). Furthermore, we examine the potential roles that these definitive hosts play in the transmission and maintenance of neglected zoonotic helminthiasis, gaining new insights into this unresolved issue.

Methods

Ethics statement

The study was approved by the Ethics Committee for Animal Experimentation (CEEA) of the Universidad de Antioquia, Colombia (AS No. 132) under collection permit No. 0524 of 2014 (IDB0321), procedures were conducted according to the Guidelines of the American Society of Mammologists for the use of wild mammals in research and education, and the EU Directive 2010/63/EU.

Study areas and sample collection

Alongside the wide distribution range of the bush dog and carb-eating fox in the Neotropical realm the sampling effort focused on the northern side of South America (Figure 1). In the environs of grass-fed cattle ranching farm, a cestode in crab-eating fox gastrointestinal tract (duodenum) was found during macroscopical examination in the Andean municipality of Ciudad Bolívar, Colombia. Unfortunately, only one specimen and a small amount of fresh faeces were successfully recovered and adequately preserved from the gut lumen after the necropsy procedure. Additionally, spontaneously released cestode proglottids in bush dog faeces were found partially dehydrated in the Amazonian municipality of Puerto Santander, Colombia. In accordance with the Köppen-Geiger classification system the Andean and Amazonian sampling areas respectively correspond to a temperate warm summer without dry season (Cfb) and tropical rainforest (Af) climate as show in Figure 1 [77].

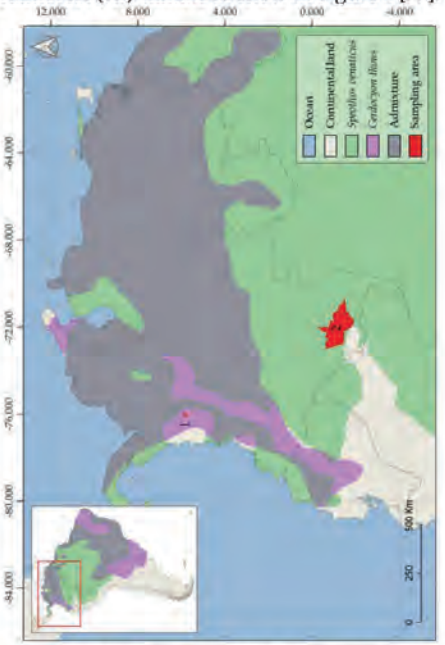

Figure 1. The geographical map depicts the historic distribution range of the bush dog (*Speothos venaticus*), the crab-eating fox (*Cerdocyon thous*), and both species admixture zone. The present study Andean (1) and Amazonian (2) sampling areas are showed in red.

After the macroscopical identification of cestode strobila and free proglottids in wild canid

faeces, the parasite specimens were gently rinsed and washed 3 times with 0.9% pre-warmed phosphate-buffered saline (PBS), and thereafter ~96% EtOH preserved until subsequent microscopical and molecular evaluation. The non-invasive methodology successfully performed for other wild mammals allows to recover adult cestode specimens without manipulate, trapping or disturb the natural behaviour of the canid species [75,78].

Phenotypic evaluation of adult cestode specimens

The general morphological and morphometrical characteristic taxonomic traits and parasite stages identification were made under microscopical analyses using an Olympus BX53 semi-motorized light microscope (Olympus Corporation, Tokyo, Japan) at 400 and 1000 X magnification. The Olympus DP74 digital camera was used to acquire eggs, adult strobila and proglottids photomicrographs. Precise measurements of parasite stages were obtained using the *cellSens* standard imaging software. Previously, some adult cestode strobila sections and free proglottids were transferred to a fixative solution (Formalin, 95% EtOH, Glacial acetic acid, Glycerine, and Milli-Q ultrapure distilled water; 10:25:5:10:50 parts respectively), thereafter lactophenol clarified, Semichon's acetocarmine stained before dehydrated in ethanol series (75, 80, 85, 90, 96, and 100%), and finally both wet- and Berlese's fluid-mounted as before described [79]. The crab-eating fox dry preserved faeces was analysed by the modified sodium acetate-acetic acid-formalin (SAF) technique [80].

Molecular phylogenetics

Complete coding sequence of the cytochrome c oxidase subunit I (COI) gene was amplified in two overlapping fragments using the primers cox1F and JB4.5, and JB3 and cox1R, respectively [81,82] using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Inc., Ipswich, USA) and the following cycling conditions: 35 cycles of 10 s at 98 °C, 15 s at 50 °C (cox1F+JB4.5) or 60 °C (JB3+cox1R), and 50 s at 72 °C. PCR products were gel-checked, purified with Exonuclease I and FastAP alkaline phosphatase (Thermo Fisher Scientific, Waltham, USA), and directly Sanger-sequenced at SeqMe (Dobříš, Czech Republic). Contiguous gene sequences were assembled, visually checked, and trimmed to the cox1 coding region in Geneious Prime 202.0.5 (http://www.geneious.com). The resulting sequences were aligned with previously published cox1 data of *Dipylidium* specimens as well as other closely related species using MAFFT's [83] L-INS-i translational align within Geneious. The use of *Nippotaenia chaenogobii* (JQ2685509) and *Nippotaenia mogurndae* (ON640728) as outgroup taxa and relevant ingroup representatives' selection was informed by previous phylogenetic estimated, most importantly by Waeschnebach *et al.*, 2012 [84] and Guo *et al.*, 2017 [85]. The phylogenetic tree was estimated under maximum likelihood criterion in IQ-TREE [86]. The best-fitting model of nucleotide evolution was selected according to the corrected Akaike information criterion in IQ-TREE [87] and nodal supports estimated through running 1000 standard nonparametric bootstrap replicates and 10000 repetitions of SH-like approximated likelihood ratio test.

Results

Morphological and morphometrical parasite identification

The morphological identification of the whitish flat barrel-shaped segments recovered from bush dog faeces (see Figure 2) shows longer-than-wider morphology, each proglottid evidence two bilateral genital pores, one in the middle of each lateral margin. The mean gravid proglottid measurements (n = 10) were 12.082 mm (SD ± 0.542 mm) in length and 3.996 mm (SD ± 0.344 mm) in width. The phenotypic evaluation corresponds well to *Dipylidium caninum* s.l. (Dipylidiidae). Thin-shelled capsules (ovigerous capsules) containing eggs were also noticed inside gravid proglottids.

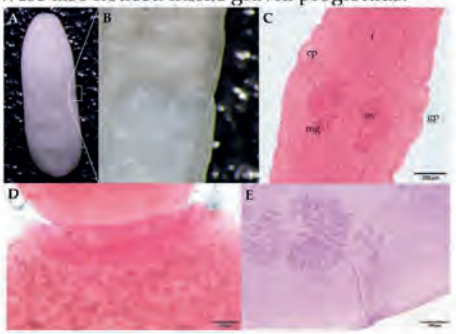

Figure 2. Proglottids of *Dipylidium caninum* s.l. (Dipylidiidae) collected from the Amazonian bush dog (*Speothos venaticus*) faeces. **A.** Wet mount unstained gravid proglottid. White square area shows: **B.** Lateral magnified view of the genital pore. **C.** Mature proglottids Semichon's acetocarmine stained; notice two sets of genital organs symmetrically distributed, the testes parenchyma (t), cirrus pouch (cp), genital pore (gp), ovaries (ov), and the Mehlis glands (mg). **D.** Close-up photograph of seed-shaped ovigerous proglottid end loaded with round to oval egg capsules (packets) with an average length range 31 - 50 µm and width from 27 - 48 µm (n = 88). **E.** Details on one of two sets of male and female reproductive organs. Scale bars: (c-e) 200 µm.

Regarding the crab-eating fox coproparasitological evaluation we evidence the presence of ascarid-type eggs which morphological traits corresponded well to *Lagochilascaris minor* (Figure 3a). Additionally, parasite stages (i. e., adult and eggs) of the diphyllobothriidean genus *Spirometra* were evidenced. A weakly muscular medium sized pinkish colour cestode (89.73 cm in length) with long distinct neck were evidenced. External segmentation throughout the specimen strobila was noticed. The cestode shows a well-developed spoon-shaped scolex with no inrolling bothrial edges. The mature and gravid proglottids were serrate and the eggs present a sightly evident unique operculum and end-pointed oval shape (Figure 3b-e). The average proglottid measurements (n = 483) were 454.14 µm (SD ± 207.16 µm) in length and 1.78 mm (SD ± 0.73 mm) in width. Strobila segments of this parasite specimen were previously molecular identified as *S. mansoni* by Brabec *et al.*, 2022 [24].

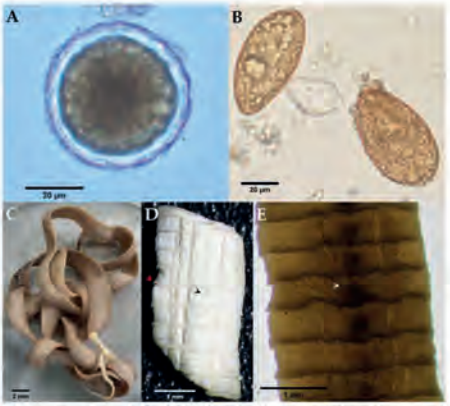

Figure 3. Morphological examination of parasite stages specimen found in free-ranging crab-eating fox (*Cerdocyon thous*) faeces. **A.** Non-embryonated *Lagochilascaris minor* egg (51.21 x 51.57 µm) with coarsely pitted surface containing multiple excavations and evident 5.79 µm thick shell. **B.** Yellowish-brown cone-shape operculated *Spirometra* eggs (61.67 x 34.97 µm). **C.** Adult *Spirometra mansoni* (Andean origin) specimen, notice spoon-shape scolex and characteristic pinkish colour due to the host vitamin B12 "plunder". **D.** Close-up photograph of the serrate gravid proglottids (red arrowhead), a genital pore is indicated with the black arrowhead. **E.** Whole mounted strobila segment where the centrally located spiralled uterus is notice (white arrowhead).

Molecular characterization of *Dipylidium caninum* specimens

Strobila segments isolated from two Andean bush dog faecal samples collected separately were molecularly characterised through sequencing of the complete cytochrome c oxidase subunit I (COI). Maximum likelihood phylogenetic analysis confirmed the species identification as *Dipylidium caninum*, placing both specimens at the base of a well-supported group composed exclusively of *Dipylidium caninum* representatives (Figure 4). The inner topology within the *Dipylidium caninum* group indicates the species consists of two genetically differentiated subgroups mutually separated by a relatively long and statistically well-supported internal node. These subgroups corresponded perfectly to the canine- and feline-specific genotypes of the parasite described previously (Labuschagne et al., 2018). The five canine-specific genotype representatives formed a non-monophyletic assemblage of specimens, although with low statistical support, relatively basal to the feline-specific genotype represented by two specimens (MG587892, OK523385). The Andean bush dog isolates grouped clearly with the canine-specific genotype (Figure 3).

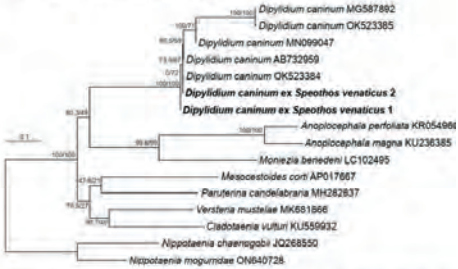

Figure 4. Phylogenetic position of *Dipylidium caninum* isolates obtained from two host specimens of Amazonian bush dog. Maximum likelihood tree from IQ-TREE based on nearly complete (1563 bp) COI gene sequences analysed as single partition using TIM+F+R5 model. Nodal values show SH-like approximated likelihood ratio test values (10000 replicates) and standard nonparametric bootstrap supports (1000 repetitions). Newly characterised specimens are shown in bold.

GenBank accessions are given after taxa names. The branch length scale bar indicates number of substitutions per site.

Discussion

In an increasingly globalizing world the recent decades anthropogenic factors have intensified the human-animal interface, thus increasing the disease spillover risk and food web collapse [25–27]. Amongst the world's carnivores, only 54.3% of species' distribution range comprised high-quality habitat, leading to landscape fragmentation and connectivity loss [28]. Wildlife infectious diseases surveillance is imperative to understand the population disease impact, eco-epidemiology and biodiversity conservation [29–31]. Synanthropic wild canids have also been reported as reservoirs of new helminth species [32–34].

On one hand bush dogs' parasitological surveys are limited due to the species elusive nature and crepuscular behaviour, thus remaining as one of the lesser-known wild canid species. Nonetheless, in 1972 the causative agent of chronic polycystic human echinococcosis (i. e., *Echinococcus vogeli*) was described for the first time in a wild bush dog captured in South America [35,36]. Furthermore, through copromicroscopical, coproantigen techniques, and post-mortem evaluation, zoonotic parasites such as *Toxocara canis*, *Lagochilascaris* and *Spirometra* have been reported in bush dogs es equal as the occurrence of *Spirocerca lupi*, *Ancylostoma caninum*, *Taenia* sp., and the apicomplexan *Cystoisospora caninum* [37,38]. On the other hand, domestic animal parasites such as *Dioctophyme renale*, *Dirofilaria immitis*, *Neospora caninum*, *Rangelia vitalii*, the cyst forming coccidia *Hammondia heydorni* as equal as the zoonotic parasites *Angiostrongylus cantonensis*, *Dipylidium caninum*, *Leishmania infantum* (syn. *L. chagasi*), *Toxoplasma gondii*, and important tick vectors have been reported for the crab-eating fox [39–47]. Thus, both wild canid species contribute to the environmental maintenance and transmission of human and domestic animal health concern parasitosis. Given the lack of information regarding the occurrence and distribution of public health concern helminthiases such as dipylidiosis, lagochilascariosis and sparganosis, the results presented here collectively provide new insight into the potential of neotropical wild canids in the emergence and transmission of zoonotic infectious diseases. Herein we successfully perform the identification of wild canid-deriver neglected zoonotic helminths harboured by the bush dog and the crab-eating fox.

The worldwide occurrence of *D. caninum* s.l., also known as the flea-, cucumber- and/or double-pored tapeworm, in domestic dogs and cats is well-documented [48–52]. Additionally, public health concern reports of the parasite on free-ranging canids and felids as a potential threat to human populations often occur [53,54]. Based on modern molecular techniques that allows to differentiate cryptic species and hidden genetic lineages, two genetically distinct variations so-called *D. caninum* canine and *D. caninum* feline genotypes have been proposed within the genus [55,56]. Non-human dipylidiosis rarely produces clinical manifestations, nonetheless animal hosts could transmit the parasite to humans, since they get infected after ingestion of metacestode (cysticercoid) containing arthropods (i. e., lice and flea) as intermediate hosts [57,58]. Wild carnivores such as dingoes (*Canis dingo*), golden jackal (*Canis aureus*), jaguar (*Panthera onca*), red fox (*Vulpes vulpes*), and spotted hyena (*Crocuta crocuta*) are also wild dipylidiosis reservoirs as definitive hosts in the parasite life cycle [57,59–

62]. The *D. caninum* infection in crab-eating fox populations has been documented as a possible consequence of anthropogenic expansion into wild hosts natural habitat [44]. A study in rural high-mountain Colombian region report the occurrence of *D. caninum* with an estimated prevalence of 20% (SD ± 8.7%) in free-roaming and peri-domestic dog populations [63]. In Colombia this parasite has only previously been reported in humans and domestic hosts mainly infected by soil contamination, thus *D. caninum* has not been detected contaminating food nor infecting intermediate hosts in the country [64]. Therefore, to the best of our knowledge results presented here expands the geographical distribution range of wildlife dipylidiosis to the Pan-Amazon and northern Andean regions, and thus constitute the first host record for bush dogs. Additionally, herein we establish that analysed cestode proglottids from bush dog correspond to the *D. caninum* canine genotype which occurred at a higher frequency in canids, have shorter prepatent period and longer lifespan than *D. caninum* feline genotype [55].

