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MONITORING ZOONOTIC AND CONSERVATION-SENSITIVE PATHOGENS AT THE HUMAN-WILDLIFE INTERFACE IN THE PERUVIAN AMAZON

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Facultat de Veterinària

Departament de Sanitat i d'Anatomia Animals

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PhD Thesis

Maria Fernanda Menajovsky Bonifaz

PhD Candidate

Dr. Pedro Mayor Aparicio

Director/ Tutor

Dr. Oscar Cabezón Ponsoda

Director

Dr. Johan Espunyes Nozieres

Director

2025

El **Dr. Pedro G. Mayor**, profesor titular e investigador del Departament de Sanitat i d'Anatomia Animals de la Facultat de Veterinària de la Universidad Autónoma de Barcelona, el **Dr. Oscar Cabezón**, investigador del Departament de Medicina i Cirurgia Animals de la Facultat de Veterinària de la Universidad Autónoma de Barcelona y el **Dr. Johan Espunyes**, investigador asociado al Grup de Recerca en Medicina de la Conservació de la Fauna Salvatge

Certifican:

Que los trabajos de investigación incluidos en la memoria de tesis doctoral "**MONITORING ZOONOTIC AND CONSERVATION-SENSITIVE PATHOGENS AT THE HUMAN-WILDLIFE INTERFACE IN THE PERUVIAN AMAZON**", presentados por la doctoranda **Maria Fernanda Menajovsky** para la obtención del Grado de Doctor en Medicina i Sanitat Animals se han realizado bajo su dirección y tutoría. Por consiguiente, autorizan su presentación a fin de ser evaluados por la comisión correspondiente.

Para que así conste y a efectos oportunos, firman el presente certificado, en Bellaterra.

Dr. Pedro G. Mayor Director / Tutor	**Dr. Oscar Cabezón** Director
Dr. Johan Espunyes Director	**Maria Fernanda Menajovsky** Doctoranda

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Abbreviations

ADV: Aujeszky Disease Virus

BSE: Bovine Spongiform Encephalopathy

CDC: Centers for Disease Control and Prevention

CMIA: Chemiluminescent Microparticle Immunoassay

CP: Collared Peccary

CSFV: Classical Swine Fever Virus

DNA: Deoxyribonucleic acid

EIDs: Emerging Infectious Diseases

ELISA: Enzyme-Linked Immunosorbent Assay

FTA: Fast Technology for Analysis of nucleic acids

GLM: Generalized linear model

GLMM: Generalized linear mixed model

HBcAbs: Antibodies against HBV core antigens

HBV: Hepatitis B Virus

HEV: Hepatitis E Virus

IgG: Immunoglobulin G

IgM: Immunoglobulin M

ILO: International Labor Organization

IUCN: International Union for Conservation of Nature

JCR: Journal Citation Reports

LEK: Local ecological knowledge

LMIC: Low- and medium-income countries

MINSA: Ministerio de Salud de Perú

NE: Neotropical echinococcosis

PBS: Phosphate-buffered saline

PCR: Polymerase Chain Reaction

PCVs: Porcine Circoviruses

PNR: Pucacuro National Reserve

PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis

RNA: Ribonucleic acid

SARS: Severe Acute Respiratory Syndrome

SARS-COV-2: Severe Acute Respiratory Syndrome Coronavirus 2

SDR: Sustainable Development Reserves

SVDV: Swine Vesicular Disease Virus

TNS: Total Number of Studies

WLP: White Lipped Peccary

YMR: Yavarí-Mirín River

Abstract

The global rise of emerging infectious diseases (EIDs) is closely tied to intensified human activities that increase contact between people, domestic animals, and wildlife, creating conditions that facilitate pathogen transmission. Human-driven changes such as biodiversity loss and subsistence hunting elevate the risk of EIDs outbreaks, particularly in biodiverse areas like the Amazon region. In the Northern Peruvian Amazon, wild meat—especially from ungulates like the white-lipped peccary (WLP)—is a vital resource for traditional rural communities. However, repeated disappearances of WLP populations have forced communities to seek alternative prey. These dynamics call for a transdisciplinary One Health approach that integrates ecological, epidemiological, and sociocultural research to address disease risks in complex social-ecological systems. The main objective of the present thesis was to improve the knowledge of health risks affecting both humans and wildlife in an Amazonian community by exploring pathogen circulation and zoonotic transmission.

In Study I, pathogens circulating among peccary populations were documented through a quantitative literature review to enhance understanding of the infectious diseases affecting this key species and to assess potential health threats. We found that information on the health status of peccaries in the Amazon is limited, geographically uneven, and primarily based on cross-sectional studies. Multidisciplinary strategies that incorporate local populations and participatory sampling techniques are necessary to bridge this knowledge gap and overcome the practical challenges of working in remote locations.

In Study II, viruses relevant to suiform health were identified—specifically classical swine fever virus and Aujeszky's disease virus—which may contribute to population collapse, particularly in large WLP groups where overabundance can facilitate pathogen transmission.

Following this, we assessed the prevalence of key zoonotic pathogens. Study III reported high *Toxoplasma gondii* seropositivity across humans (83.3% IgG, 6.1% IgM), wild mammals (30.45% across 17 species), peri-

domestic rodents (10%), and domestic animals (94.1% in dogs, 100% in cats), indicating active sylvatic and domestic cycles and continuous reinfection through the consumption of raw viscera and contact with cats and dogs in households. In Study IV, HBV and HEV exposure was found in 9.1% and 17.1% of human samples, respectively. Wildlife exposure to HBV was limited to three species and no HEV was detected in wildlife or domestic animals. Given that domestic livestock is rarely consumed in the area, there is a clear need for further investigation into transmission pathways, especially through ingestion of wild meat and contaminated water.

Finally, Study V explored the transmission risk of *Echinococcus vogeli* by examining subsistence hunting and wildlife management practices in Amazonian communities. Human behaviors, such as handling wild meat and feeding infected organs to dogs, were identified as key factors in transmission. Preventive strategies focused on safe wildlife handling and improved sanitation of domestic animals are essential to reduce the risk of *E. vogeli* infection and other zoonoses linked to the wild meat chain.

Taken together, the evidence that peccaries in the Amazon are exposed to viruses affecting swine health suggests that diseases may be a contributing factor in the recurring disappearance of WLP. The detection of pathogens across a wide range of wild, peri-domestic, and domestic animal species reflects the complexity of transmission cycles shaped by human activity and suggests that shifts in prey species may have serious implications for food security and local livelihoods. These findings underscore the urgent need for health surveillance systems in tropical forests that are both practical and culturally appropriate, as well as the importance of sustained monitoring in these remote regions.

Resumen

El aumento global de enfermedades infecciosas emergentes está estrechamente relacionado con la intensificación de las actividades humanas, que incrementan el contacto entre personas, animales domésticos y silvestres, facilitando la transmisión de patógenos. Factores influenciados por la actividad humana como la pérdida de biodiversidad y la caza de subsistencia elevan el riesgo de brotes epidemiológicos, especialmente en regiones biodiversas como la Amazonía. En la Amazonía norte peruana, la carne silvestre, en particular la del pecarí de labios blancos, representa un recurso esencial para comunidades rurales. Sin embargo, el declive poblacional de esta especie ha obligado a las comunidades a buscar presas alternativas. Estas dinámicas requieren un enfoque transdisciplinario de Una Sola Salud, combinando investigación ecológica, epidemiológica y sociocultural para abordar los riesgos sanitarios. Esta tesis buscó ampliar el conocimiento sobre los riesgos para la salud humana y fauna silvestre en una comunidad amazónica mediante el análisis de la circulación de patógenos.

En el Estudio I se documentaron patógenos que circulan en pecaríes mediante una revisión cuantitativa de la literatura para evaluar enfermedades infecciosas como posibles amenazas para la salud de esta especie. Encontramos que la información sobre el estado sanitario de pecaríes en la Amazonía es limitada, geográficamente desigual y basada principalmente en estudios transversales. Se necesitan estrategias multidisciplinarias y técnicas de muestreo participativo en las comunidades locales para superar esta brecha de conocimiento y los desafíos de trabajo en zonas remotas.

En el Estudio II, se identificaron virus relevantes para la salud de los suidos —específicamente, el virus de la peste porcina clásica y el virus de la enfermedad de Aujeszky— que podrían contribuir al colapso poblacional, especialmente en grandes grupos de pecaríes de labios blancos, donde la sobreabundancia facilitaría la transmisión de patógenos.

A continuación, se evaluó la prevalencia de patógenos zoonóticos. En el Capítulo III se reportó una alta seropositividad a *T. gondii* en humanos (83.3% IgG, 6.1% IgM), mamíferos silvestres (30.45% en 17 especies), roedores peridomésticos (10%) y animales domésticos (94.1% en perros, 100% en gatos), lo que indica ciclos silvestres y domésticos activos, con reinfección por consumo de carne y contacto con animales domésticos. En el Estudio IV, se encontró exposición al VHB y al VHE en el 9.1% y el 17.1% en humanos, respectivamente. La exposición de la fauna silvestre al VHB se limitó a tres especies y no se detectó VHE en animales silvestres. Debido al consumo limitado de ganado doméstico en la región, resulta necesario investigar a profundidad las vías de transmisión de estos patógenos a través del consumo de carne silvestre y agua contaminada.

En el Estudio V se analizó el riesgo de transmisión de *E. vogeli*, centrándose en la caza de subsistencia y prácticas de manejo de fauna en comunidades amazónicas. La manipulación de carne silvestre y alimentación de perros con órganos infectados se identificaron como principales factores de riesgo. Por tanto, estrategias preventivas enfocadas en el manejo de fauna silvestre y control sanitario de animales domésticos son fundamentales para reducir el riesgo de infección por *E. vogeli* y otras zoonosis asociadas al consumo de carne silvestre.

En conjunto, la evidencia de exposición de pecaríes a virus que afectan la salud porcina sugiere que las enfermedades podrían contribuir a la desaparición recurrente del pecarí de labios blancos. La detección de patógenos en especies silvestres refleja la complejidad de ciclos de transmisión influenciados por la actividad humana y apunta a que el consumo de presas alternatives podría tener consecuencias para la seguridad alimentaria. Estos hallazgos enfatizan la necesidad de sistemas de vigilancia sanitaria que sean culturalmente adecuados para los contextos de los bosques tropicales, así como la importancia del monitoreo continuo en regiones remotas.

Resum

L'augment global de malalties infeccioses emergents està lligat a la intensificació de les activitats humanes que incrementen el contacte entre persones, animals domèstics i salvatges, facilitant la transmissió de patògens. Factors influenciats per l'activitat humana, com la pèrdua de biodiversitat i la caça augmenten el risc de brots epidèmics, especialment a regions amb una alta biodiversitat. A l'Amazònia nord del Perú, la carn de caça, en particular la del pecarí de llavis blancs, representa un recurs essencial per a les comunitats rurals. No obstant això, el declivi poblacional d'aquesta espècie ha obligat a les comunitats a buscar preses alternatives. Aquestes dinàmiques requereixen un enfocament Una Sola Salut, integrant recerca ecològica, epidemiològica i sociocultural per afrontar els riscos sanitaris. Aquesta tesi va tenir com a objectiu millorar el coneixement sobre els riscos per a la salut humana i la fauna salvatge en una comunitat amazònica, mitjançant l'anàlisi de la circulació de patògens i la transmissió zoonòtica.

A l'Estudi I, es van documentar, mitjançant una revisió quantitativa de la literatura, els patògens que afecten les poblacions de pecarís per avaluar les malalties infeccioses com a possibles amenaces per a la seva salut. Vam trobar que la informació sobre l'estat sanitari dels pecarís a l'Amazònia és limitada, geogràficament desigual i basada principalment en estudis transversals. Calen estratègies multidisciplinàries i tècniques participatives amb les comunitats locals per superar aquesta bretxa de coneixement i las dificultats al treballar en zones remotes.

A l'Estudi II, es van identificar virus rellevants per a la salut dels suids —específicament, el virus de la pesta porcina clàssica i el virus de d'Aujeszky— que podrien contribuir al col·lapse poblacional, especialment en grups nombrosos de pecarís de llavis blancs, on una elevada densitat podria afavorir la transmissió de patògens.

Seguidament, es va analitzar la presència de patògens zoonòtics. A l'Estudi III es va trobar una alta seropositivitat a *T. gondii* en humans (83,3% IgG, 6,1% IgM), mamífers silvestres (30,45% en 17 espècies),

animals peridomèstics (10%) i domèstics (94,1% en gossos, 100% en gats), cosa que indica cicles selvàtic i domèstic actius i reinfecció pel consum de carn i el contacte amb animals domèstics. A l'Estudi IV, es va detectar exposició al VHB i al VHE en el 9,1% i el 17,1% en humans, respectivament. L'exposició al VHB es va limitar a tres espècies de fauna salvatge, i no es va detectar VHE en cap d'elles. Atès el consum limitat de bestiar de ramaderia a la regió, cal investigar a fons les vies de transmissió d'aquests patògens a través de la carn de caça i l'aigua contaminada.

A l'Estudi V es va analitzar el risc de transmissió d'*E. vogeli,* en relació amb la caça de subsistència i la gestió de fauna en comunitats amazòniques. La manipulació de carn de caça i l'alimentació de gossos amb òrgans infectats es van identificar com a factors clau. Per tant, les estratègies preventives centrades en la manipulació de fauna salvatge i el control sanitari dels animals domèstics són fonamentals per reduir el risc d'infecció per *E. vogeli* i altres zoonosis associades al consum de carn de caça.

L'exposició dels pecarís a virus que afecten la salut porcina suggereix que les malalties podrien contribuir a la desaparició recurrent del pecarí de llavis blancs. La detecció de patògens en espècies salvatges reflecteix la complexitat dels cicles de transmissió influenciats per l'activitat humana i indica que el consum de preses alternatives podria tenir conseqüències per a la seguretat alimentària. Aquests resultats subratllen la necessitat de sistemes de vigilància sanitària adaptats culturalment als contextos dels boscos tropicals, així com la importància de fer-ne un seguiment continuat en aquestes regions remotes.

Justification

Concerns about emerging infectious diseases (EIDs) have increased in recent decades (Jones *et al.*, 2008), particularly after the alleged connection between the SARS-CoV-2 virus and its spread from wildlife (Roe & Lee, 2021). The COVID-19 pandemic demonstrated how quickly infectious diseases can disrupt the world, bringing wide-ranging changes to daily life and highlighting the need for proactive and preventive measures (Canavan, 2023; Nieves & Chellappoo, 2022). Various pathogens, including zoonotic ones, have emerged in recent years (Reperant & Osterhaus, 2017), impacting both human and animal health, and generating significant economic costs. Despite substantial technological improvements over the last decades, pandemic forecasting and early warning prediction remain major challenges.