The nematode genus *Lagochilascaris* occurrence entails public health concern since human lagochilascariosis due to *L. minor* is still an extremely neglected zoonotic disease. Final hosts are carnivores (i.e., canids and felids) carrying intestinal adults which shed highly resistant ascarid-like eggs [65]. Humans acquire lagochilascariosis through the ingestion of infected intermediate hosts (i. e., agoutis, mice, rats and other rodents), however there is also evidence that humans might become infected after ingestion of embryonated eggs [66]. So far, more than 100 human lagochilascariosis cases have been recorded in the Americas [67]. Three human cases of lagochilascariosis have been documented in the Caribbean/Pacific, and Amazonian regions of Colombia [68,69]. Irrespectively, the present study constitutes the first non-human report of this parasite in Colombia. Since the amount of DNA obtained from isolated eggs was extremely low and showed partial degradation, subsequent phylogenetic analysis was non-viable. Notwithstanding, surveillance of human lagochilascariosis by local public health authorities should be recommended.

Globally distributed sparganosis is a neglected food- and water-borne zoonotic disease caused by the infection of cestodes in the genus *Spirometra* (Diphyllobothriidea) which is frequently reported in numerous wildlife species [14,18,24,70]. The sparganosis manifests as muscular and subcutaneous larvae (spargana) but brain invasion is also reported [71]. The heteroxenous parasite life cycle involves carnivores as definitive hosts where intestinal adults release eggs that subsequently pass into the environment throughout faeces during defecation. In aqueous environments eggs developed into coracidia and are ingested by copepods as the first intermediate host in which a procercoid larvae develops. These larvae are infective to second intermediate hosts (i. e., frogs, snakes, birds, and other tetrapods) were maturation into plerocercoid larvae take place [72]. Humans become infected by eating or using raw flesh of intermediate hosts in traditional poultices; or drinking water containing infected copepods [73]. In South America, a total of 16 human cases of sparganosis have been reported, only one of them located in Colombia [73,74]. Moreover, there is a presiding *Spirometra* report in Colombian wild felids as definite hosts (i. e., jaguars and ocelots) [75]. Recently, the molecular identification of here morphologically described cestode strobila retrieved from a crab-eating fox confirms the

occurrence of *Spirometra mansoni* in South America [24]. Thus, since sparganosis remains in one of the most obscure groups of cestodes, results presented here reinforce the distribution range of *S. mansoni* in the neotropics.

Therefore, here we evidence the occurrence of important zoonotic helminthiasis in two highly divergent free-ranging neotropical wild canid species, thus demonstrating the epidemiological relevance of the elusive semiaquatic bush dog and the synanthropic/peri-domestic crab-eating fox. Consequently, investigate on the potential pivotal role of wild canids into the human parasite transmission seems essential. Additionally, future ectoparasite research on different lice and flea taxa (e. g., *Felicola subrostratus*, *Trichodectes canis*, Archaeopsyllinae and Pulicinae subfamilies) infesting wild carnivore populations are urgently needed to identify the intermediate hosts which harbour extraintestinal *D. caninum* cysticercoids, and thus allows the successful lifecycle and actively favour the parasite zoonotic transmission. In conclusion, challenging parasitological approaches on elusive wildlife in remote areas, and the extremely rare opportunity to find adult cestode specimens in tropical conditions, wild canid parasitological findings must be maximized to ensure high-pitched research approaches and high-quality data. Parasitological surveys in wildlife are critical for establishing a proactive zoonotic spillover strategy to assess the threat mitigation risks in a pathogen surveillance network. As rule in all living communities' coinfections have consequences on infectious agent epidemiology and host fitness [76], thus better fundamental knowledge on wildlife associated parasitic diseases is needed to understand their role in the emergence of zoonoses such as dipylidiosis, lagochilascariosis, and sparganosis. Based on these results, we encourage further coproparasitological and ectoparasite studies both on bush dog and crab-eating fox populations, other wild carnivore species, domestic animals, and human communities to reveal the proto- and metazoan parasite infection and infestation as a public health issue to prevent zooanthroponotic parasite spillovers.

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Chapter 7 - A parasite annotated checklist for the imperilled West Indian manatee (*T. manatus*): New parasitological insights form the Neotropics.

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A parasite annotated checklist for the imperilled West Indian manatee (*T. manatus*): New parasitological insights form the Neotropics

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Abstract

The sirenians are endangered herbivorous aquatic mammals distributed along coastal areas, rivers, swamps, and wetlands in tropical and subtropical regions with a decreasing population trend. The comprehension on wildlife parasite biodiversity as reservoirs of non-zoonotic and zoonotic pathogens is of great importance in terms of conservation. Therefore, herein we conducted a parasitological survey on deceased manatees between 2002 and 2022 as part of the "Fundación Internacional para la Naturaleza y la Sustentabilidad" (FINS) manatee conservation initiative to estimate the occurrence, high-resolution morphology, and molecular identification of endoparasites in free-ranging Colombian manatees. Moreover, we present an annotated checklist on ubiquitous manatee-adapted parasite species as equal as incidental organisms reported on West Indian manatee (*T. manatus*). Thus, current study present new insights on the trematode species *Chiorchis fabaceus* for the Antillean manatee (*Trichechus manatus manatus*) subspecies as a probable concomitant cause of death. Due to the difficulty in recovering adult parasite stages from free-ranging hosts, very little morphological and molecular data are available on this trematode species. This is the first report on molecular characterization and phylogenetic position reconstruction of an adult *C. fabaceus* infecting Antillean manatees. Finally, we concisely discuss the world-wide overview reports on West Indian manatee parasites and epibionts highlighting their relevance in a one health context. Presented information contribute to the knowledge of the parasite diversity both in the West Indian manatee species and the Antillean manatee subspecies.

Keywords: Manatee, Parasites, Biodiversity, Host-parasite interaction, Sirenians, *Chiorchis fabaceus*, Marine mammals

1. Introduction

Also known as sea-cows, sirenians inhabits tropical and subtropical regions distributed in the Americas, Africa, Asia, and Oceania continents. A total of one dugong and three manatee genus comprises the extant living species in the order Sirenia (Table 1). All sirenians are listed as vulnerable to extinction by the International Union for Conservation of Nature (Hines et al., 2012; Marsh et al., 2011), are internationally protected by the Convention on International Trade in Endangered Species (CITES), and the Specially Protected Areas and Wildlife Protocol (Bertram and Bertram, 1973). These aquatic mammals naturally inhabiting rivers, estuaries, marshes, inlets, wetlands, and warm coastal waters. Also, occasionally can be found in the sea. On one hand the dugong (*Dugong dugon*) is the only extant species of the family Dugongidae and is distributed along coastal waters in limited areas of the Indian and western Pacific Oceans (Li et al., 2019). On the other hand, three species of manatee comprised the family Trichechidae: The African manatee (*Trichechus senegalensis*) is found along the western Africa coastal line, the Amazonian manatee (*T. inunguis*) which inhabits the Amazon River basin, and the most widely distributed West Indian manatee (*T. manatus*). Last species encompasses two recognised living subspecies: the Antillean manatee (*T. manatus manatus*) distributed along the Caribbean coastline and inland water bodies from northern South America, Mesoamerica and Mexico; and the Florida manatee (*T. manatus latirostris*) restricted to south-eastern Florida coast and the Mexican Gulf (Bertram and Bertram, 1973). The strong currents of the Florida's straits, deep water and the northern Gulf Coast cool winters create an effective gene flow barrier between Florida and Antillean manatee populations (Domning and

Hayek, 1986). Additionally, the Antillean manatees phenotypic plasticity demonstrate at least a coastal marine- and a riverine-ecotype within the subspecies (Castelblanco-Martínez et al., 2021).

Since the manatees are robust long-lived aquatic mammals, remarkably resilient to natural disease and anthropic maladies such as pollution, food chain collapse, and human-related trauma seems to be ideal ecosystem health sentinels under the single species approach concept (Bonde et al., 2004; Fricke et al., 2022; Wright et al., 2002). Different to other environmental and human health marine vertebrate sentinel species, non-ruminant manatees are obligate herbivores low-located on the food web, feeding both on a plethora of fresh- and salt-water plants, daily consuming up to 10% of their body weight (Bonde et al., 2004; Bossart, 2011). Thus, as equal as the case of semiaquatic endemic wildlife species in the Neotropics, manatees should be considered as potential reservoir hosts for water-, food- and gastropod-borne parasites (Uribe et al., 2021). Wildlife parasite biodiversity is of great importance in terms of conservation and to understand the wildlife reservoirs role for non-zoonotic and zoonotic parasite infection (Gómez and Nichols, 2013; Sparagano et al., 2021; Thompson et al., 2010). In a constantly changing world in which anthropic intervention undoubtedly enhance the changes' momentum, risks posed by parasites and other infectious disease are serious concerns (Shamsi, 2022). Therefore, here we describe new insights for the species *Chiorchis fabaseus* as a neglected gastropod-borne trematode and under-researched parasite disease in the Antillean manatee (*T. m. manatus*) through necropsy-based approaches, high resolution morphology and further molecular identification. The biological definition of "parasite" is a challenging notion and included all eukaryotes exclusive of fungi that derive resources and invade their hosts. Based on the historical medical concept, the term "parasite" refers to the protozoans, helminths, and arthropods with animal-like tropism (Mathison and Sapp, 2021). Additionally, current study aims to present and briefly discuss an annotated checklist for the incidental and ubiquitous manatee-adapted parasites (i. e., proto- and metazoans) and epibionts occurred in the West Indian manatee (*T. manatus*).

2. Materials and methods

2.1. *Studied area, sample collection and parasite examination*

The Antillean manatees analysed in the study were collected between 2002–2022. All animals were found dead floating in rivers, swamps, and wetlands in the departments of Antioquia and Santander, collected as part of a manatee preservation initiative, a conservation and monitoring program carried out by the "Fundación Internacional para la Naturaleza y la Sustentabilidad" (FINS) organization. During the study period, it was not always possible to analyse whole deceased animals and all their organs due to challenging tropical conditions. A total of 237 carefully gross necropsies was performed in manatees from three geographic locations in Colombia across the Andean region (Fig. 1A). Based on the Köppen-Geiger climate classification (Beck et al., 2018), manatee carcass were retrieved from sampling areas belonging to Tropical monsoon (Am), Tropical rainforest (Af), and Tropical wet and dry climate (Aw) as shown in Fig. 1B. Deceased manatees were morphologically identified and measured, thereafter assigned to 1 of 3 age classes based on body size (adults >275cm, juveniles between >175cm - <275cm. and calves <175cm)(O'Shea et al., 1985). Sex was determined, and decomposition carcasses stage classified in accordance with (Moore et al., 2020) as well as the assignment of the possible death cause was achieved by a member of the Sirenian Specialist Group for South America (IUCN-SSC) in: Cold stress, crushed/drowned, other human-related, other natural, perinatal, undetermined (other), undetermined (too decomposed), verified/not necropsied, and watercraft. A complete evaluation of the manatees' mantle was carried out searching potential ectoparasites and external symbionts. The *in situ* comprehensive parasitological (metazoan) examination of the respiratory system (i. e., Nasal cavity, trachea, bronchi, lungs, larynx), digestive tract (i. e., throat, oesophagus, stomach, small and

large intestine), and cavitary organs such as pancreas, liver and bile ducts, heart, pulmonary arteries, spleen, and kidneys took place at the environs where the manatees were found dead. The collected metazoan parasites were 70% EtOH and RNAlater™ (Invitrogen™) preserved, incubated at 4°C overnight, and stored at −20°C until further morphometrical and molecular assays were performed. Metazoans were examined using the BH-52™ light microscope equipped with a SC30™ digital camera (Olympus, Hamburg, Germany). For the morphometric analysis, the Olympus SZX7™ (Olympus Corporation, Tokyo, Japan) stereomicroscope system with Olympus DP27™ and SC30™ digital camera were used. For the photograph analysis the Olympus *CellSens*™ imaging software was used.

2.2. Ethic statemen

All animal procedures were approved by the Ethics Committee for Animal Experimentation of Universidad de Antioquia (AS No. 132) under collection permit No. 0524 of 2014 (IDB0321), performed in strict compliance with the EU Directive 2010/63/EU, in accordance to the Guidelines for the treatment of marine mammals in field research (Gales et al., 2009), and the Guidelines of the American Society of Mammologists for the use of wild mammals in research and education (Sikes, 2016). All animals included in this study were found dead, and their carcasses were collected along the main boat transits and/or by local communities.

2.3. High-resolution morphological scanning electron microscopy (SEM) assay

As previously has been successfully used in parasitological approaches on aquatic mammals such as the common bottlenose dolphins (*Tursiops truncatus*), sperm whales (*Physeter macrocephalus*) and manatees (Hermosilla et al., 2018; Vélez et al., 2019; Villagra-Blanco et al., 2017), herein we analyse the ultra-structure of trematode stages found during Antillean manatees parasitological examination. Briefly, the adult parasites were gently homogenized to lysate the body tissue and organs including the uterus and cirrus sac. Some droplets of the supernatant of the parasite macerate and whole specimens were carefully deposited on 10 mm diameter poly-L-lysine (Merck, Darmstadt, Germany) pre-coated glass coverslips (Nunc). Trematode stages were fixed in 2.5% glutaraldehyde (Merck) and thereafter post-fixed in 1% osmium tetroxide (Merck), washed in double-distilled water (ddH_2O), critical point dried by CO_2-treatment and subsequently gold particles-covered as described for manatee parasite probes (Vélez et al., 2019). Afterwards, specimens subjected to SEM analysis were examined using XL30® scanning electron microscope (Philips, Hillsboro, USA) allocated at the Institute of Anatomy and Cell Biology, Justus Liebig University Giessen, Germany.

2.4. Molecular phylogenetics

To characterize the metazoans collected during necropsy procedures, the specimens were rehydrated in descendant concentrations of EtOH, gently washed 5 times in 1X PBS solution, and thereafter lysate in ALT buffer with 20mg/ml proteinase K added incubated at 56°C. Total DNA was obtained following the manufacturer instructions for the DNeasy Blood & Tissue Kit® (Qiagen, Dusseldorf, Germany). Partial ribosomal regions of the small subunit (SSU), the large subunit (LSU) and 5.8S were amplified using the following specific primers: WormA, NF1, 18S, WormB, (for the SSU), ZX-1, NC2, Plagi 28S-r1, D3A, D3B (for the LSU) and NC1 (for the 5.8 S). A ~1600 bp partial ribosomal fragments of the small subunit (18S) region were PCR-amplified using the primers: for-5'-ggtggtgcatggccgttcttagtt-3', and rev-5'-ttagtttcttttcctccgct-3', following thermocycle profiles previously described (Vélez et al., 2018). The PCR amplicons were isolated from a preparative agarose gel using the HiYield Gel/PCR DNA Extraction Kit (Süd-Laborbedarf, Gauting, Germany). All PCR products were bi-directionally sequenced by LGC Biosearch Technologies (Berlin, Germany). Superfamily Paramphistomoidea representative 18S rDNA sequences of the recognized extant species were included to reveal the phylogenetic position of analysed specimen. SeqManPro 7.1.0 (DNASTAR

Inc., USA) was used to *in silico* edit, and finally assembled the sequence. The 18S rDNA alignment were conducted using the online version of MAFFTv. 7 (Katoh and Standley, 2013). Finally, a Neighbor-Joining algorithm analysis under 1000 bootstrap replicates was conducted in MEGAX software. Nucleotide sequence divergences were calculated using Kimura2-parameter (K2P) model for multiple substitution distance correction and were in the units of the number of base substitutions per site (Kumar et al., 2018). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of analysed helminth (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed.

2.5. The checklist structures

The current manuscript bulk consist of an annotated checklist of protozoan, helminthic, and arthropod parasite species reported in the West Indian manatee, based on extensive literature searches through November 2022. The checklist was drawn up based on publications from the period between 1838 and 2021. A systematic bibliographic search of the PubMed database, the Marine Mammals Research and Conservation Discussion (MARMAM), Scopus, ScienceDirect, Web of Science, and World Register of Marine Species; (WoRMS) was also performed using the keyword search phrases using various taxa in combination with manatee infection and case reports. Described parasite taxa have been arranged in systematic order. The list further includes information on the microhabitat, geographic distribution when it is available and organism annotations.

3. Results

3.1. Morphological, and molecular parasite identification of Antillean manatees

No ectoparasites such as cyamids nor external symbionts were evidenced in the studied manatees' mantle. During the evaluation of the manatee carcasses a detailed examination of the liver, kidneys, pancreas, and spleen was made macroscopically. A longitudinal cut along the respiratory system (i. e., nostrils, larynx, trachea, and bronchi) as equal as the heart fourth chambers and the main blood vessels do not evidence the presence of metazoan parasites. From above performed necropsies procedures the carefully gastrointestinal examination in one case draws our attention due to the abundant presence of non-segmented platyhelminths in the posterior portion of the stomach through the small intestine to the cecum and the first portion of the colon (i. e., ascendant colon). No macroscopic evidence of segmented helminths (cestodes), acanthocephalans, nor nematodes were observer. Thereafter, the metazoans examination shows two morphological types of digenean adult trematodes, one narrower and smaller than the other (Fig. 2A). Tegument folds and grooves were slightly visible around the mouth opening area in the dorsal surface view (Fig. 2B). Distinctly a circular-shape subterminal ventral acetabulum was noticed and the genital pore was located at an anterior-acetabular level (Fig. 2C). As other related amphistomes, commonly referred to as stomach- or rumen-flukes, analysed specimens was characterized by the absence of an oral sucker. The collected trematodes mean morphometric measurements were total body length of 5.64 mm, body breadth of 3.97 mm, and an acetabular opening of 1.14 µm x 1.17 µm. Based on the trematode intra-organic distribution within the host and parasite characteristic traits, the specimen described herein correspond well with the cladorchiid digenean species *Chiorchis fabaseus*.

Colombia. (**A**) Ventral view of whole specimen´s shows the two morphological body types in accordance with (Bando et al., 2014). Notice the small mouth opening (mo) and the large muscular caudal acetabular opening (ao) or ventral/caudal sucker. (**B**) Dorsal surface view. (**C**) Ventral view close-up photograph of the genital pore (gp) opening at the anterior one-third. Scale bars: (A) 2 mm, (B) 500µm, and (C) 1 mm.

The scanning electron microscopy (SEM) morphology analyses allows to evidence a bluntly rounded posterior end and slightly tapered anterior end without oral lobes trematodes (Fig. 3A). As equal as the related genus *Paraibatrema* sp., herein described specimens shows lack of genital sucker. An elongate middle to large robust body with maximum width at middle-body level and aspinose smooth tegument with folds and grooves was evidenced in the acetabular region (Fig. 3B). Adult parasite stages exhibit a double walled deeply seated acetabulum opening with post acetabular region cobble shaped but papillae absence. The depth of the acetabular sucker is about 1 mm. Additionally, to identify the digenean specimen found during manatee necropsy procedure position among the superfamily Paramphistomoidea a nearly complete 1662 bp-long fragment of 18S rRNA gene subjected to phylogenetic analysis shows that the analysed specimen clustered within the representative of *Chiorchis fabaseus* (Fig. 4).

3.2. An annotated checklist of West Indian manatee (Trichechus manatus) reported parasites

3.2.1. Metamonada

***Giardia* sp.**

Microhabitat: not mentioned (faecal examination)[1,2]

Locality: Brazil[1], Colombia[2]

References: (Borges et al., 2017a)[1], (Vélez et al., 2019)[2]

3.2.2. Apicomplexa

***Cryptosporidium* sp.**

Microhabitat: large intestinal content (Faeces)[1,2], floating faeces[3], necropsy[3]

Locality: Brazil[1,2,3]

References: (Borges et al., 2011)[1], (Borges et al., 2009)[2],(Borges et al., 2017a)[3]

Eimeria manatus (Upton, Odell, Bossart and Walsh, 1989)

Microhabitat: not mentioned (faecal examination)[1-3]

Locality: Florida[1,2], Colombia[3]

References: (Upton et al., 1989)[1], (Bando et al., 2014)[2], (Vélez et al., 2018)[3]

Eimeria nodulosa (Upton, Odell, Bossart and Walsh, 1989)

Microhabitat: not mentioned (faecal examination) [1-4]

Locality: Florida[1,2], Colombia[3,4]

References: (Upton et al., 1989)[1], (Bando et al., 2014)[2], (Vélez et al., 2018)[3] , (Vélez et al., 2019)[4]

Eimeria trichechi (Lainson, Naiff and Shaw 1983)

Microhabitat: not mentioned (faecal examination)[1]

Locality: Brazil[1]

References: (Lainson et al., 1983)[1]

***Eimeria* sp. A and *Eimeria* sp. B**

Microhabitat: not mentioned (faecal examination)[1]

Locality: Colombia[1]

References: (Vélez et al., 2019)[1]

***Eimeria* spp.**

Microhabitat: not mentioned (faecal examination)[1]

Locality: Florida[1], Puerto Rico[1]

References: (Wyrosdick et al., 2018)[1]

***Entamoeba* sp.**

Microhabitat: not mentioned (faecal examination)[1]

Locality: Colombia[1]

References: (Vélez et al., 2019)[1]

Toxoplasma gondii (Nicolle & Manceaux, 1908)

Microhabitat: not mentioned (serological assay)[1-3], Brain[4], Cerebellum[5]

Locality: Brazil [1,4], Belize[2], Puerto Rico[3], Florida[5]

References: (Attademo et al., 2016)[1], (Sulzner et al., 2012)[2], (Bossart et al., 2012)[3], (Buergelt and Bonde, 1983)[4], (Smith et al., 2016)[5]

3.2.3. Digenea

Chiorchis fabaceus (Diesing, 1838)

Microhabitat: not mentioned (faecal examination)[1,3,6,7], duodenum[3], ilium[3], colon[2,4,5], caecum[2,5], small intestine[2], stomach[2]

Locality: Florida[1,2], Mexico[3], Dominican Republic[4], Puerto Rico[5], Colombia[6,7]

References: (Bando et al., 2014)[1], (Beck and Forrester, 1988)[2], (Olivera Gómez, 2017)[3], (Mignucci-Giannoni et al., 1999b)[4], (Mignucci-Giannoni et al., 1999a)[5], (Vélez et al., 2018)[6], (Vélez et al., 2019)[7],

Chiorchis groschafti (Coy Otero, 1989)

Microhabitat: large intestine[1], not mentioned (faecal examination)[2,4], intestine[3], gastrointestinal tract[5]

Locality: Puerto Rico[1,3], Florida[2], Mexico[4], Belize[5]

References: (Bossart et al., 2012)[1], (Bando et al., 2014)[2], (Colón-Llavina et al., 2009)[3], (Olivera Gómez, 2017)[4], (Lucot et al., 2020)[5]

Moniligerum blairi (Dailey, Vogelbein & Forrester, 1988)

Microhabitat: not mentioned (faecal examination)[1,4], mucosa and submucosa of the small intestine[2,3]

Locality: Florida[1,2,3,4], Puerto Rico[4]

References: (Bando et al., 2014)[1], (Beck and Forrester, 1988)[2], (Dailey et al., 1988)[3] , (Wyrosdick et al., 2018)[4]

Nudacotyle undicola (Dailey, Vogelbein & Forrester, 1988)

Microhabitat: not mentioned (faecal examination)[1,4,5], small intestine[2,3], duodenum[2], caecum[2], colon[2]

Locality: Florida[1,2,3,5], Colombia[4], Puerto Rico[5]

References: (Bando et al., 2014)[1], (Beck and Forrester, 1988)[2], (Dailey et al., 1988)[3], (Vélez et al., 2018)[4] , (Wyrosdick et al., 2018)[5]

Pulmonicola cochleotrema (Travassos & Vogelsang, 1931)

Microhabitat: not mentioned (faecal examination)[2,8,12], nares/nostrils[1,3-7, 9-11], lungs[3,5], stomach[3], trachea[5,10, 11], bronchi[5], laryinx[11]

Locality: Puerto Rico[1, 6,11,12], Florida[2,3,12], Brazil[4,5,7], Mexico[8], Belize[9], Dominican Republic[10]

References: (Bossart et al., 2012)[1], (Bando et al., 2014)[2], (Beck and Forrester, 1988)[3], (Borges et al., 2017c)[4], (Carvalho et al., 2009)[5], (Colón-Llavina et al., 2009)[6], (Borges et al., 2017b)[7] (Olivera Gómez, 2017)[8], (Lucot et al., 2020)[9], (Mignucci-Giannoni et al., 1999b)[10], (Mignucci-Giannoni et al., 1999a)[11] , (Wyrosdick et al., 2018)[12]

Remark: Reported as current unaccepted synonymic *Cochleotrema cochleotrem*[3,10]

3.2.4. Cestoda

***Anoplocephala* sp.**

Microhabitat: small intestine[1]

Locality: Florida[1]

References: (Beck and Forrester, 1988)[1]

Remark: Infection of 1 specimen of *Anoplocephala* sp. in a single manatee likely was accidental and of equine origin[1]

3.2.5. Nematoda

***Ascarididae* gen. sp.**

Microhabitat: not mentioned (faecal examination)[1]

Locality: Mexico[1]

References: (Olivera Gómez, 2017)[1]

Cutidiplogaster manati (Fürst von Lieven, Uni, Ueda, Barbuto & Bain, 2011)

Microhabitat: Skin[1,2]

Locality: Florida[1], Japan[2]

References: (Gonzalez et al., 2021)[1], (von Lieven et al., 2011)[2]

Remark: Highly specialized free-living epibionts of the skin[1], report in captive animal[2].

Heterocheilus tunicatus (Diesing, 1839)

Microhabitat: not mentioned (faecal examination)[1,8], stomach[2,3,6,7], duodenum[2], small intestine[2], colon[2], caecum[2], gastrointestinal tract[5]

Locality: Florida[1,2,8], Puerto Rico[3,7,8], Mexico[4], Belize[5], Dominican Republic[6]

References: (Bando et al., 2014)[1], (Beck and Forrester, 1988)[2], (Colón-Llavina et al., 2009)[3], (Olivera Gómez, 2017)[4], (Lucot et al., 2020)[5], (Mignucci-Giannoni et al., 1999b)[6], (Mignucci-Giannoni et al., 1999a)[7], (Wyrosdick et al., 2018)[8]

3.2.6. Arthropoda

Aedes (Ochlerotatus) taeniorhynchus (Wiedemann, 1821)

Microhabitat: skin

Locality: Florida

References: (Reeves and Gillett-Kaufman, 2020)

Remark: Parasite capable of locating, landing on, and bite West Indian manatees.

Anopheles atropos (Dyar & Knab, 1906)

Microhabitat: skin

Locality: Florida

References: (Reeves and Gillett-Kaufman, 2020)

Remark: Parasite capable of locating, landing on, and bite West Indian manatees.

Balaenophilus manatorum (Ortiz, Lalana & Torres-Fundora, 1992)

Microhabitat: skin[1,2]

Locality: Cuba[1], Mexico[2]

References: (Ortiz et al., 1992)[1], (Suárez-Morales et al., 2010)[2]

Remark: Arthropod reported as epibiont[2].

Chelonibia manati (Gruvel, 1903)

Microhabitat: skin[1]

Locality: Puerto Rico[1], Cuba[2]

References: (Mignucci-Giannoni et al., 1999a)[1], (Ortiz et al., 2010)[2]

Remark: Reported as a commensal species[1,2]

Culex (Melanoconion) iolambdis (Dyar 1918)

Microhabitat: skin

Locality: Florida

References: (Reeves and Gillett-Kaufman, 2020)

Remark: Parasite capable of locating, landing on, and bite West Indian manatees.

***Sinelobus* sp.** (Sieg, 1980)

Microhabitat: skin

Locality: Cuba

References: (Ortiz et al., 2010)

Remark: Reported as a commensal species

4. Discussion

Parasitic trematodes have a worldwide distribution particularly affecting tropical and sub-tropical regions (Fürst et al., 2012; Robinson and Sotillo, 2022). The digenetic trematodes are a species-rich group of parasites with more than 25000 species having a considerable impact on the economy of communities as a major cause of morbidity and mortality in human and animal health (Esch et al., 2002; Keiser and Utzinger, 2009; Toledo and Fried, 2019). With a global incidence difficult to calculate, trematode global burden seems to be considerable underestimate and thus trematodiasis have been neglected for years (Chai and Jung, 2022). At least one digenetic trematode has been reported from most groups of wildlife, however the complex life

cycles which commonly involve 2 to 3 or even 4 hosts remain not fully described, and thus the majority of trematodes infecting wildlife species are neglected or still unknown (Bolek et al., 2019; Esch et al., 2002). As an example of above mentioned, the sirenian's digenean trematode life cycle of the species *Chiorchis fabaceus* and *C. groschafti* (Cladorchiidae), *Indosolenorchis hirudinaceus* (Paramphistomidae), *Moniligerum blairi* (Opisthotrematidae), *Nudacotyle undicola* (Nudacotylidae), *Pulmonicola cochleotrema* (Opisthotrematidae), *Solenorchis travassosi* (Cladorchiidae), and *Zygocotyle lunata* (Paramphistomatidae) are partially unsolved since larval stages and gastropod intermediate hosts remain undescribed (Bando et al., 2014; Blair, 1980; Vélez et al., 2019).

In current study, an Antillean manatee were identified to be extensively infected with the amphistome trematode *C. fabaceus* within the superfamily Paramphistomoidea (Cladorchiidae family). This platyhelminth was the first described parasite on sirenians (Diesing, 1838). During the past century the parasite has been sporadically identified in Belize, Colombia, Dominican Republic, Florida, Mexico, and Puerto Rico based on eggs identification, but scarce adult specimens stage descriptions have been made (Bando et al., 2014; Beck and Forrester, 1988; Bossart et al., 2012; Colón-Llavina et al., 2009; Lucot et al., 2020; Mignucci-Giannoni et al., 1999b, 1999a; Olivera Gómez, 2017; Vélez et al., 2019, 2018). Herein we describe the high-resolution morphometric morphology, and molecular identification of *C. fabaceus* adult stages parasitizing free-ranging Colombian manatees. Since the outdated *Chiorchis hawkesii* thereafter classified as *Pseudodiscus* (*Hawkesius*) *hawkesii* (Firdausy et al., 2019), to date the genus *Chiorchis* sp. is composed by two manatee species (i.e., *C. fabaceus* and *C. groschafti*) and *C. purvisi* reported in turtles (Southwell and Kirshner, 1937). As equal as other amphistomes located in the digestive tracts, liver and bile ducts of many vertebrates generating important productivity and economic losses among domestic animals (Anuracpreeda et al., 2015), the adult stages of *C. fabaceus* here found mainly located in the manatee stomach but extend along intestine to the ascendant colon. Thus, chiorchiosis may lead to extensive intestine ulcerative areas and multifocal necrotizing colitis in manatees as described in monogastric semiaquatic mammals infected with parasites of the Cladorchiidae family (Uribe et al., 2021). Likewise related amphistomes characterized for generate mucosal lesions in the stomach and intestine, chiorchiosis in manatees could manifest with loss of appetite and consequent low body condition, foetid diarrhoea, dehydration, emaciation, extreme weakness with consequent exhaustion, oedema and subnormal temperature (Conga et al., 2022; Sreedevi et al., 2017).