In response, the One Health concept has emerged as a transdisciplinary public health approach that recognizes the interconnectedness of human, animal, plant, and environmental health (Evans & Leighton, 2014). The human-animal-ecosystem health framework needs to be integrated to achieve a comprehensive approach. This integration is essential for managing exposure to pathogens through practical, sustainable, and culturally sensitive measures (Aguirre *et al.*, 2019).

The Amazon rainforest exemplifies the importance of this integrated framework due to its high biological and cultural diversity (Pimm *et al.*, 2014), which includes a vast array of vertebrate species, numerous indigenous communities, and various emerging and re-emerging infectious diseases, including malaria, Chagas disease, leishmaniasis, Yellow Fever and Oropuche. The unique interactions between humans, wildlife, and the environment in the Amazon make the One Health approach crucial for effectively understanding and managing health challenges in the region (Wilke *et al.*, 2025).

However, this region presents a substantial gap in our understanding of infectious diseases, largely due to fragile health systems and the logistical difficulties of collecting high-quality biological samples in remote areas. In consequence, despite a few scientific reports (Aston *et al.*, 2014; Dubey, 2010; Ferreiro *et al.,* 2021), there is still a lack of knowledge about the impact of infectious diseases on both wildlife (Fragoso *et al.*, 2022) and humans (MINSA, 2010), contributing to their status as neglected diseases.

Amazonian societies depend on subsistence hunting as a primary source of protein and family income (Bodmer & Pezo, 2001; Mayor *et al.*, 2022). In this context, peccaries are the most frequently hunted animal group, making up a significant portion of the harvest in the Peruvian Amazon and holding immense socioeconomic importance (Peres, 2000). However, since the 1980s, repeated episodes of white-lipped peccaries (WLP) disappearances have been documented throughout the Amazon. Although the cause remains unknown, these events are suspected to be multifactorial, with infectious diseases likely playing a significant role (Fragoso *et al.*, 2022).

The health status of Amazonian wildlife, particularly threatened species such as WLP, listed as Vulnerable by the IUCN (IUCN, 2023), remains largely unknown (Fragoso *et al.*, 2022). The increasing pig production, leading to interactions between domestic pigs *(Sus scrofa domesticus*) and peccaries (Hohnwald *et al.*, 2019; Serrao *et al.*, 1996), combined with limited data on infectious agents in suiforms, emphasizes the urgent need for thorough investigation into infectious diseases affecting these species in the Amazon.

The disappearance of peccaries could impact food security and prompt a shift in hunting pressure toward other available prey, increasing the risk of zoonotic disease transmission through contact with different species. Similar to what is observed in wild species, epidemiological data on food-borne and hunting-related diseases in the Amazon remain limited (Mayor & Bodmer, 2009).

Amazonian societies face severe health challenges, including limited access to medical facilities, a shortage of healthcare personnel, and inadequate infrastructure, challenges worsened by poverty and systemic inequalities (Mayor *et al.*, 2022; MINSA, 2010; WHO, 2020). These dynamics underscore the importance of adopting a One Health approach to strengthen prevention, detection, and response measures that address the interconnected aspects of the animal–human–environment interface.

This thesis investigates infectious diseases in an indigenous territory, focusing on various fauna species that interact with Amazonian societies, through a One Health perspective —a subject that has been overlooked in comprehensive studies. The geographic isolation of the study area, coupled with restricted human movements, provides a unique opportunity to examine a scenario characterized by minimal anthropogenic interactions. This setting could improve our comprehension of pathogen ecology and transmission dynamics to help assess the risk of human infection at the human-wildlife interface in well-preserved tropical forests.

For this purpose, this PhD thesis is structured into five studies:

- Study I: *Infectious diseases of interest for the conservation of peccaries in the Amazon: A systematic quantitative review*. In response to the recurrent disappearances observed among free-ranging WLP populations in the Amazon, this study conducts a quantitative literature review focusing on infectious diseases potentially affecting this Suiform species in the Amazon.
- Study II: *Monitoring of selected swine viral diseases in Peruvian Amazon peccaries*. This study estimates the prevalence of key swine pathogens — classical swine fever virus (CSFV), Aujeszky's disease virus (ADV), swine vesicular disease virus (SVDV), and porcine circoviruses (PCVs)— in free-ranging peccaries in two areas of the northeastern Peruvian Amazon. Since WLPs were not easily found during population declines and considering their susceptibility to similar pathogens, the collared peccary (CP) was also sampled as an indicator of virus circulation in the study areas.
- Study III: *Toxoplasma gondii in a Remote Subsistence Hunting-Based Indigenous Community* & Study IV: *A Survey of Hepatitis B Virus and Hepatitis E Virus at the Human–Wildlife Interface in the*

Peruvian Amazon of the Peruvian Amazon. These studies examine the prevalence of *Toxoplasma gondii*, Hepatitis B Virus and Hepatitis E Virus in coexisting humans, hunted wild mammals, and domestic mammals. Both studies adopt a One Health approach, involving surveys, hunting records, and field observations to document behaviors that facilitate or hinder pathogen transmission between animals and humans.

- Study V: *Risk Factors for Wildlife-Transmitted Diseases in Communities Engaged in Wildlife Consumption– A Case Study on Polycystic Echinococcosis*. This study explores the risk of *Echinococcus vogeli*'s transmission based on surveys in urban and rural societies from South America, particularly in the Amazon. It highlights how subsistence hunting, wild meat consumption, and domestic animal ownership contribute to the spread of neglected tropical diseases.

Finally, this thesis concludes with a comprehensive theoretical discussion offering a broad perspective on the situation. The general conclusions summarize the key points and implications of the research, providing a thorough understanding of infectious diseases at the Amazon's human-wildlife interface through a One Health lens.

1. INTRODUCTION

1.1. Emerging infectious diseases (EIDs)

Emerging infectious diseases (EIDs) are infections that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range. The concept of "emergence" is multifaceted, encompassing a range of phenomena such as the first instance of natural infection, expansion to new host species, spread into previously unaffected regions, reappearance after control or eradication, a rise in incidence, the development of drug resistance, or increased virulence (Karesh *et al.,* 2012). Therefore, the emergence of diseases, whether recent or historical, is often a consequence of pathogen ecology and evolution, as microbes exploit new niches and adapt to new hosts. These dynamics underline the complexity of EIDs and their growing relevance in global health, particularly in the context of intensified human–animal–environment interactions.

The emergence and global spread of infectious diseases have increased in recent decades and are expected to continue. Factors driving EIDs are primarily related to intensified anthropogenic activities such as agriculture, farming, urbanization, human and animals' movement, biodiversity loss, environmental degradation, and climate change (Baker *et al.*, 2022; Epstein, 2001). These factors increase interactions between humans and animals, thereby facilitating the transmission of pathogens in both directions (Evan & Leighton, 2014; Karesh *et al.*, 2012). Interactions between wildlife, domestic animals, and humans have been key for the emergence and dissemination of infectious diseases worldwide. These increased interactions disrupt the balance among hosts, pathogens, and the environment, resulting in disease outbreaks, increased incidence, and the geographical spread of pathogens (Figure 1.1.1) (Daszak *et al.*, 2001).

The increasing demand for food due to a growing global population has also increased the transmission of food-borne zoonoses, as many domesticated and wild vertebrates and invertebrates consumed by people can harbor zoonotic bacteria, viruses, or parasites (Delgado *et al.,* 1999; Karesh *et al.*, 2012). In that sense, intensified farming, especially with high-density animal populations, creates environmental conditions conducive to pathogen amplification and transmission (Baker *et al.,* 2022). Furthermore, changes in the abundance of animal hosts can significantly influence disease incidence in people and animals. For example, a decline in a primary host species can cause an arthropod vector to shift feeding patterns to alternative hosts, potentially triggering disease outbreaks (Karesh *et al.,* 2012; Lawler *et al.*, 2021).

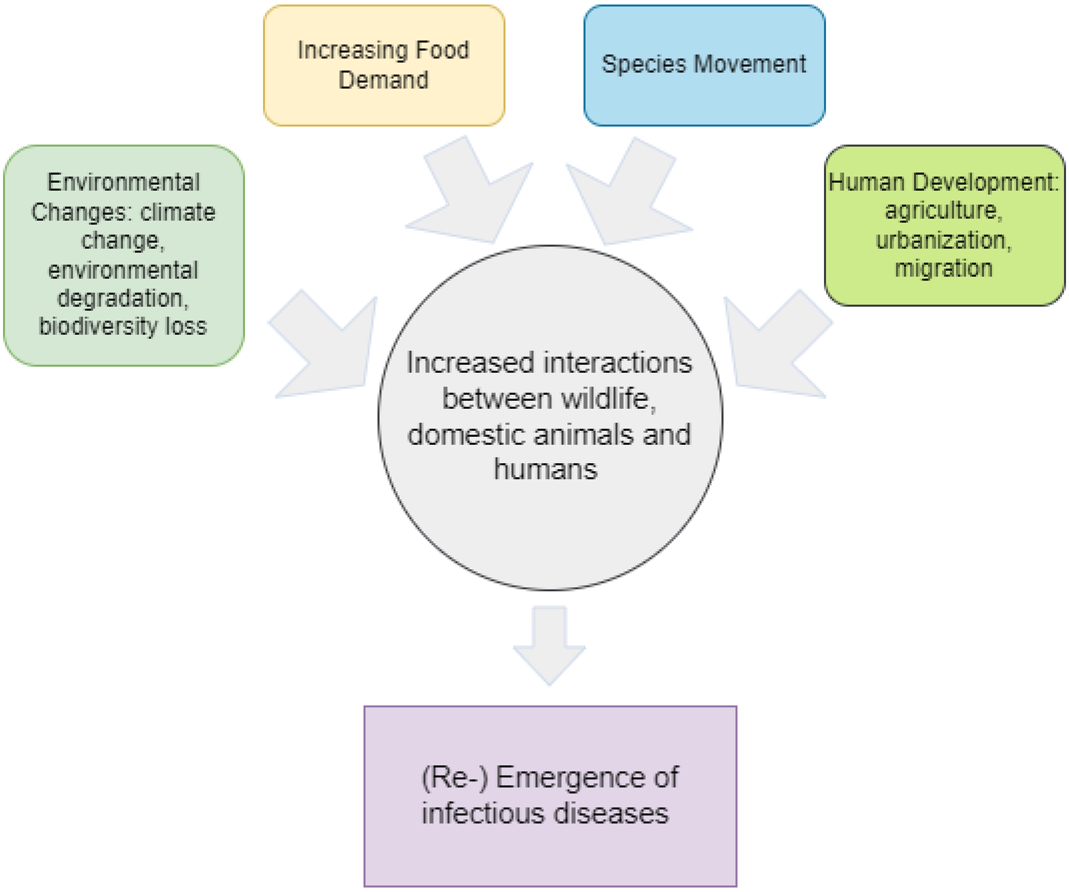

Figure 1.1.1. Diagram showing how global anthropogenic changes favor the (re)-emergence of infectious diseases. Adapted from: Destoumieux-Garzón *et al.* (2018).

However, the ability to predict epidemics is still constrained. Although there has been significant progress in understanding the drivers of disease emergence, more progress has yet to be made to effectively address pathogens that represent a serious threat to human, animal, and ecosystem health (Cunningham *et al.*, 2017; Daszak *et al.*, 2000).

1.1.1 Impact of EIDs on biodiversity

While infectious diseases have traditionally been considered natural processes affecting wildlife, it is now widely recognized that anthropogenic activities are accelerating the emergence and spread of these diseases, posing a significant threat to biodiversity (Cunningham *et al.*, 2017). Numerous pathogens can infect susceptible wild hosts, sometimes leading to severe population declines or even contributing to extinction events (Daszak, 2000).

Two major drivers of wildlife EIDs stem from human activities. First, pathogen spillover from domestic animals to wildlife can occur when contact enables the transmission of infectious agents, pushing vulnerable wildlife populations toward decline, as observed with rabies and canine distemper in wild carnivores (Acosta-Jamett et al., 2024). Second, the movement of pathogens due to global trade has facilitated the spread of diseases into novel environments through a process known as pathogen pollution. This phenomenon has introduced novel pathogens such as West Nile virus and crayfish plague into naïve environments, where they have caused significant ecological and public health damage (Cunningham et al., 2017; Daszak *et al.*, 2000). In addition, human-induced environmental changes, including habitat destruction and fragmentation, can disrupt ecological balance and alter host-pathogen interactions, increasing the risk of pathogen transmission between humans, domestic animals, and wildlife (Wilkinson *et al.*, 2018). Despite the growing recognition of these risks, significant knowledge gaps remain in understanding how these environmental changes shape disease dynamics (Cunningham *et al.*, 2017, Al Noman *et al.*, 2024).

Several examples illustrate the profound effects of EIDs on wildlife: the decimation of bird populations due to avian malaria, distemper outbreaks in marine mammals, the chytrid fungus responsible for global amphibian declines, and white-nose syndrome, which has devastated some bat populations (McCallum *et al.,* 2024). These cases underscore the urgent need to integrate wildlife health into conservation strategies, as understanding and mitigating disease impacts is essential for protecting biodiversity.

1.1.2 Zoonoses

Rudolf Virchow introduced the concept of "zoonoses" in 1855. He recognized that most human diseases originated from domestic animals, highlighting the connection between human and veterinary medicine. Virchow also argued that disease should be understood as a collective, societal phenomenon, wherein individual health is deeply connected with the health of the broader population (Nieves & Chellappoo, 2022). Since the 1950s, the emergence of zoonoses has accelerated, driven by various anthropogenic factors mentioned in previous chapters (Canavan, 2023).

Zoonotic diseases illustrate the interplay between biological and social phenomena. For example, the HIV-AIDS pandemic, which originated from chimpanzees in Central Africa, underscores the complex interactions between ecological, virological, and social factors of the emergence of infectious diseases (Canavan, 2023; Pepin, 2021).

Pathogens originating in wildlife must overcome various biological and ecological barriers before establishing themselves in human populations (Figure 1.1.2). Human activities significantly influence this process that largely depends on the regional diversity and prevalence of wildlife pathogens and the frequency of human and domestic animal contact with wildlife reservoirs of potential zoonoses (Plowright *et al.*, 2017; Wolfe *et al.*, 2005, 2007).