Furthermore, ostensibly smaller intestinal trematode species such as *M. blairi* and *N. undicola* have been associated with histological lymphoplasmacytic ulcerative enteritis with submucosal oedema and crypt atrophy, grossly visible and variably haemorrhagic, nodular, necrotic, and rugose intestinal mucosa in manatees (Arnett-Chinn et al., 2013; Dailey et al., 1988; Panike et al., 2017; Weisbrod et al., 2021). Inasmuch as the alethiological agent of chiorchiosis is a bigger parasite, a major capability of causing severe gastrointestinal associated lesions in infected host is inferred. Thus, this neglected, often undiagnosed and highly underestimated trematode infection should be considered as a concomitant cause of death in manatees. In brief, since all extant trematode parasites require either terrestrial/amphibious or aquatic obligate gastropod intermediate hosts, it would be appropriate to analyse in depth the gastropod fauna (e. g., snails, slugs, and semi-slugs) inhabiting aquatic biomes (i.e., flooded areas, rivers, natural lakes, and coastal line) shared by manatee populations, domestic animals, and humans to better understand the unknown life cycle of sirenian trematode diseases such as chiorchiosis. Moreover, trematode larval stages should be investigated in grass and seagrasses, mangrove, algae and invertebrates as main sirenian dietary sources (Allen et al., 2018; Takoukam Kamla et al., 2021). Further research is necessary to determine the trematode-gastropod associations, develop effective diagnostic tests, and

determine the parasite burden as recommended for other wildlife species (Pfukenyi and Mukaratirwa, 2018).

Regarding, the annotated check list herein we precent a total of 24 taxa records of protozoans, helminths, and arthropods reported in the West Indian manatee. Some of those reports constitute ubiquitous manatee-adapted species as well as incidental parasites, epibionts or commensal species that may disturb the manatee health condition. Highly species specific apicomplexan parasites such as *Eimeria manatus*, *E. nodulosa*, and *E. trichechi* has been reported in Brazil, Colombia, and Florida (Bando et al.. 2014: Upton et al.. 1989: Vélez et al.. 2018). Additionally, smaller oocyst sizes have been described in Colombian manatees as *E. manatus*-like type A and B (Vélez et al., 2019). Moreover, important cosmopolitan zoonotic parasites such as *Cryptosporidium*, *Giardia*, *Toxoplasma gondii* and *Entamoeba* were identified infecting manatee populations and individuals along the Neotropics. As ubiquitous protozoan parasites reported to infect a wide range of domestic and wild vertebrate hosts as well as humans, *Cryptosporidium* and *Giardia* are significant causes of diarrhoea worldwide and responsible for numerous water-borne and food-borne diseases outbreaks (Jones and Tardieu, 2021; Ryan et al., 2021). Both parasites have been identified as emerging diseases in developed and developing countries where the major wildlife role in the disease transmission to human populations is highlighted (Santin, 2020; Zhang et al., 2021). A potential pathogen pathway for wildlife infections with anthropozoonotic protozoan parasites such as *Cryptosporidium* and *Giardia* is the environmental pollution with human and domestic-animal faeces (Appelbee et al., 2005). Those parasites were the most frequently found protozoan in Latin American countries where from 1979 to 2015 a total of 16 outbreaks of waterborne-protozoa were reported (Rosado-García et al., 2017). Both *Cryptosporidium* and *Giardia* infect various species of aquatic mammals including the Antillean and Amazonian manatees in Brazil (Borges et al., 2017a). Even a zoonotic *Cryptosporidium parvum* genotype which infects humans was identified in sirenians (Morgan et al., 2000). In the case of *Giardia* there is only another report in Colombian antigen-positive Antillean manatee (Vélez et al., 2019). Public health threatening zoonotic protozoans are often recorded on aquatic-, semiaquatic mammals and marine environments (Olson et al., 2004). Despite not been fully elucidated, several *Toxoplasma gondii* transmission routes have been identified in aquatic ecosystems and numerous species of wild marine mammals around the world (Li et al., 2022). Manatees are not the exception and this obligate intracellular parasite occurred both in free-ranging Amazonian and Florida manatee populations (Mathews et al., 2012; Smith et al., 2016). Additionally, *T. gondii* antibodies have been detected in captive Antillean manatees (Attademo et al., 2016). To the best of our knowledge, potentially zoonotic *Entamoeba* / *Entamoeba*-like in manatees have only been identified in two reports from Colombia and Florida (Vélez et al., 2019; Wyrosdick et al., 2018). Since sirenian are key ecological pieces in fresh, brackish, and marine biomes, further research is needed to revel the clinical effects on manatee populations and terrestrial to aquatic dissemination way of above-mentioned water-borne zoonotic parasites as a public health issue.

The genus *Anoplocephala* is the only manatee cestode reported in literature likely as an accidental finding of equine origin and as equal as the record of Ascarididae gen. sp. could be explained because manatees share habitat with domestic animal and human populations with consequent sewage pollution and potential parasite transmission (Beck and Forrester, 1988; Olivera Gómez, 2017; Titcomb et al., 2021). To the best of our knowledge there are only two species of West Indian manatee-adapted nematodes reported. Respectively the gastrointestinal *Heterocheilus tunicatus* (Heterocheilidae) has been widely reported in Florida, Puerto Rico, Mexico, Belize, and Dominican Republic, while the skin-associated *Cutidiplogaster manati* (Diplogastridae) was first reported on a captive manatee in Japan and the body surface of free-roaming manatees in Florida (Gonzalez et al., 2021; von Lieven et al., 2011). Since the nematodes life cycle are completely unknown further research

would aid in understand their distribution, clinical manifestation, and health impact among sirenian populations.

The mosquitoes species *Aedes* (*Ochlerotatus*) *taeniorhynchus*, *Anopheles atropos*, and *Culex* (*Melanoconion*) *iolambdis* have very wide host breadth and are capable of locating, landing on, and bite the West Indian manatees (Blosser et al., 2016; Reeves and Gillett-Kaufman, 2020). These ectoparasite genus are recognized vectors of mosquito-borne pathogens such as *Dirofilaria immitis*, *Plasmodium* spp., the Group C orthobunyaviruses (GRCVs), Venezuelan equine encephalitis group viruses, eastern equine encephalitis virus, and Madariaga virus in the Americas (Blosser and Burkett-Cadena, 2017; Escobar et al., 2020; Manrique-Saide et al., 2010). Therefore, expanding the parasites spectrum that potentially can affect this imperilled aquatic mammal species. Finally, some arthropods such as *Balaenophilus manatorum*, *Chelonibia manati*, *Sinelobus* sp. located on the manatee's mantle have been recorded as commensal epibionts.

In conclusion the present study adds new morphological and molecular insights on the highly neglected *C. fabaceus* trematode species in manatees as a baseline for further parasitological research on this imperilled aquatic mammal metazoan parasite. The West Indian manatees have a pivotal role as an ideal ecosystem health sentinels (Bonde et al., 2004; Fricke et al., 2022; Wright et al., 2002), shows the potential of harbour a plethora of non-zoonotic and zoonotic parasitosis, and thus evidence their relevance to understand the pathogens interchange in the human-domestic animal-wildlife interphase as a one health concern issue. Nonetheless, the lack of knowledge on the life cycle of most manatee-adapted parasites such as trematodes and nematodes require future interdisciplinary efforts to comprehend and describe the involved hosts, transmission routes and possible pathogenic implications on the health status of manatee populations.

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Figures

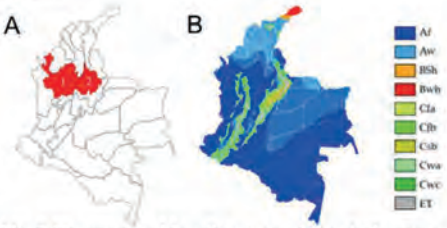


Fig. 1 Geographical location map of the study areas within Colombia. (**A**) Departments of Antioquia (1) and Santander (2). (**B**) Köppen–Geiger climate classification; Af: Tropical rainforest, Aw: Savanna, Bsh: Hot semi-arid, Bwh: Hot desert climate, Cfa: Humid subtropical, Cfb: Oceanic, Csb: Warm-summer Mediterranean, Cwc: Monsoon-influenced subpolar oceanic, Cwa: Mon-soon-influenced humid subtropical. ET: Tundra. The manatee carcasses were collected by conservation programs operated by the local governments. Detailed information on the sampling localities is refrained due to the species conservation status.

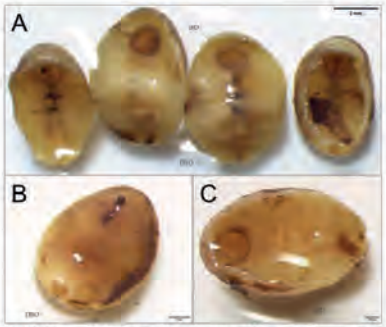


Fig. 2 Microphotograph images illustration of adult specimen of *Chiorchis fabaseus* recovery from Antillean manatee (*Trichechus manatus manatus*) gastrointestinal tract in Antioquia and Santander,

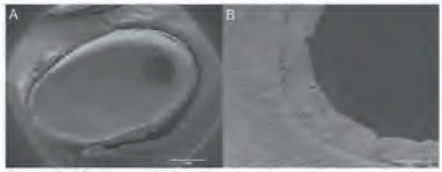


Fig. 3 Lower magnification scanning electron microscopy (SEM) micrograph of whole adult trematode specimen. Scale bar: (A) 1mm, and (B) 100µm.

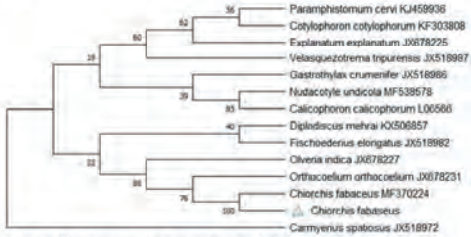


Fig. 4 Phylogenetic position of digenean trematode parasite isolate obtained during manatee necropsy is indicated with green triangle.

Table

Table 1: Extant living and extinct species in the order Sirenia.

Order	Family	Species	Subspecies	Common name	Classification §
Sirenia	Dugongidae	*Dugong dugon*		Dugong	VU
		Hydrodamalis gigas†		Steller's seacow	EX
	Trichechidae	*Trichechus senegalensis*		African manatee	VU
		Trichechus inunguis		Amazonian manatee	VU
		Trichechus manatus‡	*T. manatus manatus*	Antillean manatee	VU
			T. manatus latirostris	Florida manatee	VU
	Prorastomidae†				EX
	Protosirenidae†				EX

†Extinct taxa. ‡ Manatee species included in present study. § Based on the International Union for Conservation of Nature (IUCN) threat levels of classifications for endangered species.

General overview and Discussion - Regarding pleasant and unexpected surprises

In an increasingly globalized world, anthropogenic factors such as intensified farming with consequent agro-industrial monocultures (Barrera-Ramírez *et al*., 2019), unsustainable natural resources exploitation such as illegal mining, indiscriminate logging (Unda and Etter, 2019), wildlife hunting/trafficking (Nogueira-Filho and da Cunha Nogueira, 2018), and wildlife-meat consumption, have strengthened the human-animal interface, thus increasing the risk of bidirectional disease spillovers and food web collapses (El Bizri *et al*., 2020; Fricke *et al*., 2022; Magouras *et al*., 2020). Nowadays constant surveillance of wildlife- and domestic animal-related diseases is imperative not only for better understanding of their adverse impact on environment, human- and animal populations but also on biodiversity conservation (Day, 2016; Grogan *et al*., 2014; Karesh *et al*., 2012; Nicholson *et al*., 2020; Uribe *et al*., 2021c; Zambrano *et al*., 2014).

Notwithstanding, evidence of host-parasite co-evolution process has been demonstrated millions of years ago (Mya) in different protozoan/metazoan parasite taxa and diverse host species (de Castro Costa *et al*., 2019; Le Bailly and Araújo, 2016; Schall, 1990; Tinsley and Tinsley, 2016). As an example, the metabarcoding and high-throughput sequencing studies of ancient parasites from coprolites, cesspit sediment, mummified tissues, burial sediments and permafrost soils, provided unique scientific insights about the prehistoric human populations health and parasite communities, the ability to reconstruct rare or extinct host natural parasite fauna, and understanding on how parasites co-evolved alongside with their host populations (Wood, 2018). Although it is well-known that evolution is fundamental to all biological fields, host-parasite co-evolution is the reciprocal evolution of interacting species and over time has been a pervasive and quite diverse process. Thus, parasites exert frequency-dependent negative selection on their hosts, with the most common host genotypes having low fitness and decreasing in frequency as parasites infect them (Gibson *et al*., 2015). Therefore, co-evolution is proposed to maintain genetic diversity in host- and parasite populations (Hamilton *et al*., 1990). Based on above mentioned idea, the co-evolution process has been going for millions of years favouring the refinement of the

parasite transmission mechanisms and maintenance in diverse host populations. Therefore, the host-parasite interaction, often triggering an evolutionary adaptation in which the parasite replicates and spreads without generating a marked damage or mayor organic alteration in the affected host in a hyper-connected world (White *et al*., 2021; Wild *et al*., 2009).

There are many examples of the above-mentioned issue, since through the paleoparasitology and archaeoparasitology the occurrence of parasitic diseases both in human and animal populations in the past, have been elucidated (Sazmand, 2021). Reports on parasites coexisting within human and animal populations have been documented since ancient times both in the Old and New World. Even since the lower Cretaceous, metazoan parasite species (e. g. chewing lice) have been identified in fossils (Rasnitsyn and Zherikhin, 1999). Another example is the fossil-dated molecular phylogeny that places ancestral Cimicidae to 115 Mya as hematophagous specialists which affected bats and birds, and nowadays two major current urban pests of bedbugs, *Cimex lectularius* and *Cimex hemipterus* separated 47 Mya, parasitize humans and along the co-evolution process changed to host generalist parasites (Roth *et al*., 2019). Despite their large multi-seat public latrines with washing facilities, sewer systems, sanitation legislation, fountains and ancient Roman piped drinking water from aqueducts, parasites such as whipworm (*Trichuris trichiura*), roundworm (*Ascaris lumbricoides*), fish tapeworm (*Diphyllobothrium* sp.) and *Entamoeba histolytica* have been reported. All these parasitoses were widespread distributed among the Roman population (Johnson *et al*., 2021). Some ectoparasites such as fleas (*Pulex irritans*), head lice (*Pediculus humanus capitis*), body lice (*Pediculus humanus corporis*), pubic lice (*Phthirus pubis*) and bed bugs (*C. lectularius*) were also frequently reported to occur along the vast Roman imperium (Johnson *et al*., 2021; Mitchell, 2017; Mumcuoglu, 2008).