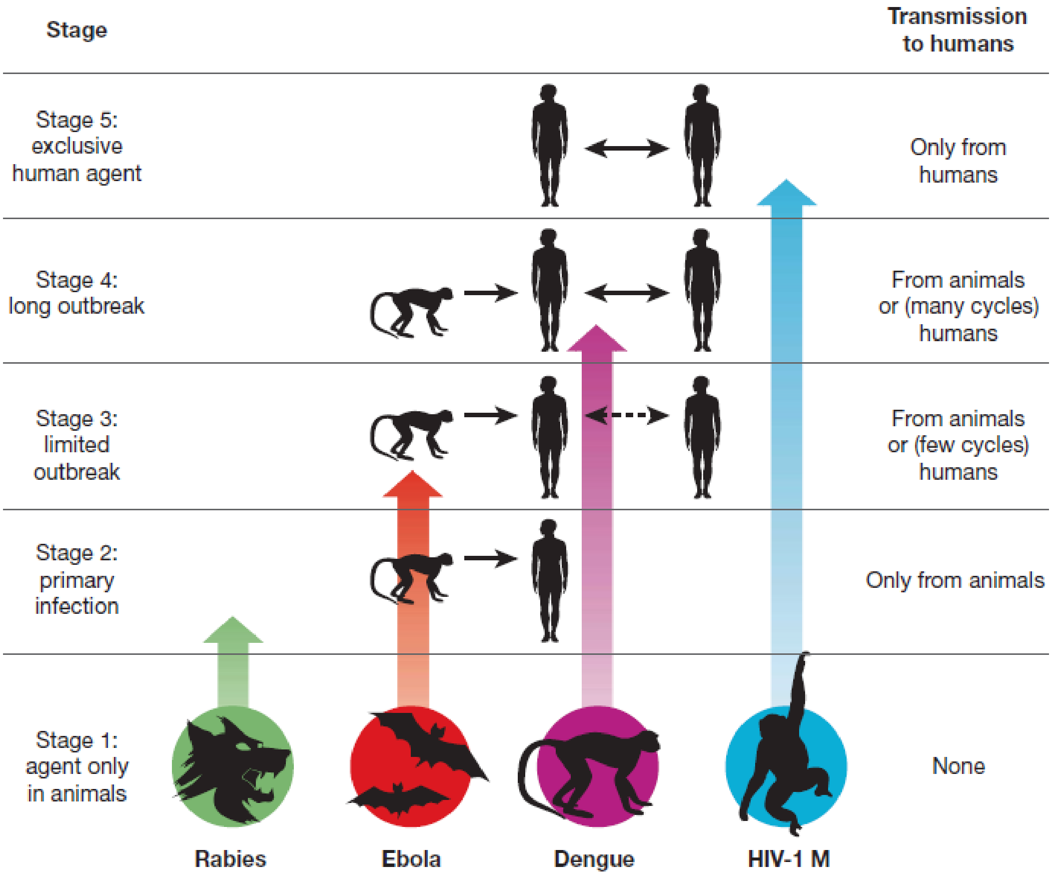

Figure 1.1.2. Diagram of the stages in the emergence of zoonotic diseases. Stage 1 (S1) involves pathogens that are exclusive to animals. In S2, the pathogen can infect humans but cannot be transmitted from human to human. S3 is marked by limited human-to-human transmission. burning out quickly. In S4, the pathogen becomes more adapted to humans, leading to sustained outbreaks and more efficient human-to-human transmission, although it may still require an animal reservoir. S5 represents the establishment of the pathogen as a specialized human disease, no longer reliant on animal hosts and capable of maintaining itself within human populations alone (Source: Wolfe *et al.*, 2007).

Once pathogens cross the species barrier, some may evolve to sustain human-to-human transmission, potentially resulting in epidemics or pandemics (Canavan,2023; Karesh *et al.,* 2012). Some diseases originated from animals, such as HIV, have become established human pathogens that now circulate independently of their animal origin. In contrast, other diseases, such as Ebola virus and Nipah virus, have not become established in human populations. This is largely due to effective local containment efforts or their limited capacity for sustained human-to-human transmission (Karesh *et al.*, 2012).

Endemic zoonoses, which are persistent regional health problems worldwide, cause the greatest impact on human health and livelihoods, resulting in roughly one billion cases of illness and millions of deaths each year (Karesh et al., 2012). However, zoonoses pose health concerns worldwide, not just in low-income countries. With the rapid expansion of global trade and travel, zoonotic diseases can spread more readily, affecting both developed and developing countries. Despite advances in understanding the underlying drivers, limited progress has been made in addressing the emergence of zoonotic pathogens from wildlife to humans (Cunningham *et al.*, 2017). Strengthening research and prevention strategies is crucial for mitigating these risks and safeguarding public health.

1.1.3 Zoonotic Spillover

In the context of public health, the term "spillover" refers to the cross-species transmission of pathogens (Lloyd-Smith *et al.*, 2009), specifically referring to Stage 2 in the Figure 1.1.2. Spillover risk is influenced by several key factors:

a) The prevalence of infection in the reservoir host.
b) The rate of human-animal contact.
c) The probability of infection upon contact.

However, not all spillover events result in the emergence of new diseases or outbreaks. For a pathogen to successfully establish and spread in human populations, biological, social, and environmental conditions must align to support replication within the human host and favor transmission (Figure 1.1.2: Stages 3, 4 and 5). Therefore, the dynamics of zoonotic diseases involve multiple stages, including transmission within the animal reservoir, spillover to humans, and potentially stuttering or sustained transmission among humans (Lloyd-Smith *et al.*, 2009; Wolfe *et al.*, 2007). Because spillover events require a convergence of specific circumstances, they remain rare. However, certain factors can increase the density of humans, vectors, and reservoir hosts, thereby enhancing contact among these groups and facilitating the emergence and spillover of zoonotic diseases (Plowright *et al.*, 2017). Key factors that promote spillovers include:

1. Close contact with domestic animals (e.g., pets transmitting rabies; Chomel, 2014) or wild species (e.g., through bushmeat consumption or hunting) can facilitate transmission of pathogens such as HIV, Nipah, Ebola, and Hendra (Hahn *et al.*, 2000; Wolfe *et al.*, 2005). Occupational exposure, especially in poorly regulated environments, also heightens transmission risk among individuals working with livestock like poultry, swine, or cattle (Klous *et al.*, 2016).
2. Phylogenetic distance: Spillover is more likely between phylogenetically similar species (Han *et al.*, 2016). For instance, transmission is more probable between non-human primates and humans than between humans and reptiles. However, with sufficient adaptation, interactions can still facilitate spillovers between evolutionary distant species (Ellwanger & Chies, 2021).
3. Type of pathogen: Generalist pathogens, particularly RNA viruses, which have high mutation rates, are more likely to cross species barriers and cause outbreaks (Johnson *et al.*, 2015). Following spillover, pathogens that transmit through direct contact, cause chronic infections, or infect the respiratory tract are more likely to spread within a population (Geoghegan *et al.*, 2016).
4. Biodiversity loss: Highly biodiverse environments can reduce zoonotic spillover risk through the "dilution effect", where a greater variety of host species lowers pathogen prevalence in key reservoir hosts (Civitello *et al.*, 2015). Thus, loss of biodiversity may increase the probability for pathogens reaching and spreading among humans (Gibb *et al.*, 2020; Ostfeld & Keesing, 2017).
5. Social and economic factors: Land-use changes, such as deforestation and agriculture, disrupt ecosystems and increase human-wildlife interactions, thereby elevating zoonotic risk (Daszak *et al.*, 2007; Han *et al.*, 2016). For example, Amazonia plays a key role in climate regulation, and deforestation in this region raises temperatures and exacerbates extreme weather.

These changes, along with deforestation-related activities, facilitate the spread of disease vectors and drive the emergence of infectious diseases (Ellwanger *et al.*, 2020).

6. Climate change: Global climate shifts —including warming oceans, increased atmospheric vapors, ice melt, and extreme events like prolonged droughts or heavy rainfall— can alter species abundance and distribution and might impact infectious diseases cycles (Epstein, 2011; Pfenning-Butterworth *et al.,* 2024). For instance, certain pathogens and their vectors, particularly mosquitoes, expand their geographic and host range in response to increased temperature and precipitation (Bengis & Frean, 2014).
7. Human and animal movements: Another significant factor contributing to disease emergence is the relocation of wild animals from their natural habitats, as seen with Monkeypox in the United States due to the importation of rodents from Africa (Daszak & Jones, 2008).

Understanding the complexity of zoonotic spillovers is challenging due to their stochastic nature and limited empirical data. Examining the biological and environmental barriers that usually either inhibit or permit pathogen transmission is crucial. This knowledge can guide strategies to decrease pathogen spillover from wildlife to humans, thereby helping prevent future disease outbreaks, epidemics, and pandemics (Ellwanger & Bogo, 2021).

1.1.4 The One Health Initiative

As mentioned previously, EIDs pose a complex threat to biodiversity, human health, and well-being. Historically, some perspectives focused mainly on EIDs as a human health issue, while others emphasized the equal importance of animal health and ecosystem sustainability. Today, the interconnected nature of these challenges—often called "wicked problems"—requires a transdisciplinary and integrated approach (Evans & Leighton, 2014).

The current global health landscape is marked by complexity, interconnectedness, and the convergence of various factors, such as globalized disease spread, food insecurity, population growth, evolving animal production systems, and climate change. These realities emphasize the need to focus more on the connections between ecosystem, animal, and human health (Evans & Leighton, 2014).

The One Health concept represents a reconceptualization of health management, driven by the exponential environmental changes and population growth of the past century. It emphasizes the interconnectedness of human, animal, and ecosystem health and the need for integrated, collaborative efforts to address global health challenges. This transdisciplinary approach has been endorsed by global institutions such as the World Health Organization (WHO), particularly in addressing food safety, zoonosis control, and antibiotic resistance (Nieves & Chellappoo, 2022).

The idea of interconnected health is not new. Hippocrates (460 BCE–367 BCE) alluded to such connections, and many ancient civilizations and modern indigenous peoples have long recognized the interdependence of humans, animals, land, and water. Archaeology and anthropology have shown that infectious diseases emerging in human populations from animal sources —often triggered by environmental changes— have occurred repeatedly throughout history (Evans & Leighton, 2014).

In recent history, major international infectious disease events such as Zika, Ebola, SARS, and MERS have fueled the shift towards the One Health perspective, underscoring the urgent need for new global health management approaches (Evans & Leighton). By 2005, the avian flu crisis among wild birds and chickens significantly accelerated interest in the One Health approach (Woods *et al.*, 2017). Most notably, the COVID-19 pandemic starkly revealed how swiftly infectious diseases can disrupt societies worldwide, making emerging diseases a primary focus of current research and highlighting the need for proactive and preventive measures.

Implementing the One Health approach requires a deep understanding of the complex ecology of zoonotic diseases at the human-animal-environmental interface. It demands collaboration across fields such as animal and human medicine, ecology, sociology, microbial ecology, evolution, and probably all (at least most) Earth, Health, and Social Sciences. From the perspective of an interdependent world, this holistic vision postulates that these objectives are also interdependent and should be pursued as a unified objective (Cardinale *et al.*, 2012) (Figure 1.1.3).

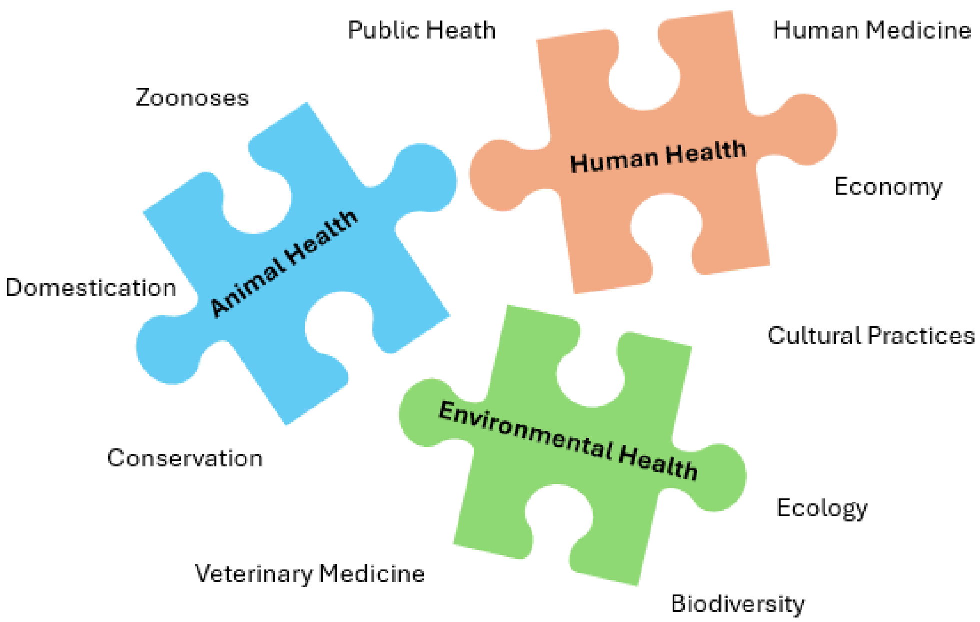

Figure 1.1.3. The holistic, transdisciplinary, and multisectoral approach of the One Health concept. Adapted from: Destoumieux-Garzón *et al.* (2018).

Effective solutions require a comprehensive approach that manages human exposure to animals carrying zoonotic pathogens with practical, sustainable, and culturally sensitive measures (Aguirre *et al.*, 2019). This includes ensuring the health of domestic animals through appropriate healthcare practices, vaccination protocols, and prophylactic antiparasitic treatments. It also involves addressing the health of peri-domestic animals and wildlife by implementing mitigation measures such as restricting wildlife access to pathogen sources like production facilities and landfills (Gamble *et al.*, 2023). Therefore, a robust One Health approach must also incorporate ethnographic, anthropological, and sociological research to understand how social and cultural factors shape human-animal interactions, as these behaviors may either hinder or facilitate pathogen transmission (Canavan, 2023). Ultimately, this comprehensive approach aims to manage health proactively, anticipating and mitigating risks before they escalate into broader public health threats.