Moreover, some examples of ancient parasites coexisting millions of years ago with humans and animals in the New World (i. e., Neotropical region) has been widely reported along the South American subcontinent. Parasitological analysis of coprolites has allowed exploring ecological relationships in ancient times (Petrigh *et al*., 2019). Major parasitological

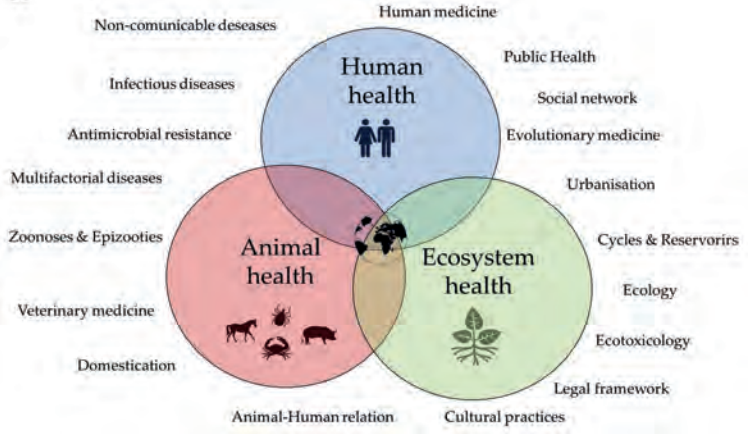
discoveries have occurred on human remains along South America archaeological sites (i. e., Brazil, Argentina, Chile, and Peru); some of those parasite diseases went out of Africa due to European and Asian slave traffics and dispersed around the world. Furthermore, in the process of animal domestication, humans also acquired pathogens from domesticated animals (e. g. cattle, sheep, goats, swine, birds, reindeers, yaks, camels, New World camelids, rodents, rabbits, fishes, and bees), since humans constantly altered their consumption habits and changed dramatically natural environments for livestock activities (Novo and Ferreira, 2016). Thus, animal coprolite parasitological evaluation is an important tool to promote knowledge about different zoological groups of parasites infecting ancient populations and indirectly helping to understand persistence of certain zoonotic-relevant parasites of prehistoric animals and humans (Sianto *et al*., 2014). The oldest molecular parasite record was documented in the Argentinean southern Puna, where some Pleistocene large mammalian carnivore coprolites, i. e. pumas (*Puma concolor*) were found. Recovered puma coprolites from the paleontological and archaeological site Peñas de las Trampas 1.1 dated to 16573–17002 calBP (calibrated years before the present), reported the occurrence of *Toxascaris* sp. morphologically compatible eggs, thereafter, confirmed by ancient mitochondrial DNA (mDNA) analysis as *Toxascaris leonina* (Petrigh *et al*., 2019). Also, other study analysed 30 feline coprolites from a south-eastern Piauí archaeological sites used by human groups in the past where eggs of *Spirometra* sp., *Toxocara cati*, Spirurida, Oxyuroidea, *Calodium cf. hepaticum*, *Trichuris cf. muris*, *Trichuris* sp., and other Trichuridae, *Oncicola* sp., and nematode larvae were found (Sianto *et al*., 2014). Some of these parasitological findings are reported nowadays in different species of South American wild felids (Uribe *et al*., 2021c). Thus, the role of animal populations in the transmission of zoonotic parasites in the region should be here discussed. Parasitic agents had a greater proliferation since the end of the Pleistocene and the beginning of the Holocene, throughout the so-called Neolithic revolution, in the fertile crescent area of Mesopotamia where ideal sedentary conditions were given with the consequent increase in human population density and animal domestication, leading to tight contact between animals and humans. Finally, we had the beginning of the "technification" of agriculture, favouring the settlement of nomadic

populations which also generated ideal conditions for parasitic proliferation and transmission by conglomerating around a plantation or post-harvest product multiplicity of vertebrate and invertebrate species. Despite this boom given throughout the Neolithic revolution, parasites have been reported in various coprolites throughout history with further development of techniques for their identification between 1955 and 1969 (Camacho *et al*., 2018).

Thus, One Health (or Global Health) is not a new concept and thus recognizes the constantly evolving relationship between animals, humans and plants within the planet they share and this interaction has shaped, and continues to shape the course of human events and history (Evans and Leighton, 2014). The series of strategic objectives known as the "Manhattan Principles" derived from a meeting of the Society for the Conservation of Wildlife in 2004, in which the existing interdependency between human and animal health was recognized as pivotal influencing factor on global-public health, -food supply and -economy. The novel incorporation of ecosystem health, including wildlife in the "One Health" initiative constitutes a global strategy that highlights the need for a holistic and transdisciplinary approach that incorporates multi-sectoral expertise in investigating wildlife-, human-, and animal-health (please see Figure 3) as equal ecosystems and considering the different relationships between them (Destoumieux-Garzón *et al*., 2018). During the last decade a significant increase of infectious agents affecting both humans and domestic animals, has been observed, with consequent spread and appearance of multiple epizootics in animal populations, epidemics in humans with effective zoonotic transmissibility between both populations, making the risks of pandemics increasingly critical and more frequent as in past centuries (Han *et al*., 2016; Ihekweazu *et al*., 2021; Pfeffer and Dobler, 2010; Rahman *et al*., 2020). Human- and animal-health have also been threatened by anti-microbial resistance (Iwu *et al*., 2020). These principles were a vital step towards the recognition of the critical importance of collaborative and interdisciplinary approaches to adequately and comprehensively respond to emerging and re-emerging infectious diseases, but particularly for the inclusion of wildlife health as an essential component of global infectious disease prevention, surveillance, control, and mitigation (Mackenzie and Jeggo, 2019).

Wildlife fauna is normally defined as animals that roam freely, whether they are mammals, birds, fish, reptiles, amphibians, or invertebrates without any type of direct human intervention in their domestication and/or submission to zootechnical practices of any kind. Wildlife individuals carrying infective agents will always pose certain degree of risk to human and domestic animal health, so the human-animal-ecosystem interface will be of great importance in the evolution and appearance of pathogens, and therefore demanding constant monitoring or surveillance. Thus, it is essential to know the causes and consequences of human activities for a rigorous interpretation of disease dynamics and to promote effective public health policies. As a valuable asset at a worldwide level, health security must always be understood on a global scale and from a transversal perspective integrating human-, animal-, plant-, ecosystem-health and biodiversity (Destoumieux-Garzón *et al*., 2018).

Figure 3: The One Health concept as a holistic, transdisciplinary, and multi-sectoral approach



Adapted from: (Destoumieux-Garzón *et al*., 2018).

In a rapidly changing world with growing concern on biodiversity loss and an increasing number of animal and human diseases arising from wildlife, the need for effective wildlife health research is now widely recognized. However, the applicable procedures and insights gained from studies related to human and domestic animal health can be partially

extrapolated to wildlife. Therefore, difficulties in wildlife health research are largely related to the zoological, behavioural, and ecological intrinsic characteristics of elusive free-living populations and "hard-to-reach" study areas (Ebmer *et al*., 2020; Hermosilla *et al*., 2015; Ryser-Degiorgis, 2013; Uribe *et al*., 2022a). Although clinical evaluation in domestic animals can be challenging, the situation becomes even more complex when we refer to wildlife (Kull, 2014; Myers, 2006). Because it is common that among WA manifestation of clinical signs are the result of co-infections and considering that many parasitoses might rather result in sub-clinical manifestations, parasite-induced disorders will most likely occur in weak, new-borns and elderly individuals dying in the field or becoming prey of apex predators.

In line, emerging infectious diseases of zoonotic potential, including bacteria, virus, fungi, and parasites, will frequently have WA as natural reservoir hosts representing an important risk factor for public health, and being reported from all continents. Various pathogens and various routes of transmission are here being involved, just as multiple epidemiological and environmental risk factors, increasing exchange of parasitic zoonoses between populations of wildlife, humans, and domestic animals. The total number of infectious diseases transmissible between animals and humans is still unknown, but there are currently records of approximately 1415 zoonotic pathogens in human populations (Taylor *et al*., 2001), of which 62% originate from wildlife (Childs *et al*., 2007; Kruse *et al*., 2004; Taylor *et al*., 2001). The close contact that currently exists between populations of wild animals and human populations is becoming more frequent due to dramatic anthropogenic changes in ecosystems that have been taking place for decades around the globe (Ihekweazu *et al*., 2021; Petrigh *et al*., 2019; Rahman *et al*., 2020). Some of these changes are directly mediated by the development of agro-industrial monocultures (Barrera-Ramírez *et al*., 2019) (e. g. pigs, chickens, ducks, turkeys, fish, gastropods, crustaceans, amphibians, and reptiles), the expansion of agricultural frontier due to extensive commercial cattle ranching, illegal mining, indiscriminate logging (Unda and Etter, 2019), hunting (Nogueira-Filho and da Cunha Nogueira, 2018) and illegal trafficking of wildlife, worldwide. Finally, all above-mentioned factors will cause the food web chain collapse as reported elsewhere (Fricke *et*

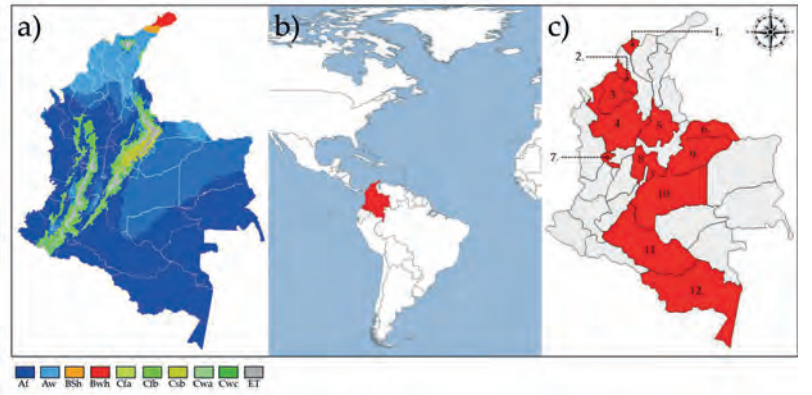
al., 2022). Given that many of these WA share their biomes with rural or suburban human communities, they are considered ideal sentinels of public health and it is therefore why the evaluation of their health homeostasis and disease aetiology can be used indirectly to determine not only ecosystem health, but also the health status of other individuals with whom they share ecological niches. Particularly rare and neglected protozoan and metazoan zoonotic diseases have been documented to occur in free-ranging WA throughout history with multiple examples in various continents.

Additionally, WA-associated infectious diseases emergence or re-emergence highlights the need for better understanding on the mechanisms and factors involved in eco-epidemiology of parasitoses (Martinez, 2018; Uribe *et al*., 2022a). Accordingly, One Health concept will be expanded, further developed, to subsequently give a breakdown of parasitological findings obtained in current doctoral thesis. Consequently, sampling efforts of present doctoral research proposal covered a wide range of biological regions from the Andean to the Amazon, the Caribbean and included the vast Orinoco basin. Based on the Köppen-Geiger climate classification (Beck *et al*., 2018) diverse weather patterns such as humid subtropical (Cfa), oceanic (Cfb), semiarid/hot semi-arid (BSh), tropical monsoon (Am), tropical rainforest (Af), tropical wet and dry (Aw), tundra (ET), and warm-summer Mediterranean (Csb) climate were included along the study analysis as shown in Figure 4a. Moreover, WA and domestic animals' species as diverse as the Antillean manatee (*Trichechus manatus manatus*), the bush dog (*Speothos venaticus*), the capybara (*Hydrochoerus hydrochaeris*), the puma (syn. cougar), the crab-eating fox (*Cerdocyon thous*), the jaguar (*Panthera onca*), the jaguarundi (*Herpailurus yagouaroundi*) and the ocelot (*Leopardus pardalis*); additionally a nationwide survey on angiotropic nematode *A. vasorum* was conducted including serological analyses of 955 domestic dogs (*Canis familiaris*) and a worldwide analysis of neglected angio-neurotropic *Gurltia paralysans* in wild and domestic felids were herein included and/or sampled across previously described study areas.

Based on current Colombian geopolitical division, we carried out different type of sampling and/or parasitological analysis in 12 of the 32 departments reported for the country (Figure

4b and c), covering a total of 524.970 Km², thus constituting half (~50.3%) of the continental national territory area of Colombia.

Figure 4: Total sampling areas covered during current study within the Colombian national territory



Descriptive geographical map of the areas sampled during the doctoral thesis. **a**) Köppen–Geiger climate classification for Colombia; Af: Tropical rainforest, Aw: Savanna, Bsh: Hot semi-arid, Bwh: Hot desert climate, Cfa: Humid subtropical, Cfb: Oceanic, Csb: Warm-summer Mediterranean, Cwc: Monsoon-influenced subpolar oceanic, Cwa: Mon-soon-influenced humid subtropical, ET: Tundra. **b**) Colombia location within the South America subcontinent. **c**) Different departments in accordance with the geopolitical division of Colombia; Sampled areas are show in red: (1) Atlántico, (2) Sucre, (3) Córdoba, (4) Antioquia, (5) Santander, (6) Arauca, (7) Risaralda, (8) Cundinamarca, (9) Casanare, (10) Meta, (11) Caquetá, and (12) Amazonas.

Since free-living WA are considered as excellent bioindicators of ecosystem health, particularly for water-borne, food-borne, soil-borne and gastropod-borne parasites, WA-related investigations will help to generate strategies to avoid zooanthroponotic transmissibility to human populations and transmission to domestic and/or synanthropic animals (Bossart, 2011; Cunningham *et al*., 2017; Uribe *et al*., 2021a, 2021c). Regarding expected parasitological abundance in the tropical region, it is considered as one of the most biodiverse areas worldwide, thus current doctoral thesis identified a plethora of parasites both in WA and domestic animals throughout the Colombian national territory (Table 2.), bringing to discussion eco-epidemiological importance of animals´ based on One Health concept thereby furthering continues efforts to control and eradicate infectious

protozoan/metazoan parasitoses (Balakrishnan, 2017; Dantas-Torres, 2021; Waikagul, 2006). Although this doctoral thesis was carried out in Colombia as unique Central and South American hinge joining key territory, the parasitological results obtained here can be extrapolated to other tropical regions and other latitudes considering ground-breaking information herein reported.

Table 2: Summarized list of identified parasites and respective hosts throughout current doctoral thesis

Parasites	Hosts	Zoonotic	Reference
Spirometra sp.	*L. pardalis* *P. onca*	✓	(Uribe *et al*., 2021c)
Toxocara cati	*L. pardalis* *P. onca*	✓	
Oncicola sp.	*L. pardalis* *P. onca*		
Cystoisopora sp.	*L. pardalis* *P. onca*		
Taenia omissa	*P. concolor* *H. yagouaroundi*†		
Protozoophaga obesa	*H. hydrochaeris*		(Uribe *et al*., 2021a)
Strongyloides sp.		✓	
Echinocoleus hydrochoerid			
Trichostrongyloidea			
Monoecocestus sp.			
Hippocrepis hippocrepis			
Plagiorchis muris		✓	
Taxorchis schistocotyle			
Neobalantidium coli		✓	
Cryptosporidium sp.		✓	
Entamoeba sp.		✓	
Eimeria trinidadensis			
Cycloposthiidae			
Gurltia paralysans	*F. catus*		(Uribe *et al*., 2021b)
Angiostrongylus vasorum	*C. familiaris*		(Uribe *et al*., 2022b)
Spirometra mansonoides	*C. thous*	✓	(Brabec *et al*., 2022)
Lagochilascaris minor	*C. thous*	✓	(Uribe *et al*. Chapter 6)
Dipylidium caninum	*S. venaticus*	✓	
Chiorchis fabaceus	*T. m. manatus*		(Uribe *et al*. Chapter 7)

✓: Parasites with zoonotic potential. †: *Taenia omissa* sister linage.