1.2 The Amazon Region

The Amazon region is the world's largest expanse of tropical forest, boasting high levels of both biological and cultural diversity (Pimm *et al.*, 2014). It occupies the largest hydrographical basin on the planet, containing approximately 20% of the world's liquid freshwater and covering 44% of the South American subcontinent (Aguilar *et al.*, 2007). Over 30 million people reside in the Amazon region, most of whom live in traditional, predominantly rural societies (Cunha & De Almeida, 2000). These rural communities are often geographically isolated, with distinct languages, cultures, and institutions often differing from mainstream society (Sarivaara *et al.*, 2013). They inhabit ecosystems characterized by low anthropogenic impact and high biodiversity and rely on hunting and fishing for subsistence (Aston *et al.*, 2014; Mayor *et al.*, 2022). In these areas, wild meat is a critical source of protein and income due to the scarcity of domestic meat in tropical forest areas (El Bizri *et al.*, 2020a; Mayor *et al.*, 2022).

The recognition of hunting as a key factor in traditional livelihoods is enshrined in the universal definition of subsistence economy and traditional activities in Convention 169 of the International Labor Organization (ILO 1989). Moreover, traditional and indigenous people in Latin America have the right to participate in decisions regarding the use, management, and conservation of natural resources, integrating local knowledge and experiences (Berkes *et al.* 2000). This framework grants inhabitants of Sustainable Development Reserves the right to use natural resources and engage in management decisions.

In tropical forests, hunting typically involves the use of bows and arrows or shotguns, although snares and dogs are also commonly employed. After shooting an animal, hunters may either wait to see if it dies or track it down to finish the kill. Larger preys are preliminary butchered in the forest, where their entrails are removed and discarded on the forest floor or in rivers. The main butchering, usually performed by the hunter, takes place at a camp near a water source, using *machetes* as the primary tool and usually without safety equipment. Animals weighing less than 30 kg are transported to the village, where women begin preparations for further butchering and cooking (Milstein *et al.*, 2020; Van Vliet *et al.*, 2022).

In the Amazon region, mammals are the most commonly hunted animals, followed by reptiles and birds. Among mammals, ungulates are the primary source of meat due to their significant biomass contribution (Bodmer & Pezo, 2001; El Bizri *et al.*, 2020b). The most hunted species include the white-lipped peccary (*Tayassu pecari*), the collared peccary (*Pecari tajacu*), the red brocket deer (*Mazama americana*), the tapirs (*Tapirus terrestris*), and the lowland pacas (*Cuniculus paca*), all of which represent the largest biomass extracted (Mesquita & Barreto, 2015; El Bizri *et al.*, 2020b). These species are highly sought after and valued for their taste and size.

This thesis specifically focuses on the northeastern Peruvian Amazon, one of the most biologically diverse regions in the Amazon Basin. This area, largely represented by the Department of Loreto, hosts the highest diversity in the Amazon, with particularly rich communities of primates and amphibians. It is also home to Yagua Indigenous communities, some of whom live in voluntary isolation (Aston *et al.,* 2014).

In this region, subsistence hunting is regulated across community conservation reserves, nationally co-managed reserves, and Indigenous territories. Management strategies include harvest limits for non-vulnerable species, hunting bans on vulnerable species and wildlife habitat conservation (Bodmer *et al.*, 2023).

Additionally, pelts from certain subsistence-hunted species can be legally exported for commercial profit, as long as they are not listed in

Appendix I of the Convention on International Trade in Endangered Species. The State plays a key role in managing wildlife and oversees a pilot certification program for peccary pelts in Loreto (Fang *et al.*, 2018; Bodmer & Pezo, 2001). Despite the presence of major urban wild meat markets in Loreto (Mayor *et al.*, 2021), the region remains relatively remote, with high mammal diversity, low human population density, and thus, comparatively low hunting pressure (Mayor *et al.*, 2015).

1.2.1 Peccaries

Peccaries (Cetartiodactyla: Tayassuidae) are omnivorous ungulates that primarily feed on fruits and nuts. They belong to the suborder Suiformes and are therefore related to pigs (Grubb, 2005). There are three species of peccaries: the Chacoan peccary (*Catagonus wagneri*), the white-lipped peccary (WLP), and the collared peccary (CP). The Chacoan peccary, the largest and heaviest of the three species, measures 96-177 cm in length and weighs between 29.5 and 40 kg. It is characterized by longer dorsal pelage and elongated head, tail, and legs. The collared peccary, the smallest, measures 78.8-106 cm and weighs 30-51 kg. The white lipped peccary falls in between, measuring 90-139 cm and weighing 25-40 kg (Figure 1.2.1). All three species are native to Latin America and occur in various ecosystems and bioregions across the American continent, distributed throughout tropical forests from the Southwestern United States to northern Argentina (Figure 1.2.2) (Fragoso, 1999; Sowls, 1997).

Figure 1.2.1. Species of peccaries. a) Adult Chacoan peccary (photo by Juan M. Campos Krauer), b) Collared peccary (photo by Pedro Mayor), c) White- lipped peccary (photo by Pedro Mayor).

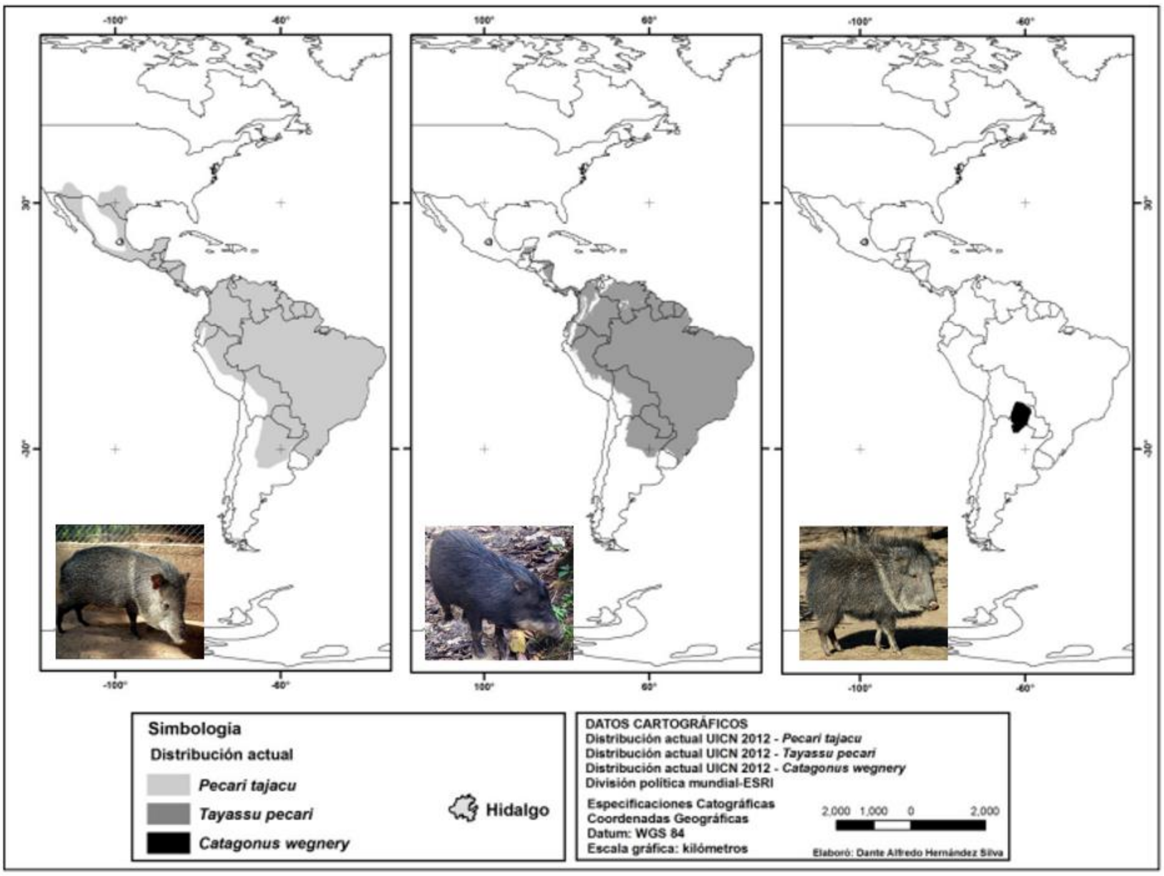

Figure 1.2.2. Distribution areas of the three species of peccaries (*Tayassu pecari*, *Pecari Tajacu* and *Catagonus wegnery*) present on the American continent. (Source: Hernández Silva, 2013; Photos: Juan M. Campos Krauer & Pedro Mayor).

Peccaries play a crucial role as ecosystem engineers, significantly shaping both abiotic and biotic communities. By digging soil, spreading seeds, and being prey for top predators, they contribute to forest regeneration and ecological balance (Peres, 2000; Ripple *et al.*, 2016; Sobral *et al.*, 2017). Their actions influence nutrient cycling, plant growth, and animal diversity. As a result, they are considered key species whose presence contributes meaningfully to the overall dynamics and resilience of ecosystems (Altrichter *et al.,* 2012). In the Amazon, two species of peccaries coexist: the WLP and CP (Fragoso *et al.*, 2016). Both species constitute a significant portion of the harvested animals in the Peruvian Amazon. In the early 2000s, the annual harvest of peccaries was estimated to be approximately 14,000 WLP and 20,000 CP individuals in rural areas of the Peruvian Amazon (Bodmer & Pezo, 2001) and 611,527 WLP and 551,949 CP individuals in the Brazilian

Amazon (Peres, 2000). Moreover, peccaries hold immense socioeconomic importance, being the most traded wild meat species in urban markets. In 2017 and 2018, WLP meat contributed an average of 79 tons annually, representing approximately 18% of the total 442 tons of wild meat traded. CP meat accounted for 152 tons, making up about 40% of the total meat traded in urban wild-meat markets in Iquitos, the largest market in the Peruvian Amazon (Mayor *et al.*, 2022). Additionally, WLP and CP hides are exported to Europe, supported by a Certification Program developed in collaboration with indigenous communities in the Peruvian Amazon. This initiative aims to increase the added value of hides obtained by communities that manage hunting sustainably, adhering to an allowed annual extraction rate (Fang *et al.,* 2008).

1.2.2 Disappearances of the White-lipped Peccary

Since the 1980s, repeated disappearances episodes of WLP populations have been observed, and numerous events have been documented throughout the Amazon, indicating a periodicity in this phenomenon (Fang *et al.,* 2008; Fragoso, 1998). The WLP population cycles typically span 20 to 30 years, characterized by a rapid population decline within 1 to 5 years, followed by a period of absence or low abundance lasting 7 to 12 years, and concluding with a slow recovery phase over approximately 20 years (Fragoso *et al.*, 2022). This phenomenon has been occurring in vast, unfragmented regions spanning millions of hectares, impacting ecosystem dynamics and jeopardizing food security for rural communities in the Amazon. In some cases, WLP populations have failed to fully recover, now persisting in a fraction of their historical range (Figure 1.2.6) (Keuroghlian *et al.,* 2013). The escalating number of local extinctions across multiple countries prompted the IUCN to elevate the species' conservation status from "Near Threatened" to "Vulnerable", designating it as "high risk of extinction in the wild" since 2012 (IUCN, 2023).

Figure 1.2.3. Locations of white-lipped peccary (*Tayassu pecari*) disappearances. Brown dots mark disappearances of small or unknown areal extent. Yellow lines mark the estimated extent for disappearances over larger regions. The 686-million ha western Amazon region, the source area for pelt and hunting data, is delineated in red. The blue line demarcates the Amazon biome. The disappearance site in Guatemala is not shown. Base map provided by the United States Geological Survey (USGS) (Source: Fragoso *et al.*, 2022).

Early studies initially attributed these disappearances to migrations (Vickers, 1991). However, this hypothesis was later discounted, as the disappearances lasted at least 10 years, an abnormally long duration for typical ungulate migrations (Fragoso, 2004). The dynamics of ungulate populations are generally influenced by factors such as weather, interspecific interactions, and food availability (Forchhammer *et al.*, 1998; Imperio *et al.,* 2012; Owen-Smith, 2000). Still, none of these factors exhibit the distinct population cycles observed in WLP (Fragoso *et al.*, 2022).

Overhunting has also been considered a contributing factor to WLP population declines (Wolfe *et al.*, 1998). While subsistence hunting is common in tropical forests, its impact on vertebrate communities in the Amazon has become more pronounced and widespread due to rapid rural population growth in some areas. However, the Peruvian Forestry and Wildlife Service establishes an annual subsistence hunting quota for peccaries in accordance with CITES II regulations, as pelts obtained from subsistence hunters are sold on the international market (Fang *et al.,* 2008). Additionally, studies indicate that sustainable hunting of species like brocket deer, peccaries, and large rodents is viable in the Peruvian Northern Amazon, and that primate populations, which are sensitive to hunting, have not shown declines due to this activity (Mayor *et al.*, 2015; Bodmer *et al.*, 2024). Currently, approximately 60% of the Northern Peruvian Amazon incorporates subsistence hunting management practices (Bodmer *et al.*, 2024).

Despite WLP and CP coexisting in the Amazon and being closely related taxonomically, CP populations have not shown similar declines (Fragoso, *et al.*, 2022). Both species are influenced by the conservation status of the forest, are hunted in similar numbers, and have comparable reproduction cycles (Mayor *et al.*, 2017; Perez-Peña *et al.,* 2018). Nevertheless, significant ecological differences between the species could influence the epidemiology of shared pathogens.

White-lipped peccaries form herds of over 100 individuals, with home ranges between 20 and 110 km^2 (Fragoso, 1998; Keuroghlian *et al.*, 2004). In contrast, CP gather in smaller groups of 3 to 20 individuals and have home ranges between 1 to 3 km^2 (Fragoso, 1999; Fragoso, 2004; Jori *et al.*, 2009; Keuroghlian *et al.,* 2004; Perez-Peña *et al.*, 2018). These differences in social structure and home range size may influence each species' response to pathogens and disease outbreaks. Large herds of

WLP with extensive home range may create favorable conditions for pathogen maintenance or suffer mass mortalities if a highly pathogenic agent is introduced into a naïve population (Fragoso *et al.,* 2022).

Given these observations, the density-dependent overcompensation theory has gained prominence. This theory suggests that WLP overpopulation triggers density-dependent effects, such as increased pathogen transmission, leading to disease outbreaks and subsequent population decline (Fragoso *et al.*, 2022). Although disappearances of WLPs threaten the food security of rural Amazon communities reliant on wild meat, the role of disease in these declines—and how these may interact with other stressors—remains an open question. There is still limited knowledge regarding the impact of pathogens on WLP disappearances (Fragoso *et al.,* 2022).

1.2.3 Zoonoses in Amazonian rural societies

Biodiversity loss significantly affects forest ecosystems and can disrupt pathogen transmission pathways, potentially increasing the likelihood of new pathogens establishing in human populations (Duffy, 2003; Keesing & Ostfeld, 2021). One example is the disappearance of the WLP in the Amazon region, prompting hunters and community members to increase interactions with other animal species to find alternative food sources, mainly rodents and primates. This can further disrupt ecological balances and increase (or at least, change) pathogen transmission risks.