Globally, a total of twenty-three (23) different parasite taxa were here reported through different methodologies such as various coproparasitological examination techniques, enzyme-linked immunosorbent assays (ELISA), scanning electron microscopy (SEM), and both molecular identification and thereafter phylogenetic analyses. Some of these parasites, particularly within wild felid jaguars, ocelots, pumas and jaguarundis, were brought back from oblivion. Some species constitute new host records or have enlarged previously known distribution areas in Colombia. Consequently, current compendium generated new insights and highlighted future perspectives to parasitological research as a still-expanding field in the Americas. It is important to emphasize that a total of nine parasite species (n = 9) have zoonotic relevance and originating from different taxonomic groups (i. e., apicomplexan-, ciliated- and ameboid-protozoans, trematodes, acanthocephalans, nematodes and cestodes) were herein successfully identified. Therefore, only some parasite species of major public health concern are described in more detail whereas others are discussed under a rather general point of view to avoid repetition of already published articles.

The first of these surprising and unexpected findings was the occurrence of *Spirometra* sp. and *Spirometra mansoni* in wild felids and canids, respectively. Sparganosis is a globally distributed neglected water- and food-borne disease caused by larval stages (plerocercoid) of tapeworms belonging to the *Spirometra* genus located in various organs and tissues (Brabec *et al*., 2022; Galán-Puchades, 2019; Kuchta *et al*., 2021). Humans become infected by eating or using raw flesh of intermediate hosts (e. g. amphibians, reptiles) in traditional poultices; or by drinking water containing infected copepods (Kuchta *et al*., 2015). The occurrence of *Spirometra* infections across South America has been reported since the beginning of the 20th century (Mueller *et al*., 1975). This cestode genus has been previously recorded in South American wild felids (Acuña-Olea *et al*., 2020; Almeida *et al*., 2016; Arrabal *et al*., 2020). To the best of our knowledge, herein we report *Spirometra* sp. for the first time in Colombian free-ranging jaguars and ocelots (Uribe *et al*., 2021c). Moreover, herein through molecular identification of an adult tapeworm retrieved from a crab-eating fox in the locality of Ciudad Bolívar, Antioquia, Colombia, we confirmed the occurrence of the more frequent causative agent of human sparganosis (i. e., *Spirometra mansoni*), commonly

reported from Southeast Asia, and representing the second congeneric species with known zoonotic potential in the Americas (Brabec *et al*., 2022). Thus, phylogenetic analysis under maximum-likelihood criterion resolved the position of the tapeworm nested deep within the clade of *S. mansoni*, thus proving the presence of this causative agent of human sparganosis in the American continent. This was an unexpected and quite novel result that provides new data on global distribution of different genotypes of this important cestode since the concurrent presence of the second congeneric species with zoonotic potential urges deeper investigations into the parasite's life cycle and the epizootiology of a disease that could affect public health in the Americas.

On the other hand, toxocariasis caused by *T. canis* and *T. cati* is a zoonotic helminthiasis where eggs passed in the faeces of canids and felids may remain infective for years in the environment and capable to infect a large range of vertebrate species including humans (Wu and Bowman, 2020). Based on the increasingly prominent and more frequent insidious impact of zoonotic toxocariasis on human health, key research gaps and better understanding of biology of little studied *T. cati* are essential for guiding urgently needed actions in support and promotion of public health, particularly in children (Maciag *et al*., 2022). The overall *Toxocara* sp. prevalence in domestic dogs and cats might range from 0 to >87% and/or from 0 to >60%, respectively along subpopulations in North America, Mesoamerica and South America, while *T. cati* shows higher prevalence occurrence in animals with less than 1 year of age similarly to prevalence of *T. canis*-infected young dogs (Ketzis and Lucio-Forster, 2020). Therefore, toxocariasis is considered the most ubiquitous gastrointestinal helminth disease in domestic canids and felids (Wu and Bowman, 2020). Conversely, research data on wild felid toxocariasis is very scarce. Herein we report the occurrence of zoonotic *T. cati* in jaguars and ocelots during faecal examination, highlighting again the potential role of wildlife in parasite transmission to local human communities (Uribe *et al*., 2021c). This ascarid nematode is the dominant species in most felids due to its complex life cycle, including lactogenic transmission and a wide array of paratenic hosts, such as rodents (mouse and rat), macaques (*Macaca mulatta*), chickens, quails (*Coturnix japonica*), piglets, earthworms (*Eudrilus eugeniae*) and even by the ingestion of raw duck,

cattle, and/or sheep liver (Bowman, 2020; Peng *et al*., 2020). Despite worldwide distribution of *T. cati* and its endemicity in most American countries (Bolivar-Mejia *et al*., 2014), human- and felid toxocariasis are still poorly understood in Colombian rural areas, since most studies have been conducted in urban areas, including large cities of the country (López-Osorio *et al*., 2020). Once again this shows the importance of constant parasitological surveys not only in wild felids (final hosts) but also in other wild animals' populations acting as paratenic hosts.

Besides identification of cestode *Spirometra* sp. and of ascarid nematode *T. cati* in wild felids, the first report in the Americas of *S. mansoni* in a crab-eating fox were here discussed. Additionally, we recorded occurrence of soil-borne zoonotic nematode genus *Strongyloides* in endemic populations of wild large rodents such as capybaras as potential wild reservoir host for strongyloidiosis of domestic animals and humans inhabiting the Orinoco basin (Uribe *et al*., 2021a). Despite being a relevant helminthiasis with an estimate of 30–100 million *Strongyloides*-infected humans worldwide (Byard, 2019; Krolewiecki and Nutman, 2019); this zoonotic nematode remains largely neglected due to its chronic and longstanding auto-infective process associated with its unique and complex life cycle (Nutman, 2017). Human strongyloidiosis is mainly caused by the species *Strongyloides stercoralis* or other *Strongyloides* spp., which have both free-living and endogenous parasitic stages that can persist for decades, usually with host and parasite reaching amicable agreement of mutual toleration (Grove, 1996). Such as other helminthic diseases, the diagnosis of *Strongyloides* sp. infection may sometimes be difficult and the treatment of strongyloidiosis may be problematic particularly given recent mass migrations or population movements (Byard, 2019). The parasite has a board spectrum of clinical illness, may cause a brief period of acute symptomatology and signs after initial infection, and then lapse into a chronic asymptomatic carrier stage for decades due to the nematode's capability to auto-infect hosts (Byard, 2019). Human strongyloidiosis is one of the most neglected diseases, and as such its occurrence in pregnancy and lactation period has even been more neglected and understudied, despite epidemiological importance of lactogenic transmission for neonates (Wikman-Jorgensen *et al*., 2021). As soil-borne parasitosis, infective *Strongyloides*-larvae can

penetrate the human skin causing *larva migrans cutanea* (LMC). Thus, *Strongyloides*-infected synanthropic capybara herds may actively contribute to widespread LMC and originate parasite spillover among domestic animals and human populations and should be constantly monitored as a feasible transmission source of this tylenchid parasite in the Orinoco basin (Uribe *et al*., 2021a).

Furthermore, in the same giant rodent species abovementioned (i. e., *H. hydrochaeris*) we reported for the first time the trematode *Plagiorchis muris* as a new host record (Uribe *et al*., 2021a). This zoonotic-relevant food-borne disease, known as plagiorchiosis still remains as a major worldwide public health concern, despite changes in eating habits, alterations in social and agricultural practices, health education, industrialization, environmental alteration, and broad-spectrum available anthelmintic treatments (Fried *et al*., 2004). Plagiorchiosis is considered as an intestinal trematode disease that occurs in humans as well as rodents. Interestingly, the family Plagiorchiidae contains so far ten species capable to infect humans, i. e., *Plagiorchis harinasutari, Plagiorchis javanesis, Plagiorchis philippinensis, Plagiorchis elegans*, *Plagiorchis koreanus*, *Plagiorchis maculosus*, *Plagiorchis muelleri*, *Plagiorchis muris*, *Plagiorchis neomidis*, and *Plagiorchis vespertilionis*) (Chai *et al*., 2009). These intestinal trematodes have a highly complex origin, distribution, and divergence that apparently start in eastern regions of Laurasia (modern Southeast Asia territory), from where these parasites spread through the Amur paleomicrocontinent and the Beringia to North America and further expanding their distribution through Central and South America (Bogatov and Vainutis, 2022). It is important to highlight that within the taxonomically complex digenean Plagiorchiidae family, *P. muris* is the more frequent species capable to infect humans and it has been reported across continents (Suleman *et al*., 2019). This neglected intestinal trematode is considered as "rodent-borne disease" with endemicities in Asia and North America via an effective transmission route from rodents to humans (Asada *et al*., 1962; Chai and Lee, 2002; Hong *et al*., 1996; McMullen, 1937; Youn, 2009). Conversely, information regarding plagiorchiosis epidemiology, life cycle and human reports, still remain scarce for Africa, the Americas and Europe (Catalano *et al*., 2019; Franssen *et al*., 2016; Rogan *et al*., 2007). Thus, description presented here expands previously known geographic distribution

range of *P. muris* and constitutes the first host record in capybaras. Therefore, we strongly recommend future activities on this euryxenous zoonotic trematode in South American domestic animals and humans sharing habitats with synanthropic capybaras. More studies are needed on *P. muris* life cycle, the spectrum of obligate gastropod intermediate host species, second intermediate hosts, and other final hosts, and possible public health impact of human plagiorchiosis in rural populations.

In addition, in Colombian capybaras herein we reported the presence of water-borne apicomplexan and anthropozoonotic parasite *Cryptosporidium*. The World Health Organization (WHO) estimates that 91% of human population has regular access to sources of treated drinking water either through public tap water aqueduct networks, artisan wells or ground perforations but still at least 1.8 billion of people continues to use contaminated drinking water sources with faeces, thus causing at least 502000 deaths due to *Cryptosporidium*-mediated diarrhoea per year (Lim and Nissapatorn, 2017). Cryptosporidiosis is being the second cause of death, especially among children under 5 years of age (Checkley *et al*., 2015; Platts-Mills *et al*., 2015). Cryptosporidiosis has been identified as one of the main etiological agents of diarrheal outbreaks in human populations (Lim and Nissapatorn, 2017). In line, the genera *Cryptosporidium* and *Giardia* are extensively recognized worldwide as neglected etiological water-borne agents responsible for multiple outbreaks in human populations, epizootics among both domestic and wild animals, and effective transmissibility at the animal-human interface (Dixon, 2009; Kutz *et al*., 2009). These parasitic agents have been extensively studied, which is why they are not always considered as neglected tropical diseases (NTDs) and, additionally, they are not included in the list of tropical diseases eligible for a priority revision voucher (Priority Review Voucher - PRV) from the US Food and Drug Administration (FDA) (Archer *et al*., 2020; Choy and Huston, 2020). Despite not being considered as NTDs by the WHO or the CDC, cryptosporidiosis is still highly prevalent in rural areas of Africa, Asia, America, Oceania and Europe (Becker *et al*., 2015; Kantzanou *et al*., 2021; Korpe *et al*., 2018). Thus, cryptosporidiosis is one of the etiological agents responsible for multiple outbreaks of gastroenteric disorders in poor human populations worldwide and considered as poverty-

related disease (PRD) (Ryan *et al*., 2021). Human cryptosporidiosis, mainly caused by *Cryptosporidium parvum* has become one of the main causes of diarrheal diseases worldwide, posing a significant threat to infants and immunosuppressed patients (Kantzanou *et al*., 2021). The genus *Cryptosporidium* has already been described in 10 rodent families (Feng *et al*., 2020; García-Livia *et al*., 2020; Lv *et al*., 2009; Perz and Le Blancq, 2001; Stenger *et al*., 2015; Ziegler *et al*., 2007). Accordingly, a rather low prevalence of *C. parvum* natural infections was already described for capybaras (Hydrochoeridae) in Brazil (Meireles *et al*., 2007). Additionally, *Cryptosporidium* was already described for other free-living semiaquatic mammals species like South American sea lion, North American river otter, neotropical river otters, and giant otters (Borges *et al*., 2018; Ebmer *et al*., 2020; Gaydos *et al*., 2007). These two last otter species share their natural habitat in Colombia with capybaras implying possible transmission with highly infective and environmental resistant sporulated *Cryptosporidium*-oocysts. Since *Cryptosporidium*-subtype found in capybaras had 100% genetic similarity to bovine *C. parvum*-isolate subtype IIaA15G2R1 (Meireles *et al*., 2007), it raises the importance of bidirectional way in which thick-walled *Cryptosporidium* oocysts could spread with ease in aquatic ecosystems among semiaquatic mammals like capybaras, domestic animals, and humans (Uribe *et al*., 2021a).

Moreover, in Colombian capybaras we reported two other potentially zoonotic species of protozoan parasites (*Neobalantidium coli* and *Entamoeba* sp.) (Uribe *et al*., 2021a). Previously known as *Balantidium coli* but recently renamed as *Neobalantidium coli* based on polymorphism of SSrDNA sequences, this intestinal protozoan species has been recorded in domestic pigs as main reservoir hosts and many other mammalian species, including primates (Pomajbíková *et al*., 2013). Various animals such as rodents, camels, cattle, donkeys, sheep and goats have been also proposed as reservoir hosts for human neobalantidiosis (Nilles-Bije and Rivera, 2010; Ponce-Gordo and García-Rodríguez, 2021). The neglected ciliate *N. coli* is an opportunistic parasite that can be found throughout the world affecting a variety of hosts, including domestic pigs that are the main reservoir, while humans become infected through direct or indirect contact with pigs and other infected hosts passing infective cysts (Giarratana *et al*., 2021). In rural areas and in some developing

countries where hosts faeces matter contaminates the water supply, there is a greater likelihood that neobalantidiosis may develop in humans (Schuster and Ramirez-Avila, 2008). Despite the fact, that neobalantidiosis is predominantly occurring in tropical and subtropical regions, also *N. coli*-infections have been reported from cooler climate geographic region (Yu *et al*., 2020). This unattended cosmopolitan water-borne disease may be subclinical in humans, as it is mostly seen in pigs, or may develop as a fulminant typhlocolitis with bloody and mucus-containing diarrhoea which finally might lead to colon perforation (Schuster and Ramirez-Avila, 2008).

Regarding the detection of *Entamoeba* sp. in capybaras, it is remarkable that within the genus the species *Entamoeba histolytica* is a parasite frequently found contaminating vegetables and fruits, and directly associated with diarrheal disease in humans as a major food-borne public health problem across the world (Li *et al*., 2020). Thus, this ameboid protozoan is one of the main species of intestinal parasites reported in humans, particularly causing acute diarrhoea, dysentery, amoebic colitis, and even amoebic liver abscesses (Dacal *et al*., 2020; Li *et al*., 2021). As the fourth leading parasitic cause of human mortality, *E. histolytica* mainly infect children in developing countries, transmitted by food and/or water contamination with infective and highly resistant *E. histolytica* cysts (Li *et al*., 2021). Once again, the possibility of wildlife playing a key role in transmission and maintenance of this zoonotic parasitosis is most likely to occur (Li *et al*., 2021). Nevertheless, as in the case of wild non-human primates this issue requires to be clarified further as different *Entamoeba* species might be interpreted as *E. histolytica* due to scarce morphological and molecular data and gaps in recently published data on *Entamoeba* species (Elsheikha *et al*., 2018). Consequently, free-ranging capybaras should be considered as natural reservoir hosts for various zoonotic-relevant water-, food-, soil- and gastropod-borne parasites of public health concern (Uribe *et al*., 2021a).