Indeed, pathogen reservoirs are frequently found among species in Rodentia, Cetartiodactyla, Carnivora, and Primate orders (Keesing & Ostfeld, 2021). These same groups also serve as primary meat sources in the Amazon region (Bodmer & Pezo, 2001; Mesquita & Barreto, 2015). While pathogens are generally more likely to spread between phylogenetically similar species, with primates posing a particularly high risk (Han *et al.*, 2016), interactions across distantly related species can still lead to spillover if the pathogen adapts to a new host (Ellwanger & Chies, 2021).

Pathogen spillover in rural communities may be driven by subsistence hunting and related activities, such as wildlife trade, which heighten human-animal contact and the risk of zoonotic disease transmission (Dobson *et al.*, 2020; Ribeiro *et al.*, 2022; Sangkachai *et al.*, 2025). These rural populations are vulnerable, since subsistence hunting is essential to traditional livelihoods, providing a critical source of protein and income for rural communities (Bodmer & Pezo, 2001; Mayor *et al.,* 2022).

Hunters and meat processors face the highest risk of pathogen transmission (Wolfe *et al.,* 1998). Hunting activities —including tracking, capturing, handling, and sometimes basic field killing and transportation of carcasses— expose individuals to zoonotic disease risks through contact with freshly killed or dead animals, which may sometimes result in injuries. Additionally, the consumption of wild meat poses a particular threat of foodborne diseases, either from pathogens the animal carried when hunted or from post-mortem cross-contamination caused by poor hygiene conditions.

Amazonian societies also face significant challenges, including poverty, inequality, and social vulnerability, which are reflected in barriers to healthcare access, limited educational opportunities, and underreporting of health problems (Mayor *et al.*, 2022; Montenegro, 2006). These barriers to medical care exacerbate health risks and hamper accurate health assessments (Badanta *et al.*, 2020; PAHO, 2007). Medical facilities in these remote areas are often distant, poorly equipped, and culturally inappropriate, resulting in inadequate healthcare (PAHO, 2009).

In Peru's rural areas, healthcare coverage is notably incomplete (MINSA, 2007), and the health system's response to local needs is insufficient. For instance, vaccination coverage for three-year-old children is only 21.1%, and there is a critical shortage of healthcare personnel (OPS, 2002). Indigenous populations in isolated Amazonian villages have been hit twice as hard by COVID-19 compared to other areas, with severe vulnerabilities exacerbated by inadequate healthcare infrastructure (ILO, 2020; WHO, 2020). The pandemic highlighted the region's severe vulnerabilities, including limited ICU availability, overcrowded housing, and strained healthcare systems, complicating efforts to contain the virus (Amazon Regional Observatory, 2022; Abizaid *et al.,* 2024).

Indigenous and rural populations also struggle with poverty and underdevelopment, with 34.6% of Peru's Northern Amazonian population living in poverty—above the national average—and many lacking basic services such as water (53.8%) and sanitation (63.7%) as of 2017 (Oriuela & Contreras, 2021; Viceministerio de Gobernanza Territorial,2018).

These conditions, coupled with close contact with highly biodiverse forested areas in peri-domiciliary environments and poor hygiene practices, heighten the risk of contracting infectious diseases (Ferreiro *et al.,* 2021; Vitaliano *et al.,* 2015). Despite the increased risk of infectious diseases in the Amazon, health data from rural Amazonian communities is severely underreported (OEI, 2005), likely due to limited and unreliable data collection and cultural diversity, which creates differences in health perceptions and reporting practices.

The neglect of these populations in terms of health services and poverty may contribute to the underreporting and limited research on certain diseases, perpetuating a cycle where both the diseases and the affected communities remain understudied. Low-cost, sustainable health strategies that consider biogeography and cultural context are crucial for addressing the health impacts and conservation challenges of wild meat consumption (Franco-Paredes *et al.*, 2017). These strategies require data collection, regular monitoring of wildlife, food security, and public health monitoring, alongside community-based wildlife management and effective communication to raise awareness of ecosystem protection and zoonotic risks (FAO, 2024; Sangkachai *et al.*, 2025).

2. HYPOTHESIS

The Amazon region hosts an exceptionally high level of biodiversity, particularly among medium- and large-sized mammals, birds, and reptiles. However, this ecosystem is increasingly threatened by climate change and anthropogenic pressures. While the drivers of biodiversity loss are diverse, the potential role of pathogens remains largely understudied in this context. This thesis hypothesizes that infectious diseases may play a role in the population dynamics of white-lipped peccaries, a species that has experienced unexplained large-scale population declines across the Amazon.

This biodiversity is not only ecologically important but also underpins the livelihoods of many Amazonian communities, who rely on wild meat as a primary source of animal protein and income. Despite this reliance, the public health implications of wild meat consumption under shifting ecological conditions remain largely unknown. The collapse of WLP populations—key to the subsistence hunting practices of Indigenous communities—has led to shifts toward alternative prey species. A secondary hypothesis of this thesis is that these shifts may increase human contact with new or previously infrequent wildlife hosts, thereby heightening the risk of zoonotic disease transmission and the emergence of new public health threats.

The lack of epidemiological data in the Amazon region hinders effective surveillance and control of communicable diseases in rural and remote Amazonian communities. To address this critical gap, this thesis proposes the analysis of biological samples obtained through subsistence hunting as a culturally appropriate and logistically feasible method for disease surveillance. This approach enables the collection of valuable data on pathogens circulating at the wildlife-human interface—particularly from species that are both commonly consumed and ecologically impacted—thus supporting integrated efforts in conservation and public health.

3. OBJECTIVES

The main objective of the present thesis was to improve the knowledge of health risks affecting both humans and wildlife in an Amazonian community by exploring pathogen circulation and zoonotic transmission, while supporting the development of integrated approaches and innovative tools for disease surveillance in remote areas.

The specific objectives were:

1. To identify currently reported health threats for white-lipped peccaries and detect gaps of knowledge for future research directions in Amazonian peccaries' conservation.

2. To improve the knowledge about the circulation of selected infectious diseases in areas of WLP population fluctuations by assessing the seroprevalence and presence of major swine pathogens in CP and WLP populations in the Peruvian Amazon.

3. To describe the circulation and transmission risks of *Toxoplasma gondii* at the human–wildlife–domestic interface in an isolated community that relies on subsistence hunting in a well-preserved forest in the Peruvian Amazon.

4. To evaluate Hepatitis B Virus (HBV) and HEV circulation in the human–wildlife interface and identify risk factors and behaviours through sociological analysis in an indigenous community that relies on subsistence hunting in a well-conserved and isolated area of the Peruvian Amazon.

5. To describe the risk of *Echinococcus vogeli* infection in humans by examining subsistence hunting practices and wildlife management as potential factors for zoonotic disease exposure in Amazonian communities.

4. STUDIES

Study I

Infectious diseases of interest for the conservation of peccaries in the Amazon: A systematic quantitative review

Biological Conservation, 2023, 277, 109867.

Introduction

Over the past decades, infectious diseases have been increasingly emerging and are likely to continue to emerge and spread globally, mostly due to the intensification of anthropogenic activities such as agriculture, urbanization, and the movement of species, along with environmental degradation and climate change (Baker *et al.*, 2022; Keusch *et al.*, 2022). Many of these pathogens can affect susceptible wild hosts, decreasing their populations and, in some cases, leading to their local or, even, global extinction (Smith *et al.*, 2006).

Peccaries are key species of the Amazon ecosystem, favoring the maintenance and regeneration of forests and animal habitats through seed dispersion (Altrichter *et al.*, 2012). However, repeated episodes of disappearances of local populations of white-lipped peccaries (*Tayassu pecari*; WLP) have been observed since the 1980s in the Amazon region. These disappearances have been followed by a full recovery of the original population after more than a decade (Fragoso, 2004) or even after almost three decades (Fragoso *et al.*, 2022; Taber *et al.*, 2008). This phenomenon has been occurring in exceptionally large nonfragmented regions of millions of hectares, affecting ecosystem cycles and even food security in indigenous and campesino people in the Amazon; but still remains poorly understood (Fragoso *et al.*, 2016). Although hunting and habitat destruction have been the most studied threats, the large populations and the ecology of the species suggest that diseases may be involved in their dynamics (Altrichter *et al.*, 2012; Fragoso *et al.*, 2022). In addition, although white-lipped peccaries inhabit the Amazon region sympatrically with collared peccaries (*Pecari tajacu*; CP), no decline in CP populations have been observed (Fragoso, 1998).

In recent decades, serological studies in wild peccaries have evidenced the presence of infectious diseases that could impair their health and population dynamics (De Castro *et al.*, 2014; Karesh *et al.*, 1998; Morales *et al.*, 2017; Romero Solorio, 2010), including diseases usually present in domestic livestock (Herrera *et al.*, 2008; Freitas *et al.*, 2009). As such, the lack of a thorough evaluation of the health status of this species, the increase in pig production in the Amazon promoting the interaction between domestic pigs (*Sus scrofa domesticus*) and peccaries (Hohnwald *et al.*, 2019; Serrao *et al.*, 1996), and the scarce information on infectious agents in the suiform interface highlight the need for a detailed review of the infectious diseases affecting these species in the Amazon region.

The present study aims to perform a systematic quantitative literature review on infectious diseases affecting peccaries and domestic pigs in the Amazon region, in order to identify currently reported health threats for white-lipped peccaries and detect gaps of knowledge for future research directions in Amazonian peccaries' conservation.

Materials and methods

We conducted a search in English-language in the online databases Scopus and Pubmed, with a date range from the 1st of January 1990 to the 28th of February 2022. We used keywords related to the host species (peccaries, swine, and pigs), the political units of each country composing the Amazon region (Brazil, Perú, Colombia, Venezuela, Ecuador, Bolivia, Suriname, Guyana, and French Guyana), and pathogen or disease-associated terms. Given that the bibliography related to diseases in the Amazon is not broad, we conducted an additional search through Google Scholar in English language. This allowed us to identify studies published in journals not indexed in the Journal Citation Reports (JCR), academic theses, dissertations, and congress presentations or summaries, using the same previous search terms combined or as a search refining term to improve result outputs. Since non-English language studies are numerous in biodiversity and conservation topics in South America, we also conducted additional searches in Spanish and Portuguese in Google Scholar. The search algorithms are presented in Annex 1.

We evaluated each entry obtained in the different searches based on the title, abstract, and details of the document to evaluate the research topic, correct location in the Amazon region and correct host species, in order to decide the inclusion in our database. We also excluded general review papers from the database to avoid reporting duplicated data (Figure I.1). We reviewed the content of each study and used it to construct a database by recording the following general information: species reported, sample size, location, year of study, year of publication, pathogens studied, laboratory and diagnostic methods, results, and additional notes when needed. We also classified the selected bibliography into the category 'Conservation Medicine' if they investigated the impact of infection or disease on wildlife populations, the category 'Zoonosis' when they were focused on the transmission or affection of pathogens to humans, and 'Production' when investigating the impact of diseases on livestock.

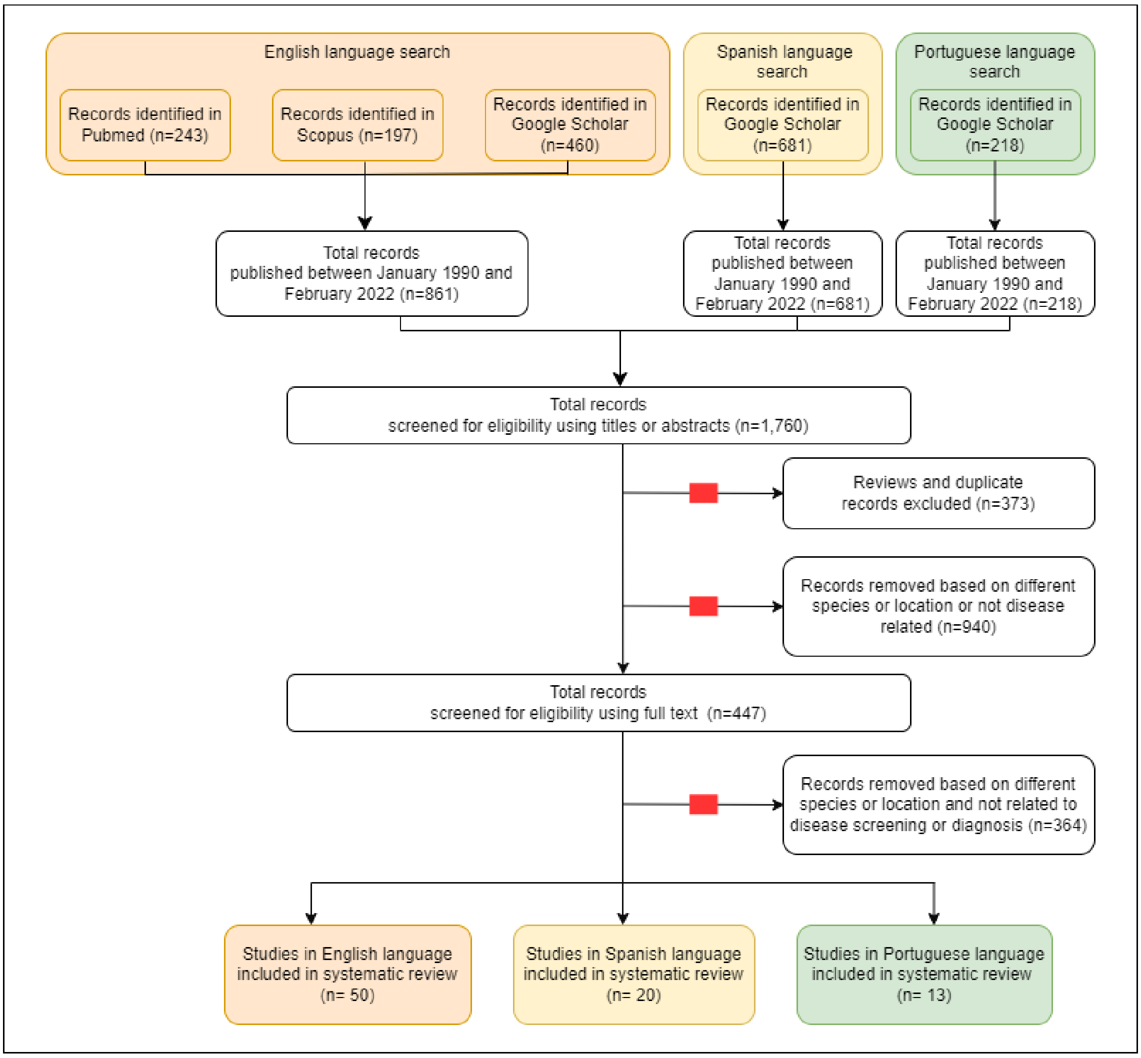

Figure I.1. Conceptual diagram of the identification of studies on infectious diseases affecting suids in the Amazon region, and selection process for the inclusion in the systematic review using PRISMA (Preferred Reporting Items for Systematic review and Meta-Analysis).