In the same way, herein we identified the occurrence of the nematode genus *Lagochilascaris* which entails public health concern. Human lagochilascariosis due to *Lagochilascaris minor* is an extremely neglected zoonotic disease and limited to the American continent. This is a

rare human ascarid parasite (order Rhabditida) and mostly diagnosed as a chronic disease persisting for several years (Campos *et al*., 2017; Neves, 2016). In chronic lagochilascariosis, the parasitic larvae burrows into subcutaneous tissues of the neck, paranasal sinuses, and mastoids (Campos *et al*., 2017). Additionally, *L. minor* may cause lesions in the region of the head and neck, including the tonsils, ocular globe, nasal sinuses, middle ear, dental alveolus, rhinopharynx, lungs, sacral areas, and less frequently the central nervous system (CNS). Humans become infected after ingestion of raw or undercooked meat of game animals (acting as either intermediate- or paratenic hosts) which contain encysted *L. minor* third-stage larvae (Tanowitz and Machado, 2013. In the Americas, human lagochilascariosis have been reported in more than 100 cases (Campos *et al*., 2017). Human cases of lagochilascariosis have been reported across Central and South American countries like Bolivia (Ollé-Goig *et al*., 1996), Brazil (Neves, 2016), Colombia, Costa Rica, Ecuador (Calvopiña *et al*., 1998), Mexico (Barrera-Pérez *et al*., 2012), Paraguay (Roig O. R *et al*., 2010), Perú, Surinam (Oostburg, 1992), Trinidad and Tobago (Draper, 1963), and Venezuela (Orihuela *et al*., 1987). Only three lagochilascariosis human cases have been documented in Colombia (Little and Botero, 1984; Moncada *et al*., 1998). To the best of our knowledge here we present first non-human case report of *L. minor* in a Colombian crab-eating fox. Thus, current doctoral thesis constitutes the first report of this parasite in non-human hosts of Colombia.

Finally, regarding the last zoonotic parasite identified in current doctoral thesis we reported the occurrence of the neglected causative agent of dipylidiosis in wild canids. Dipylidiosis is a cosmopolitan underrated disease caused by the cestode *Dipylidium caninum* which belongs to the order Cyclophyllidea (family Dipylidiidae) and been identified in several sylvatic species, namely foxes, wolves, jackals, hyaenas, coyotes, racoon dogs and cheetahs (Rousseau *et al*., 2022). Recently two genetically distinct variations have been proposed within this species, i. e. the *D. caninum* canine genotype and the *D. caninum* feline genotype (Beugnet *et al*., 2018; Labuschagne *et al*., 2018). While *D. caninum* infection typically occurs in canids and felids as main definitive hosts it may infect humans after the ingestion of adult fleas/lice harbouring infective cysticercoids, and reported particularly in children living in

close proximity to *D. caninum*-infected definitive hosts (Hogan and Schwenk, 2019). There are reported cases of human dipylidiosis from several countries and it is distributed worldwide (Gutema *et al*., 2020). As seen for other PRDs, human dipylidiosis mostly affects children of poor countries with low hygiene standards, nonetheless very little is so far conducted by WHO to prevent or control this parasitosis. Clinical manifestations of human dipylidiosis often consist of perianal pruritus with mild dermatitis of perineal area and linear excoriations thought to be secondary to the patient scratching. Moreover, diarrhoea, vomitus and abnormal weight gain have been described (Chong *et al*., 2020). Transmission of this intestinal cestode is bidirectional (i. e. WA to domestic animals and domestic animals to WA) and possible to occur due to shared habitats, particularly at night, when wild animals come close to human populations in their forage for food (Rousseau *et al*., 2022). Herein we expand the geographical distribution range of *D. caninum sensu lato* to the Pan-Amazon and northern Andean regions and constituting the first host record of this anthropozoonotic cestode in bush dogs. Analysed bush dog cestode corresponded well to recently proposed *D. caninum* canine genotype which occurs at a higher frequency in canids, has a shorter prepatent period and longer lifespan than the *D. caninum* feline genotype (Beugnet *et al*., 2018).

Despite not having clinical nor epidemiological relevance in human populations, other non-zoonotic parasites were here identified both in WA and domestic animals. However, these etiological agents are important to understand parasite dynamics and the role they play in host health. Additionally, some of these parasite reports also constitute new host records, or parasitological findings that for many decades have been forgotten in literature. One of these cases was the acanthocephalan genus *Oncicola* identified both in ocelots and jaguars (Uribe *et al*., 2021c). This acanthocephalan has been known circulating in South American felines for almost 9000 years (Amin, 2013; Orrell, 2017; Sianto et al., 2014), and we brought it back from oblivion (Uribe *et al*., 2021c). Another unexpected parasitological finding that is limited to both carnivorous and herbivorous animal species is the precise phylogenetic identification of *Taenia omissa* collected from a dead puma and a road-killed jaguarundi (Uribe *et al*., 2021c). To date *T. omissa* molecular data is restricted to reports in natural

intermediate hosts such as domesticated alpacas (*Vicugna pacos*) and free-living red brockets (*Mazama americana*) (Gomez-Puerta *et al*., 2017). Thus, we enlarged the sequence data for this tapeworm species in felids, expanding its geographical distribution range to Colombia and adding the jaguarundi as a new definitive host for this taeniid cestode. Similarly in the same hosts species above mentioned, *Cystoisopora*-like oocysts were identified at a higher taxa level and thus we recommend to further identify if these oocysts belonged either to *Cystoisospora rivolta*, *Hammondia hammondi* or *Besnoitia* sp., as one of the more frequently reported cyst-forming coccidian in wild- and domestic felids (Dubey, 2018). Moreover, herein we reported occurrence of highly underestimated protozoan species such as *Eimeria trinidadensis*, the nematodes *Protozoophaga obesa*, the capillarid *Echinocoleus hydrochoerid* and the metastrongyloid lungworms *A. vasorum* and *G. paralysans*, the cestode of the genus *Monoecocestus* sp., and neglected trematodes *Hippocrepis hippocrepis*, *Taxorchis schistocotyle*, and *Chiorchis fabaceus*. For all these unattended parasites we tried to bring more attention on possible consequences for WA health and hopefully will derive in more awareness on these cryptic parasites. Finally, free-ranging WA should be considered not only as natural reservoirs for various pathogens but also to be affected by them; the same holds true for peri-domestic and synanthropic WA within Colombian national territories. Thus, emblematic WA species here investigated can be a good way to draw attention of the importance of parasite investigations under the concept of One Health, particularly for unattended species of vertebrate and invertebrate animals.

Conclusion

As rule in all living communities' coinfections have consequences on infectious agent epidemiology and host fitness, thus better fundamental knowledge on wildlife associated parasitic diseases is needed to understand their role in the emergence of zoonoses (Hoarau *et al*., 2020). Based on these results, we encourage further parasitological studies on wildlife, domestic animals, and human population to reveal parasitoses of public health concerns as an important issue in favours to prevent potentially zooanthroponotic parasite spillover events.

Summary

Wild animals (WA) have shown to be excellent bioindicators of important zoonotic-relevant pathogens in incrementally anthropogenic environments. As a result of continuous increase of anthropogenic pressure on fragile ecosystems, the contact of human populations with WA is constantly increasing. In an increasingly globalized world, anthropogenic factors such as intensified farming with consequent agro-industrial monocultures, unsustainable natural resources exploitation such as illegal mining, indiscriminate logging, wildlife hunting/trafficking, and wildlife-meat consumption, have strengthened the human-animal interface, thus increasing the risk of bidirectional disease spillover. The WA are indirect indicators of ecosystem health since they are sentinels of some neglected anthropozoonotic ecto- and endoparasitic diseases. Therefore, it is important to know the parasite fauna occurring in Neotropical wildlife, not only to strengthen conservation plans for threatened species, but also for the generation of valuable public health information to avoid potential human infections. Since Neotropics is an extensive and highly heterogeneous region, here we selected the hinge joining key territory of Colombia because remains as a poorly investigated area for wildlife parasitology and is the second most biodiverse country of the globe. Thus, there are scarce and old dated literature reports on various infectious agents that Colombian wildlife may harbour.

Herein we presented a first nationwide approach on neglected parasite fauna occurring in Neotropical wild- and domestic animal populations closely related to human populations in different habitats or biomes in which they inhabit. Additionally, WA-associated infectious diseases emergence or re-emergence highlights the need for better understanding on the mechanisms and factors involved in eco-epidemiology of parasitoses. Consequently, sampling efforts of present doctoral research proposal covered a wide range of biological regions from the Andean to the Amazon, the Caribbean and included the vast Orinoco basin. Covering a total of 524.970 Km^2, thus constituting half of the continental national territory area of Colombia. Moreover, WA and domestic animals' species as diverse as the Antillean manatee (*Trichechus manatus manatus*), the bush dog (*Speothos venaticus*), the

capybara (*Hydrochoerus hydrochaeris*), the puma (syn. cougar) (*Puma concolor*), the crab-eating fox (*Cerdocyon thous*), the jaguar (*Panthera onca*), the jaguarundi (*Herpailurus yagouaroundi*) and the ocelot (*L. pardalis*). Additionally, a nationwide survey on angiotropic nematode *A. vasorum* was conducted including serological analyses of 955 domestic dogs (*Canis familiaris*) and a worldwide analysis of neglected angio-neurotropic *Gurltia paralysans* in wild and domestic felids were herein included and/or sampled across previously described study areas.

Globally, a total of twenty-three (23) different parasite taxa were here reported through different methodologies such as various coproparasitological examination techniques, enzyme-linked immunosorbent assays (ELISA), scanning electron microscope (SEM), and both molecular identification and thereafter phylogenetic analyses. Some of these parasites were brought back from oblivion, constitute new host records, or have enlarged previously known distribution areas in Colombia. The first of these surprising and unexpected findings was the occurrence of *Spirometra* sp. and *Spirometra mansoni* in wild felids and canids, respectively. Additionally, herein we report the occurrence of zoonotic *T. cati* in jaguars and ocelots during faecal examination, highlighting again the potential role of wildlife in parasite transmission to local human communities. Additionally, the occurrence of soil-borne zoonotic nematode genus *Strongyloides* in endemic populations of wild large rodents such as capybaras as potential wild reservoir host for strongyloidiosis of domestic animals and humans inhabiting the Orinoco basin was reported. Furthermore, in the same giant rodent species (*H. hydrochaeris*) abovementioned we reported for the first time the trematode *Plagiorchis muris* as a new host record. This zoonotic-relevant food-borne disease, known as plagiorchiosis still remains as a major worldwide public health concern. In addition, in Colombian capybaras herein we reported the presence of water-borne apicomplexan and anthropozoonotic parasites *Cryptosporidium*, *Neobalantidium coli* and *Entamoeba* sp.

In the same way, herein we identified the occurrence of the nematode genus *Lagochilascaris* which entails public health concern. Human lagochilascariosis due to *Lagochilascaris minor*

is an extremely neglected zoonotic disease and limited to the American continent. To the best of our knowledge here we present first non-human case report of *L. cf. minor* in a Colombian crab-eating fox. Thus, current doctoral thesis constitutes the first report of this parasite in non-human WA of Colombia. Finally, regarding the last zoonotic parasite identified in current doctoral thesis we reported the occurrence of the neglected causative agent of dipylidiosis in wild canids. Herein we expand the geographical distribution range of *Dipylidium caninum sensu lato* to the Pan-Amazon and northern Andean regions and constituting the first host record of this anthropozoonotic cestode in bush dogs. Analysed bush dog cestode corresponded well to recently proposed *D. caninum* canine genotype which occurs at a higher frequency in canids, has a shorter prepatent period and longer lifespan than the *D. caninum* feline genotype.

Nowadays constant surveillance of wildlife- and domestic animal-related diseases is imperative not only for better understanding of their adverse impact on environment, human- and animal populations but also on biodiversity conservation. Despite not having clinical nor epidemiological relevance in human populations, other non-zoonotic parasites were here identified both in WA and domestic animals. One of these cases were the acanthocephalan genus *Oncicola* identified both in ocelots (*Leopardus pardalis*) and jaguars and the precise phylogenetic identification of *Taenia omissa* collected from a dead puma (*P. concolor*) and a road-killed jaguarundi. Similarly in the same hosts species above mentioned, *Cystoisopora*-like oocysts were identified at a higher taxa level. Moreover, herein we reported the occurrence of highly underestimated protozoan species such as *Eimeria trinidadensis*, the nematodes *Protozoophaga obesa*, the capillarid *Echinocoleus hydrochoerid* and the metastrongyloid lungworms *A. vasorum* and *G. paralysans*, the cestode of the genus *Monoecocestus* sp., and neglected trematodes *Hippocrepis hippocrepis*, *Taxorchis schistocotyle*, and *Chiorchis fabaceus*. For all these unattended parasites we tried to bring more attention on possible consequences for WA health and hopefully will derive in more awareness on these cryptic parasites. Free-ranging WA should be considered not only as natural reservoirs for various pathogens but also to be affected by them; the same holds true for peri-domestic and synanthropic WA within Colombian national territories. Thus, emblematic WA species

here investigated can be a good way to draw attention of the importance of parasite investigations under the concept of One Health, particularly for unattended species of vertebrate and invertebrate animals. Finally, current compendium generated new insights and highlighted future perspectives to parasitological research as a still-expanding field in the Americas.

Zusammenfassung

Wildtiere (WT) haben sich als hervorragende Bioindikatoren für wichtige zoonotisch relevante Krankheitserreger in einer, zunehmend durch anthropogene Einflüsse geprägten Umwelt erwiesen. Infolge des kontinuierlichen anthropogenen Drucks auf empfindliche Ökosysteme nimmt der Kontakt zwischen Menschen und Wildtieren ständig zu. In einer zunehmend globalisierten Welt haben anthropogene Faktoren wie zum Beispiel die Intensivierung der Landwirtschaft mit den daraus resultierenden industriellen Monokulturen, die Ausbeutung natürlicher Ressourcen wie illegaler Bergbau, wahlloser Holzeinschlag, die Jagd auf Wildtiere und der Handel mit denselben sowie der Verzehr von Wildtierfleisch die Schnittstelle zwischen Mensch und Tier verstärkt. Damit wird permanent das Risiko einer Pathogenübertragung sowohl von Tier auf Mensch als auch umgekehrt erhöht.