When a thesis or congress presentation was later published as an article, the information was considered only from the research article. When information was partially published in an article (for example, some but not all pathogens were published in a research article), the unpublished information was considered directly from the thesis.

Results

A total of 84 publications met our inclusion criteria, including 62 research articles, 16 research theses and six congress/conference presentations. Of these 84 publications, five theses and two congress presentations were discarded because their results were also present in other publications, reducing the total number of publications assessed in this systematic review to 77 (Annex 2).

Species and sampling design

Domestic pigs were the most studied suiform species, being reported in 35 (45.5 %) of the retrieved studies. The CP were included in 31 (40.3 %) of these studies, and WLP in 23 (29.9 %), including 12 mixed-species studies. Overall, 47 (61.0 %) reports involved captive animals (35 studies on domestic pigs, ten on CP, one on WLP, and one involving both peccary species); while 29 (37.7 %) involved free-ranging animals (ten on WLP, nine on CP, and ten on both peccary species). Only one study (1.3 %) involved both captive and free-ranging animals and considered CP and WLP.

The number of animals sampled varied depending on the species. Studies involving peccaries presented sampling sizes ranging from one to 125 individuals, with a median sample size of eleven individuals (Q1: 3; Q3: 42); while the sample size in studies involving domestic pigs ranged from six to 1070 individuals, with a median size of 109.5 (Q1: 63; Q3: 179). *3.2*.

Pathogens

Eighty pathogens were studied in domestic pigs and peccaries, including 35 (43.8 %) parasites, 25 (31.3 %) bacteria and 20 (25.0 %) viruses. *Toxoplasma gondii* (15.6 %, 12/77 studies) and *Leptospira* spp. (11.7 %, 9/77 studies) were the most studied pathogens. Other reported pathogens were *Streptococcus* spp. (6.5 %, 5/77 studies), Hepatitis E virus (6.5 %, 5/77), Aujeszky disease virus (5.2 %, 4/77 studies), *Brucella* spp. (5.2 %, 4/77 studies) and *Trypanosoma* spp. (5.2 %, 4/77 studies), among others.

Of the 42 studies including peccaries, the most studied pathogens were *Leptospira* spp. (14.3 %, 6/42 studies), *T. gondii* (14.3 %, 6/42 studies), *Brucella* spp. (9.5 %, 4/42 studies), Aujeszky disease virus (9.5 %, 4/42 studies), and *Trypanosoma* spp. (9.5 %, 4/42 studies). While regarding the 35 studies in domestic pigs, the most studied pathogens were *T. gondii* (8.6 %, 6/35 studies), Hepatitis E virus (14.3 %, 5/35 studies), *Leptospira* spp. (8.3 %, 3/35 studies), and *Taenia solium* (8.3 %, 3/35 studies). Thirty-seven pathogens were reported in CP, 29 in WLP, and 19 in domestic pigs. In CP, *Leptospira* spp. was the most reported pathogen (10.8 %, 4/37 studies). In WLP, the most reported pathogens were *T. gondii* (13.8 %, 4/29 studies) and Aujeszky's disease virus (10.3 %, 3/ 29 studies). In domestic pigs, the most reported pathogens were Hepatitis E virus (26.3 %, 5/19 studies) followed by *T. gondii*, *Leptospira* spp., and *T. solium* (each 15.8 %, 3/19 studies). The total list of pathogens and prevalence/seroprevalence compiled in this literature review can be found in Table A1.

Of the 80 pathogens studied, 24 pathogens were not found in suiform species in the Amazon region, including African swine fever virus, Classical swine fever virus, Foot and Mouth disease virus and *Mycobacterium tuberculosis*, among others (see Table A1 for the full list of undetected pathogens). Out of the 77 evaluated studies, 43 (55.8 %) focused on the ‘Zoonosis’ category, 26 (33.8 %) on the ‘Conservation Medicine’ category, and 22 (28.6 %) on the ‘Production’ category. The combination of two or all three focus of study was detected in 13 (16.9 %) studies.

Diagnostic testing performed and study types

Diverse methodologies were reported for pathogen diagnosis and surveillance over the last three decades, showing a notable increase in the use of molecular biology techniques (Figure I.2). Twenty-eight (36.4 %) studies used molecular methods for pathogens' RNA/DNA identification and characterization (for example PCR and sequencing), while 25 (32.5 %) included serologic diagnostic testing (for example Enzyme-Linked Immunosorbent Assay, microscopic agglutination tests and Immuno-fluorescence antibody tests). Seventeen (22.1 %) studies contained diagnosis of pathogens based on direct microscopy, histopathology and diverse culture techniques. Complementary clinical evaluations were only reported in 2 (2.6 %) studies. Seven (9.1 %) studies included a combination of two or more methodologies. Specific laboratory techniques are shown in Table A2.

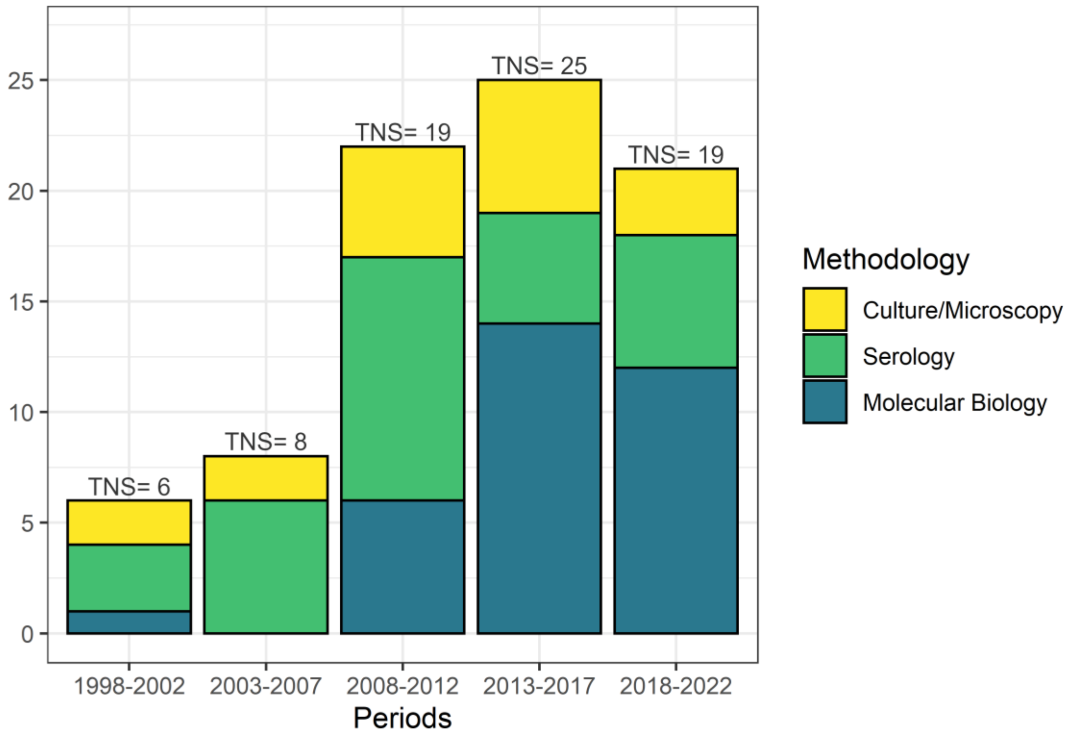

Figure I.2. Diagnostic methods and techniques used in the studies included in the review (n = 77) on infectious diseases affecting suids in the Amazon region, distributed by period between 1998 and 2022 (not mutually exclusive). TNS: Total number of studies.

Most of the studies (56/77; 72.7 %) were cross-sectional, while two were longitudinal (2.6 %), and nineteen (24.7 %) did not report research period information. Ten studies (13.0 %) encompassed two different sampling periods, and six (7.8 %) were based on long periods of sampling (>three years).

Study location

The reviewed studies were performed in the Amazonian regions of Brazil (63.3 %, 49/77), Peru (28.6 %, 22/77), Bolivia (2.6 %, 2/77), French Guyana (2.6 %, 2/77), Colombia (1.3 %, 1/77), and Ecuador (1.3 %, 1/77). The most sampled provinces/states were Pará (Brazil, 29.9 %, 23/77), Mato Grosso (Brazil, 29.9 %, 23/77), and Madre de Dios (Peru, 14.3 %, 11/77) (Figure I.3).

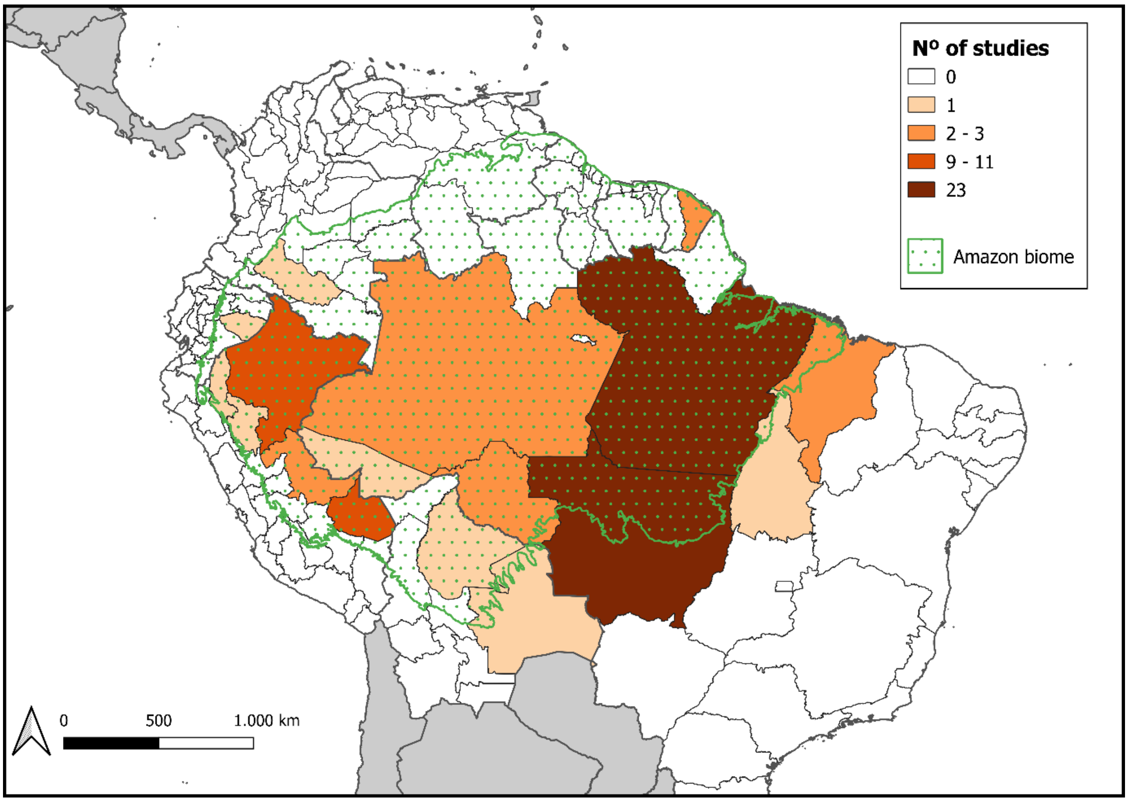

Figure I.3. Locations of studies on infectious diseases affecting swine and peccaries in the Amazon region. Provinces or states are colored according to the number of the studies included in the review (n = 77).

Publication format

Forty-nine (63.6 %) studies were published in English, including 45 articles and 4 congress presentations. Sixteen (20.7 %) were published in Spanish, comprising 10 articles and 6 theses, and 12 (15.6 %) in Portuguese, including 7 articles and 5 theses. The 62 research articles were published in 41 different journals; in particular, 44 (70.9 %) articles were published in journals indexed in the JCR and 18 (29.5 %) articles were published in journals not indexed in the JCR. The reviewed articles were mostly published in the journals '*Revista Brasileira de Parasitologia Veterinaria*' (9.7 %, 6/62), '*Revista de Investigaciones Veterinarias del Perú*' (6.5 %, 4/62), *'Acta Scientiae Veterinariae'* (4.8 %, 3/62), and '*International Journal for Parasitology: Parasites and Wildlife*' (4.8 %, 3/62). Moreover, eleven theses were defended in eight different universities, being the Universidade de Sao Paulo (Brazil) (18.2 %, 2/11) the highest representative. Four theses were from undergraduate studies (36.4 %), four from master studies (36.4 %), and three from doctoral dissertations (27.3 %) (Table A3).

Discussion

In the lasts 50 years, the increase of human population and anthropogenic activities, including deforestation, intensive farming, illegal and poorly regulated wildlife trade, and climate change, have promoted unprecedented habitats' destruction and biodiversity loss worldwide (Mace, 2005). Wildlife population declines may be caused by single or multiple synergic elements, and diseases should always be considered as potential synergic factors (Preece *et al.*, 2017). In the present work, we reviewed the scientific literature on infectious diseases of domestic pigs and peccaries in the Amazon region, aimed to the identification of health threats for peccaries, especially WLP, and to identify future research directions for the Amazonian peccaries' conservation.

The available information related to diseases in free-ranging wild species listed as Vulnerable or Endangered by the IUCN is still very limited and has been focused on few pathogens in selected wild host species (Martinez-Gutierrez and Ruiz-Saenz, 2016; Scheele *et al.*, 2019). At the view of the results of our review, studies on diseases affecting the health of wild peccaries have received limited attention. We also detected a lack of inter and multi-disciplinary studies encompassing infectious diseases. Preventing and managing emerging diseases in wildlife demand an interdisciplinary approach such as the One Health initiative, which recognizes health issues at the complex human, animal and environmental interface (Harrison *et al.*, 2019; Trilla, 2020). Studies that consider different approaches such as ecology, environment, human population and activities are mandatory to face the conservation of WLP (Jori *et al.*, 2009; Romero Solorio, 2010).