Die WT sind indirekte Indikatoren für die Gesundheit des Ökosystems, da sie Träger vernachlässigter anthropozoonotischer ekto- und endoparasitärer Krankheiten sind. Dies macht deutlich wie wichtig es ist, die in der neotropischen Tierwelt vorkommende Parasitenfauna zu kennen, nicht nur, um die Erhaltungspläne für bedrohte Arten besser konzipieren zu können, sondern auch, um so viele Informationen wie möglich über die menschliche Gesundheit zu erhalten. Dieses Wissen kann potenzielle Infektionen beim Menschen vermeiden. Da es sich bei den Neotropen um eine ausgedehnte und sehr heterogene Region handelt, haben wir hier das Schlüsselgebiet Kolumbien ausgewählt. Kolumbien stellt als eines der artenreichsten Länder der Welt, ein noch wenig erforschtes Gebiet für die Wildtierparasitologie dar. Es gibt nur wenige Studien über die diversen Pathogene, die in kolumbianischen Wildtieren vorkommen und die bereits existierende

Literatur ist zum Teil veraltet. In der vorliegenden Dissertation ist die erste landesweite Studie über die vernachlässigte Parasitenfauna neotropischer Wild- und Haustierpopulationen erfolgt. Dafür haben die Autoren Populationen ausgewählt, die mit Menschen in verschiedenen Lebensräumen oder Biomen in engem Kontakt stehen. Darüber hinaus verdeutlicht das Auftreten oder Wiederauftreten von WT-assoziierten Infektionskrankheiten die Notwendigkeit eines besseren Verständnisses der Mechanismen und Faktoren, die an der Öko-Epidemiologie von Parasitosen beteiligt sind. Folglich umfasste die Beprobung im Rahmen dieses Dissertationsvorhabens ein breites Spektrum an geologischen Regionen mit unterschiedlichsten Ökosystemen. Untersucht wurden sowohl Räume in den Anden als auch im Amazonas und in der Karibik bis hin zum großen Orinoco-Becken. Die Untersuchungen umfassten insgesamt 524.970 km², was der Hälfte des Staatsgebiets Kolumbiens entspricht. Darüber hinaus wurden unterschiedlichste Wild- und Haustierarten untersucht wie zum Beispiel die Antillen-Seekuh (*Trichechus manatus manatus*), der Buschhund (*Speothos venaticus*), das Wasserschwein (*Hydrochoerus hydrochaeris*), den Puma (*Puma concolor*), den krebsfressenden Fuchs (*Cerdocyon thous*), den Jaguar (*Panthera onca*), den Jaguarundi (*Herpailurus yagouaroundi*) und den Ozelot (*Leopardus pardalis*). Zusätzlich wurde eine landesweite Studie über den angiotropen Fadenwurm *Angiostrongylus vasorum* durchgeführt, einschließlich serologischer Untersuchung von 955 Haushunden (*Canis familiaris*). Des Weiteren wurde eine weltweite Analyse zum vernachlässigten angio-neurotropen Parasiten *Gurltia paralysans* bei Wild- und Hauskatzen erstellt.

Insgesamt wurden hier 23 verschiedene Parasitentaxa durch verschiedene Methoden wie koproparasitologische Untersuchungstechniken, Enzymimmunoassays (ELISA), Rasterelektronenmikroskopie (SEM) und molekulare Identifikationsmethoden sowie anschließende phylogenetische Analysen erfasst. Einige dieser Parasiten wurden dabei aus der Vergessenheit geholt, stellen neue Wirtsbeschreibungen dar oder haben zuvor bekannte Verbreitungsgebiete in Kolumbien vergrößert. Die erste dieser überraschenden und unerwarteten Entdeckungen war das Vorkommen von *Spirometra* sp. und *Spirometra mansoni* bei wildlebenden Feliden bzw. Kaniden. Darüber hinaus berichten wir über das

Auftreten von dem zoonotisch relevanten Parasiten *Toxocara cati* beim Jaguar und dem Ozelot, im Rahmen einer Analyse von Losungen dieser Prädatoren, was erneut die potenzielle Rolle von WT bei der Übertragung von Parasiten auf die lokale Bevölkerung verdeutlicht. Darüber hinaus wird über das Auftreten der zoonotischen Nematoden der Gattung *Strongyloides* in endemischen Populationen wild lebender großer Nagetiere wie Wasserschweine berichtet, die potenziell als Reservoirwirte für Strongyloidose bei Haustieren und Menschen im Orinoco-Becken dienen könnten. Außerdem haben wir bei *H. hydrochaeris* zum ersten Mal den Trematoden *Plagiorchis muris* nachgewiesen. Die durch Lebensmittel übertragbare Krankheit, die als Plagiorchiose bekannt ist, hat zoonotische Relevanz. Zusätzlich haben wir bei kolumbianischen Wasserschweinen das Vorhandensein von im Wasser lebenden apikomplexen und anthropozoonotischen Parasiten wie *Cryptosporidium*, *Neobalantidium coli* und *Entamoeba* sp. nachweisen können. In gleicher Weise haben wir hier das Vorkommen der Nematodengattung *Lagochilascaris* identifiziert, die ein Problem für die öffentliche Gesundheit darstellen kann. Die durch *Lagochilascaris minor* verursachte Lagochilascariose des Menschen ist eine äußerst vernachlässigte Zoonose und beschränkt sich auf den amerikanischen Kontinent. Soweit uns bekannt, wird hier der erste nicht-menschliche Fall von *L. cf. minor* bei einem kolumbianischen Krabbenfuchs/Maikong (*C. thous*) vorgestellt. In der vorliegenden Dissertation ist somit der erste Bericht über diesen Parasiten bei WT in Kolumbien beschrieben. Bei dem letzten zoonotische Parasiten, der in der aktuellen Dissertation identifiziert und vorgestellt wird, handelt es sich um den vernachlässigten Erreger der Dipylidiose bei wildlebenden Kaniden. In dieser Arbeit erweitern wir das geografische Verbreitungsgebiet von *Dipylidium caninum sensu lato* auf den Pan-Amazonas und die nördlichen Andenregionen. Des Weiteren wird der erste Wirtsnachweis dieses anthropozoonotischen Zestoden bei Buschhunden beschrieben und dem kürzlich vorgestellten Genotyp von *D. caninum* canine zugeordnet, der bei Kaniden häufiger vorkommt, eine kürzere Präpatenzzeit sowie längere Patenz aufweist als *D. caninum* feline.

Heutzutage ist die ständige Überwachung von Wild- und Haustierkrankheiten nicht nur für ein besseres Verständnis ihrer nachteiligen Auswirkungen auf Tier, Mensch und

Umwelt, sondern auch für die Erhaltung der biologischen Vielfalt unerlässlich. Im Rahmen dieser Dissertation wurden auch andere, für Wild- und Haustiere wichtige Parasiten detektiert, die für die menschlichen Bevölkerung weder klinisch noch epidemiologisch relevant sind. Einer dieser Fälle war die Gattung *Oncicola* aus dem Stamm der Kratzwürmer/Acanthocephala, die sowohl beim Ozelot als auch beim Jaguar nachgewiesen wurde, sowie die genaue phylogenetische Identifizierung von *Taenia omissa*, die aus einem toten Puma und einem Jaguarundi stammt. Des Weiteren wurde bei den oben genannten Wirtsarten Cystoisopora-ähnliche Oozysten auf Taxa-Ebene identifiziert. Darüber hinaus berichteten wir über das Vorkommen von stark unterschätzten Protozoenarten wie *Eimeria trinidadensis*, den Nematoden *Protozoophaga obesa*, *Echinocoleus hydrochoerid* und den metastrongyloiden Lungenwürmern *A. vasorum* und *G. paralysans*, die Zestoden der Gattung *Monoecocestus* sp., und die Trematoden *Hippocrepis hippocrepis*, *Taxorchis schistocotyle* und *Chiorchis fabaceus*. Bei all diesen bisher wenig beachteten Parasiten haben wir versucht, die Aufmerksamkeit auf mögliche Folgen für die Gesundheit von Wildtierpopulationen zu lenken, und hoffen, dass diese kryptischen Parasiten dadurch stärker ins Bewusstsein gerückt werden. WT sollten nicht nur als natürliches Reservoir für verschiedene Krankheitserreger betrachtet werden, sondern auch als von denselben beeinträchtigt; das Gleiche gilt für peri- und synanthrope WT auf kolumbianischem Staatsgebiet. Daher können und sollten die hier untersuchten ausgewählten WT-Arten ein guter Ausgangspunkt sein, um auf die Bedeutung von Parasitenuntersuchungen im Rahmen des One-Health-Konzepts aufmerksam zu machen, insbesondere für bisher unbeachtete Wirbeltiere und wirbellose Tiere. Zusammenfassend hat das vorliegende Werk neue Erkenntnisse und Zukunftsperspektiven für die parasitologische Forschung aufgezeigt, die sich auf dem amerikanischen Kontinent noch immer im Aufbau befindet.

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Appendix

Supplementary Materials Chapter 1
The following material is available online at https://www.mdpi.com/article/10.3390/pathogens10070822/s1, Video S1: 3D model of *T. omissa* rostellar large hook, Video S2: 3D model of *T. omissa* rostellar small hook.

Supplementary Materials Chapter 2
The following are available online at https://www.mdpi.com/article/10.3390/pathogens10091152/s1, Figure S1: Adult specimen of *Hippocrepis hippocrepis* (Trematoda: Notocotylidae) found in capybara manure pellet piles collected in a flooded area of La Maporita. Scale bar: 2 mm, Figure S2: Cycloposthiidae cyst (47.28 µm × 33.03 µm), notice adoral ciliary zone (white arrowhead), vestibulum (red arrowhead), and cytoproct (black arrowhead). Scale bar: 10 µm, Video S1: Biopercular plugged *Echinocoleus hydrochoeri* egg, Video S2: Biflagellate egg of *Hippocrepis hippocrepis*.

Supplementary Materials Chapter 3
The following are available online at https://www.mdpi.com/article/10.3390/pathogens10121601/s1, Table S1: Gastropod species as potential intermediate hosts for *G. paralysans* in Colombia.

Supplementary Materials Chapter 4
The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms10081565/s1, Table S1: Dog serum database.

Supplementary Materials Chapter 5
The following supporting information can be downloaded at: http://doi.org/10.3201/eid2811.220529, Appendix and Methods.

Supplementary Materials International Conference
This supplementary material is based on the international poster presentation entitled "Wide Gastrointestinal Parasite Survey in World`s Largest Extant Semiaquatic Rodent *Hydrochoerus hydrochaeris* (Linnaeus 1766)" following attached:

WAAVP Dublin 2021 28th International Conference of the World Association for the Advancement of Veterinary Parasitology

A Wide Gastrointestinal Parasite Survey in World`s Largest Extant Semiaquatic Rodent *Hydrochoerus hydrochaeris* (Linnaeus 1766)

Manuel Uribe [1,2], Juan Vélez [1,2], Arlex Rodríguez-Durán [3], Jesús Cortés-Vecino [3], Sara López-Osorio [1,2], Anja Taubert[1], Carlos Hermosilla [1], Jenny Chaparro-Gutiérrez [2]

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Free-ranging capybaras (*Hydrochoerus hydrochaeris*) are affected by wide range of proto- and metazoan, including zoonotic water-, food- and gastropod-borne parasitoses as opportunistic neozoan infections in semi-aquatic ecosystems. Overlapping of capybara's natural ecological habitats with human/domestic animal activities, as seen in the Orinoquía region, have unfortunately increased in last decades. Three wild capybara populations located in Colombian Orinoco savannah were studied for occurrence of gastrointestinal parasite infections. A total of 46 faecal samples were collected from free-ranging capybaras close to cattle farms. Applying standard parasitological techniques, coproELISAs for detection of *Cryptosporidium*- and *Giardia*-specific antigens, PCRs and macroscopical analyses, the study revealed infections of 16 different parasite taxa.

Six zoonotic parasites were identified, i. e. *Cryptosporidium* sp., *Entamoeba* sp., *Neobalantidium coli*, *Lagochilascaris cf. minor*, Strongyloides sp., and *Plagiorchis muris*). Identified *P. muris* trematode represent the first report within South America and likewise constituting the first host record. Alongside, presence of the ascarid species *Lagochilascaris cf. minor* expands previous distribution range of emerging lagochilascarosis in the Americas. Overall, parasitological findings include four new host records (*Lagochilascaris* sp., *Plagiorchis muris*, *Neobalantidium coli*, and *Entamoeba* sp.). Present findings constitute a baseline data for future monitoring studies targeting impact of anthropogenic changes on capybara's population health conditions and thereby contributing to protection of these semi-aquatic giant rodents tightly linked to activities of domestic animals and humans.

Figure 1. Precise geographic location of sampling zones.

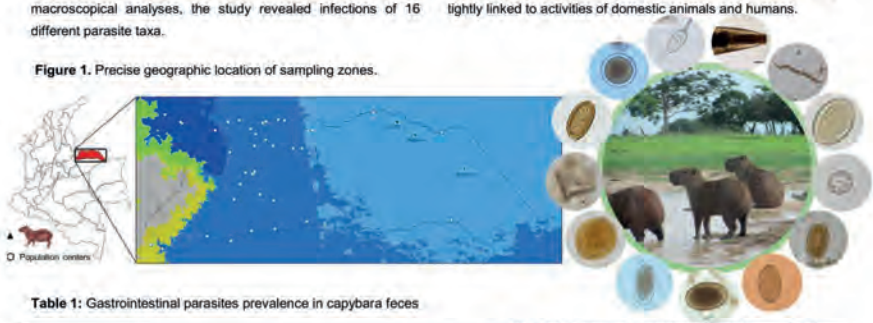

Table 1: Gastrointestinal parasites prevalence in capybara feces

	Phylum	Parasite	Stage	Technique	Bocas del Arauca *n* = 15	Cinaruco *n* =8	La Maporita *n* = 23	Total Prevalence
Protozoa	Apicomplexa	*Cryptosporidium* sp.	Oocysts	coproELISA	13.3	75	34.8	34.8 (16/46)
		Eimeria trinidadensis	Oocysts	SAF	13.3	25	26.1	21.7 (10/46)
	Amoebozoa	*Entamoeba* sp.	Cysts	SAF	26.7	25	8.7	17.4 (8/46)
	Ciliophora	*Neobalantidium coli*	Cysts	SAF	6.7	-	4.3	4.3 (2/46)
		Cyclophosthium sp.	Cysts	SAF	40	-	17.4	21.7 (10/46)
Metazoa	Platyhelminthes Class: Nematoda	Ascarididae	Eggs	SAF	20	25	34.8	28.3 (13/46)
		Lagochilascaris-like	Eggs	SAF	13.3	-	-	4.3 (2/46)
		Echinocoleus hydrochoeri	Eggs	CF/SAF	46.7	87.5	56.5	58.7 (27/46)
		Protozoophaga obesa	Eggs/Larvae/Adult	SS/CF/SAF	20	12.5	17.4	17.4 (8/46)
		Strongyloides sp.	Larvae	SAF	46.7	25	43.5	41.3 (19/46)
		Ancylostomatidae	Eggs	CF/SAF	53.3	25	39.1	41.3 (19/46)
	Class: Cestoda	*Monoecocestus* sp.	Eggs	CF/SAF	6.7	-	8.7	6.5 (3/46)
		Taeniid	Eggs	CF/SAF	-	12.5	-	2.2 (1/46)
	Class: Trematoda	*Hippocrepis hippocrepis*	Eggs/Adult	SF/SS/SAF	26.7	12.5	13	17.4 (8/46)
		Plagiorchis muris	Adult	Sequencing	6.7	-	-	2.2 (1/46)
		Taxorchis schistocotyle	Eggs	SS/SAF	40	75	34.8	43.5 (20/46)

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Declaration / Erklärung

I completed the submitted dissertation independently and without unauthorized outside help and made only with the aids that I have given in the dissertation. All text passages, which literally or analogously from published or not published writings are taken, and all information is based on oral based on information are identified as such. At mine carried out and mentioned in the dissertation I have the principles of good scientific practice, as laid down in the "Statutes of the Justus Liebig University Giessen to ensure good scientific practice" complied with.

Ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

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Manuel Uribe Soto

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