Our review highlighted a notably higher number of studies in captive animals than in free-ranging wild individuals. In fact, domestic pigs were the most studied suiform species. Collaborative working with local hunters have been proven to be a useful strategy, allowing the access to a larger biological sampling in terms of species and individuals (Aston *et al.*, 2014; Morales *et al.*, 2017). In addition, more inexpensive strategies, such as filter paper, allows a convenient sample collection and preparation, especially when working in remote areas such as the Amazon region (Aston *et al.*, 2014). Considering the difficulties of sampling procedures and logistics in wild species in the Amazon, studying the declines in WLP population represents a challenge. Additionally, in areas where population declines occurred, individuals may hardly be detected due to the consequent low animal density.

From the perspective of disease prevention, studies on infectious diseases in captive peccaries and domestic pigs from the Amazon region can be a cornerstone for the evaluation of the risk of disease spreading from captive suiforms to free-ranging peccaries. In the rural Amazon, pigs raised for subsistence are usually not confined, housed in the backyard without biosecurity conditions, and having direct access to the natural environments of the Amazon rainforest (Hohnwald *et al.*, 2019; Labruna *et al.*, 2002), therefore increasing the probability of contact with populations of wild peccaries.

Although WLP disappearances have been documented in several Amazon regions of Brazil, Peru, Ecuador, Bolivia, French Guyana, Guyana, and Colombia (Fragoso *et al.*, 2022), most studies on infectious diseases were performed in only three of these regions, in Brazil and Peru. This uneven and disproportionate localization of studies depicts an incomplete representation of the Amazon region and limits the knowledge on the variability of diseases in the current distribution of peccaries. In some regions, studies reported the presence of specific pathogens in all three suiform species, providing initial information on the diseases at the interface of these species in the Amazon region. This knowledge facilitates sampling procedures and logistics in areas where sampling wildlife is highly complicated, or in locations where WLPs have disappeared. However, in other regions, information on a specific pathogen was only available for one species.

Regarding the type of studies carried out, most of the studies were cross-sectional surveys, offering a snapshot of a single moment in time, and not providing enough information on causes, effects, and risks in a disease-population relationship (Thelle and Laake, 2015; Wobeser, 2007). Cohort, case-control, and longitudinal studies may be more relevant to understanding the impact of infectious diseases on WLP populations. Moreover, we reviewed a notable number of studies that did not report information on the sample period, hampering the association of the results with a specific event or conditions of the population.

In this review, the difference between the number of studies focused on the conservation of both peccaries and those describing the presence of zoonotic agents was also striking. The higher number of studies focusing on zoonotic agents is probably driven by the fact that subsistence hunting is a wide-spread practice in tropical forests, and, in the Amazon, wild meat represents a significant source of animal protein and income for rural and indigenous communities (El Bizri *et al.*, 2020a; Torres *et al.*, 2018) and can even be found in urban markets (Mayor *et al.*, 2022). This condition increases the probability of exposure for Amazonian societies to threads related to food security and zoonosis.

All research studies that carry out biological collections need to have prior authorizations from ethics committee form institutional participants, and collection authorizations from each corresponding public sectors. For instance, in Brazil, the responsible public institutions for the authorizations of wildlife biological collection is the Instituto Chico Mendes de Conservaçao da Biodiversidade of the Ministry of the Environment, and in Peru, the Servicio Nacional Forestal y de Fauna Silvestre belonging to the Ministry of the Agriculture, and the Servicio Nacional de Areas Naturales Protegidas of the Ministry of the Environment. In addition, the conduction of comprehensive and holistic studies within the One Health framework frequently requires the integration of other interfaces (humans, domestic animals and/or environment) that will each need authorizations from public sectors. To these must be added the permits for the export of biological samples when needed, and the parallel importation authorizations by the receptor government, which includes compliance with the requirements of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity, authorizations by national Animal Health departments, and finally the particular requirements of conveyors and courier companies.

We argue that export permits could be avoided by improving the availability of appropriate technology with comparable costs in local countries. Overall, obtaining prior authorizations is highly expensive in terms of time, economy and human efforts. Definitely, although it is essential to control the activities that are carried out with local biodiversity, from the point of view that research should help improve the sustainability of that biodiversity, we claim a smart process for the obtention of all required authorizations.

Among the pathogens studied in the reviewed studies, *T. gondii* and *Leptospira* spp. were the most reported. Although these diseases do not pose a threat to animal populations, they are important foodborne pathogens. *Toxoplasma gondii* has an average prevalence of infection of 30 % in human Amazon communities, even reaching 100 %, and Leptospirosis is among the main zoonotic causes of morbidity throughout the world, especially in tropical regions (Costa *et al.*, 2012; Dubey, 2010; Sobral *et al.*, 2005). Additional pathogens reported in domestic pigs are related to livestock production or zoonosis (e.g. Hepatitis E and *Taenia sollium*). These pathogens represent major public health and food safety problems worldwide especially in rural tropical and subtropical regions, where they also affect the economy of producers (Dalton *et al.*, 2008; Del-Brutto *et al.*, 2017).

Aujeszky disease virus and *Brucella* spp. were also reported in peccaries (Mayor *et al.*, 2006; Romero Solorio, 2010). Aujeszky's disease causes a wide array of organ disorders, generating mortalities of 100 % in newborn pigs and 30 % in older piglets (Zuckermann, 2002). Brucellosis is one of the most common zoonoses in the world and is also present in wild species (Paulin and Ferreira Neto, 2003). In Brazil and Peru, bovine brucellosis is endemic in several states and regions (Megid *et al.*, 2005; Pappas *et al.*, 2006; Poester *et al.*, 2009), and causes important reproductive problems, including infertility and abortions that can reach up to 80 % (Duncan, 1990). These diseases can impair the population dynamics of large herds of WLP. However, due to the small number of animals sampled and the lack of longitudinal studies, the potential impact of Aujeszky's disease or brucellosis on peccaries has not yet been assessed (Martins De Castro *et al.*, 2014; Romero Solorio, 2010).

Finally, we found that studies in local official languages (Spanish or Portuguese) accounted for a third of the studies. This information generated by local professionals and in the local language has a crucial role in providing information on biodiversity and conservation, and advising local policies (Amano *et al.*, 2021). This evidences that non-English-language studies must be considered when reviewing topics related to biodiversity and conservation in the Amazon.

Study II

Monitoring of selected swine viral diseases in Peruvian Amazon peccaries

EcoHealth, 2025, 22:69-78

Introduction

Peccaries (Cetartiodactyla: Tayassuidae) are native to Latin America and are distributed from Mexico to northern Argentina (Sowls, 1997). They play a pivotal role in ecosystem dynamics by rooting soils, dispersing seeds and seedlings, consuming plant and animal matter, and serving as prey for top predators (e.g., Panthera onca and Puma concolor) (Porfirio *et al.*, 2017; Sobral *et al.*, 2017; Weckel *et al.*, 2006). In the Amazon, two species of peccaries coexist: the white-lipped peccary (WLP, *Tayassu pecari*) and the collared peccary (CP, *Pecari tajacu*). Both species are an enormous source of protein and socioeconomic importance for local communities (Mayor *et al.*, 2022).

Since 1980s, researchers have reported recurrent local disappearances of WLP populations across their Latin American range, spanning at least 50 million hectares (Fragoso *et al.*, 2016; 2022; Richard-Hansen *et al.*, 2014), and affecting the ecosystem dynamics, as well as the food supply and economic activities of indigenous and rural communities (Fragoso *et al.*, 2016; 2022). Early studies attributed these populations' oscillations to migrations, overhunting, and environmental changes (such as land-use changes), but none have reached consistent conclusions (Altrichter *et al.*, 2012; Fragoso *et al.*, 2022).

The density-dependent overcompensation theory has recently gained prominence, suggesting that WLP population overabundance triggers density-dependent effects, such as increased pathogen transmission, resulting in disease outbreaks and population decline (Fragoso *et al.*, 2022). Even though both WLP and CP are influenced by the same environmental conditions, have similar reproduction cycles (Mayor *et al.*, 2017), and are similar hunting preferences for subsistence hunters (Pérez-Peña *et al.*, 2021), such disappearance cycles have not been reported for CP.

However, there are several ecological differences between the two species. In general, WLP occupy densities ranging from 3.7 to 25 individuals/km2, while CP occupy densities ranging from 2.8 to 9.8 individuals/km2 (Fragoso, 1998; Keuroghlian *et al.*, 2004). These differences may influence how animals respond to pathogens and disease outbreaks (Fragoso *et al.*, 2022).

Several swine viruses, including classical swine fever virus (CSFV), Aujeszky's disease virus (ADV), and swine vesicular disease virus (SVDV), as well as various porcine circoviruses (PCV), have a significant impact on swine health, affecting the reproductive, nervous, respiratory, and gastrointestinal systems, with high associated mortality rates that can reach 100% (Postel *et al.*, 2018; WOAH, 2023; Zuckermann, 2000).

However, to our knowledge, there is a significant gap in information about the occurrence and health impact of infectious diseases on wildlife in the Amazon region. Indeed, diseased WLP and carcasses have been observed during periods of population decline in some regions, but further examination was not carried out (Fragoso *et al.*, 2022).

In the Southern Peruvian Amazon, studies have reported the occurrence of antibodies against ADV in free-living WLP populations (Romero Solorio, 2010). Also, ADV and porcine circovirus 2 (PCV-2) have been reported in CP and WLP populations in the Bolivian and Brazilian Amazon but ignoring their impact on their population dynamics (Karesh *et al.*, 1998; De Castro *et al.*, 2014). PCV-2 has also been documented among domestic pigs in the Southern Brazilian Amazon (Dutra *et al.*, 2013).

WLP populations can experience explosive population growth (Fang *et al.*, 2008) and due to the risk and rate of infectious disease transmission is higher in dense populations (Tarwater & Martin, 2001), we hypothesized that highly virulent infectious diseases may be involved in the severe population declines of WLP in the Amazon.

The present study aimed to improve the knowledge about the circulation of selected infectious diseases in areas of WLP fluctuations by assessing the seroprevalence and presence of major swine pathogens in CP and WLP populations in the Peruvian Amazon.

Materials and methods

Study Area

The study was carried out in two areas of the Peruvian Amazon: the Yavarí -Mirín River (YMR) basin and the Pucacuro National Reserve. The Yavarí-Mirín River basin (YMR; 04º19’53’’S; 71º57’33’’W) is a remote area on the Peru-Brazil border, composed of a diverse landscape that ranges from upland forests with nutrient-poor sandy soils to flooded forests with relatively nutrient-rich soils (Ter Steege *et al.*, 2003).

The only village in YMR is Nueva Esperanza, a Yagua indigenous community of around 300 people. The main human activities of this community are traditional small-scale agriculture, fishing, logging, and subsistence hunting (Mayor *et al.*, 2015). There is no largescale agriculture in the study area; therefore, the crops are subsistence-based. In the YMR basin, WLPs have shown extreme population fluctuations over the last 25 years, including a decline from a high of 15 ind./km2 in 2000 to 2 ind./km2 in 2004 and a complete disappearance between 2005 and 2015 (Fang *et al.*, 2008; Bodmer *et al.*, 2024).

The Pucacuro National Reserve (PNR), located on the border with Ecuador (2º26’53’’S; 75º20’29’’W), is composed of high terrace forests, non-flooded habitat with dissected relief in a humid tropical forest. PNR has a game species management plan that includes CP, WLP, and *Cuniculus paca*. Due to their large population sizes and relatively high reproductive capacity, management groups hunt these species for consumption and commercialization to support local indigenous hunters’ economies (SERNANP, 2014). There have been no reports of WLP or CP population declines in PNR (Perez Peña *et al.*, 2016).

Both study areas are highly biodiverse and well-preserved, with low human impact (Pitman *et al.*, 2003b; SERNANP, 2019), and the nearest pig farms are 160 and 170 km away, respectively, from YMR and PNR (Figure II.1).

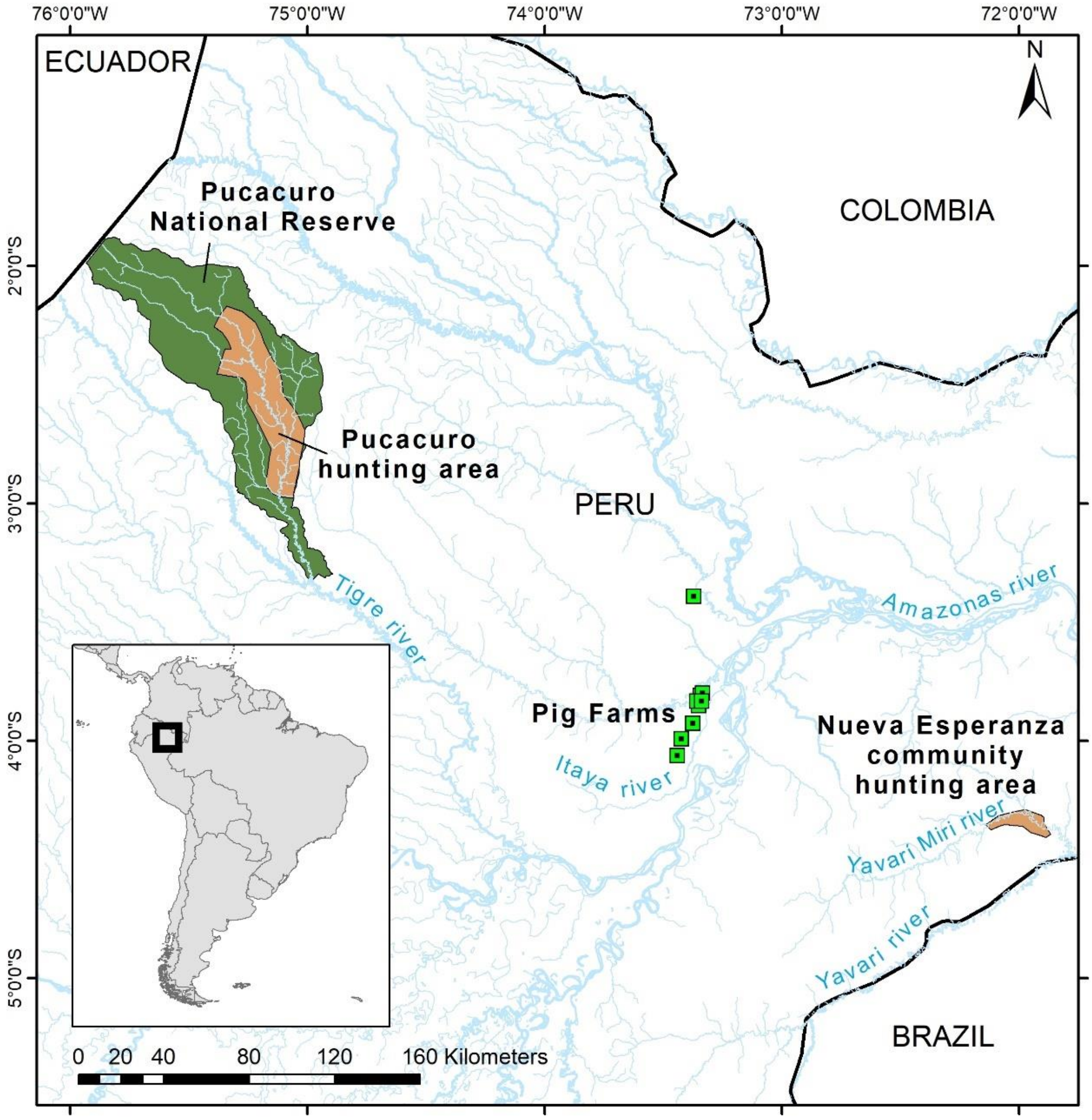

Figure II.1. Map of the study area, including the hunting areas of Nueva Esperanza community in the Yavarí Mirin River basin and the Pucacuro National Reserve. The map also shows all pig farms registered in the region (squares; SENASA, pers. comm.).

Biological Collection

Blood samples were obtained from both study areas, totaling samples from 98 WLP and 140 CP. The samples were collected by subsistence hunters and park rangers as part of a wildlife conservation program, taking advantage of the discarded material from legal subsistence hunting. Hunters impregnated blood from the cranial or caudal cava veins on either Whatman filter paper n. 3 or FTA cards (Scheilcher &

Schuell, Germany). The YMR basin was sampled from 2008 to 2015 and 2019 to 2020, while the PNR was sampled from 2012 to 2014. Samples were collected in all seasons, and hunters recorded the species, date, location, and sex. The samples were kept at room temperature in a sheltered environment in the study areas for 15 to 100 days before being stored at – 20ºC until analyses (Aston *et al.*, 2014; Morales *et al.*, 2017).

Serological Analyses

A 132 mm^2 area of the filter paper with blood was cut with a sterile punch and diluted in 400 lL of sterile PBS (phosphate-buffered saline). These samples were vortexed for 20 s, stored at 4_C for 24 h before being vortexed for 20 s, and frozen at - 20_C until analysis. As serum represents 40% of total blood volume (Nobuto, 1966) and the blood concentration on filter paper is 40 lL/cm^2, we obtained a serum dilution of 1:20. The elutions were tested for antibodies against CSFV, ADV, and SVDV using the ID Screen Classical Swine Fever E2 Competition ELISA kit, the ID Screen Aujeszky gB Competition ELISA kit, and the ID Screen Swine Vesicular Disease Competition (IDvet, Montpellier, France), respectively.

Molecular Analyses

RNA was extracted from 200 lL of the elutions using the commercial kit IndiMag_ Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany) according to the manufacturer's instructions. RNA concentrations were determined using the Qubit Fluorometric Quantification High Sensitivity Assay (Invitrogen, California, USA). Molecular detection of porcine circoviruses 1, 2, 3, and 4, as well as CSFV, was performed by conventional and RT-PCR, following previously described protocols (Vilcek *et al.*, 1994; Quintana *et al.*, 2002; Oliver-Ferrando *et al.*, 2016; Franzo *et al.*, 2020; Saporiti *et al.*, 2021). The 95% confidence intervals for antibodies and pathogen prevalence were calculated using the Wilson score method (Thrusfield, 2018).

Statistical Analysis

We used Fisher's test to compare seroprevalences of CSFV and ADV between all the animals analyzed in YMR and PNR, as well as between the two analyzed periods in YMR.

Ethics

Field and laboratory procedures were previously authorized by Peruvian and International Authorities: Dirección General de Flora y Fauna Silvestre from Peru (041–2007- DGGFS-DGEFFS, 0350–2012-DGFFS-DGEFFS, 258–2019- MINAGRI-SERFOR-DGGSPFFS), the Head of the National Reserve of Pucacuro (03–2012-SERNANPRN Pucacuro), and the Institutional Animal Use Ethics Committee from the Universidad Peruana Cayetano Heredia (029–03- 19, protocol #102,142) and the Universitat Autònoma de Barcelona (protocol #4829). Export permits were as follows: 003258-CITES-Perú, 003260-CITES-Perú, BB-00017 20ISpain, and BB-00018 20I-Spain.

Results

Six specimens presented antibodies against CSFV, three CP and three WLP, for an overall seroprevalence of 2.14% (CI95%: 0.70–6.11%) in CP and 3.06% (CI95%: 1.05–8.62) in WLP (Table II.1). Seropositive CPs were only detected in YMR in 2009, 2013, and 2014, during the WLP population crash in the area. One seropositive WLP was detected in YMR in 2020 and two seropositive individuals in PNR in 2014. Antibodies against ADV were detected in 3.06% WLPs (3/98; CI95%: 1.05–8.62). Two seropositive individuals were hunted in YMR in 2019, during WLP population recovery, and one in PNR in 2014 (Table II.1). All samples were negative for antibodies against SVDV. Molecular analyses for PCV-1, PCV-2, PCV-3, PCV-4, and CSFV were all negative.

Fisher tests did not reveal any significant difference in the seroprevalence between both areas or both periods.

Table II.1. Results for the Detection of Antibodies Against Classical Swine Fever Virus (CSFV) and Aujeszky's Disease Virus (ADV) in White-Lipped Peccary (WLP; *Tayassu pecari*) and Collared Peccary (CP; *Pecari tajacu*) in the Yavarí-Mirín River Basin and the Pucacuro National Reserve (Peruvian Amazon).

Area	Species	Period	CSFV	ADV
YMR	WLP	2008 to 2015	0/6 (0%) [0.00-39.03%]	0/6 (0%) [0.00-39.03%]
		2019 to 2020	1/49 (2.04%) [0.36-10.69%]	2/49 (4.08%) [1.13-13.71%]
		Total	1/55 (1.82%) [0.32-9.61]	2/55 (3.64%) [1.00-12.32%]
	CP	2008 to 2015	3/98 (3.06%) [1.05-8.62]	0/98 (0%) [0.00-3.77%]
		2019 to 2020	0/39 (0%) [0.00-8.97%]	0/39 (0%) [0.00-8.97%]
		Total	3/137 (2,20%) [0.75-6.24]	0/137 (0%) [0.00-2.73]
PNR	WLP	2012 to 2014	2/43 (4.65%) [1.28-15.46]	1/43 (2.33%) [0.41-12.06]
	CP	2012 to 2014	0/3 (0%) [0.00-56.15]	0/3 (0%) [0.00-56.15]

Discussion

Wildlife population declines are usually multifactorial processes, even if one cause can be identified as predominantly responsible (Sodhi *et al.*, 2009). Many species are already threatened due to habitat fragmentation, declining genetic diversity, or overexploitation (Frank *et al.*, 2006), and their combination with infectious diseases increases even more the risk of local extinctions (Smith *et al.* 2006).

Throughout the Amazon, at least 28 independent WLP disappearance events have been recorded (Fragoso *et al.*, 2022). Since there is no published data on the infectious diseases' impact in peccaries, and it is not clear which pathogens may be present in peccary populations, their assessment should be carefully addressed. In this sense, a recent study highlighted the necessity of performing longitudinal studies on infectious diseases in peccaries to address this gap of knowledge (Study I).

The present study confirmed the exposure of Peruvian peccaries to CSFV and ADV. These viruses cause disease in domestic and feral pigs, being the latest ones considered as reservoir hosts for domestic pigs worldwide (Postel *et al.*, 2018; Zuckermann, 2000). Classical swine fever (CSF) has not been previously reported in the Amazon region, but it is considered an endemic disease in domestic pigs from other regions in Brazil and Peru (De Oliveira *et al.*, 2020; Pereda *et al.*, 2005), and its circulation has also been reported in CP in non-Amazon areas in Colombia (Montenegro *et al.*, 2018).

Our results present evidence of peccaries contact with the virus in the Amazon region, as both WLP and CP were exposed to CSFV in both study areas. However, our results are inconclusive in establishing whether or not these viruses drove the WLP population to collapse. On the one hand, WLP were almost absent in the YMR basin between 2008 and 2015 (n = 6; 0% seroprevalence), when antibodies against CSFV were detected in collared peccaries (n = 98; 3.06% seroprevalence), tipping the balance in favor of the hypothesis of CSFV as a modulator of population dynamics of WLP. Antibodies against CSFV were only detected in WLP during the following years (2019–2020) when the population had recovered. On the other hand, antibodies against CSFV were detected in WLP from the PNR, where populations did not decline. Since CSFV is highly contagious and can cause large mortality outbreaks in domestic pigs and wild boars, a significant role of CSFV in the population declines of peccaries cannot be completely ruled out and warrants deeper investigation.

As with CSFV, the susceptibility and impact of ADV on peccaries are still unknown but potentially significant in case of virulent strains. Seropositivity for antibodies against ADV has been previously observed in WLP from the Southern Peruvian and Bolivian Amazon, but not in CP (Karesh *et al.*, 1998; Romero Solorio, 2010). In our study, antibodies were detected only in WLP and in both study areas, with similar low seroprevalences as in the previous studies. As with CSFV, antibodies were not detected in the YMR basin during the decline in the WLP population and were only detected in the following years. Similarly, these findings are inconclusive in establishing the role of ADV in the declines of WLP populations.

However, they deserve further attention as the impact of the virus on peccaries is still unknown. In addition, ADV does not only affect swine species but can also cause significant mortality in carnivorous mammals, for example, in dogs and in wild species such as *Puma concolor* (Cunningham *et al.*, 2021; Zhang *et al.*, 2015). Thus, the presence of ADV in peccaries may represent a conservation issue for both peccaries and endangered carnivore species.

We consider it unlikely that the pathogens studied were introduced through pig farms. Feral pigs have not been reported in either YMR or PNR, and pig farms are located near Iquitos, at least 160 km away from both sampling areas (Fig. 1). The maximum home range of CP is 7km2 and of WLP is 100–200km2 (Fragoso, 1998; Kiltie & Terborgh, 1983; Taber *et al.*, 1994), and the presence of large rivers in the area as ecological barriers hinder contact with the aforementioned farms. Additionally, in front of our study, area lies the Brazilian territory, which includes Brazil's largest Indigenous Reserve. These are areas where farming is prohibited (Verissimo *et al.*, 2011).

Consequently, we can infer that there is no spillover of pathogens from domestic pigs in the frontier area. However, the presence of traditional small-scale pig farming cannot be ruled out and should be further evaluated to understand the potential role of small-scale farming in the introduction of swine viruses in the free-living Amazon. In fact, it is possible that previous contacts with backyard pigs could have introduced these viruses within the free-living peccary population, where they have been naturally transmitted between and within their populations.

Antibodies against SVDV and genome from PCV-1, PCV-2, PCV-3, PCV-4, and CSFV were not detected in any sample from the study. Swine vesicular disease virus and the four porcine circoviruses are associated with different degrees of disease in pigs. From these, only PCV-2 has previously been reported in wild peccaries and domestic pigs in the Colombian Amazon (Montenegro *et al.*, 2018) and the Brazilian Amazon and Pantanal (De Castro *et al.*, 2014; Dutra *et al.*, 2013). SVDV and PCV have not been reported in peccaries nor domestic pigs in the Amazon basin (Study I), supporting our findings and evidencing their improbable role in the population dynamics of WLP.

Some technical constraints, such as sample conservation, must be considered when interpreting our results. Filter paper is a convenient technique when working with local communities in logistically challenging remote areas because it simplifies sample collection and storage, especially since an adequate cold chain is unavailable. Local communities rely on subsistence hunting for food and could become active samplers of valuable biological material that is often discarded. In previous studies, these same samples have already been used successfully to perform serological and molecular DNA analyses (Aston *et al.*, 2014; Morales *et al.*, 2017); however, it is important to consider that storage at room temperature under ambient conditions could potentially impact sample stability. This can lead to a higher likelihood of false negative results compared to analyzing frozen biological samples (Bevins *et al.*, 2016), particularly when working with RNA viruses.

Therefore, our results must be considered conservative, and the reported seroprevalence may be lower than reality. Furthermore, it is worth noting that PNR was sampled more than 10 years ago, and since then, the health status of the pecaries' populations in the area might have changed. Additionally, we obtained few samples in the CP sampling in PNR and during the WLP population crash in YVR, these negative results may be underestimating circulating antibodies against CSFV, ADV or SVDV.

Determining infectious diseases' impact on WLP disappearance episodes is challenging. The presence of a pathogen in a declining population does not imply that the pathogen played a significant role in the decline (McCallum, 2012). Baseline data still needs to be included to model the epidemiology and the impact of viruses like CSFV and ADV on peccaries (Study I). Due to viral strains with different severity of disease (De Oliveira *et al.*, 2020; Pereda *et al.*, 2005), isolation and phylogenetic analysis of circulating strains in free-ranging peccaries and experimental infections are required to evaluate and understand their pathogenesis and potential effects.

Expanding the geographical coverage of studies on infectious diseases in peccaries to include other areas where population declines have been reported would assist in determining whether pathogens are associated with population declines. Because the fluctuations of WLP populations are likely to be multifactorial, it is also essential to include strategies for monitoring population dynamics that allow anticipating the population decline to develop surveillance before and during a WLP disappearance event.

Study III

Toxoplasma gondii in a Remote Subsistence Hunting-Based Indigenous Community of the Peruvian Amazon

Tropical Medicine and Infectious Disease, 2024, 9, 98

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects a wide variety of wild and domestic warm-blooded animals (Dubey, 2021). This ubiquitous protozoan is one of the world's most common parasites, infecting an estimated two billion people (Pappas *et al.*, 2009; Petersen & Dubey, 2001). Felids are the definitive hosts of *T. gondii*, while warm-blooded species (birds and mammals, including humans) act as intermediate hosts and become infected with asexual forms (Dubey, 2021). The most common route of infection is oral, which involves ingesting undercooked contaminated meat, raw vegetables, or water contaminated with oocysts (Cook *et al.*, 2000; Montoya, 2002).

Toxoplasmosis is usually asymptomatic in healthy people, but it can be fatal in young, immunocompromised, or congenitally infected individuals (Dubey, 2004; Tenter *et al.*, 2000). Understanding the factors that contribute to the maintenance and spread of *T. gondii* in the environment is critical for developing effective animal and human health management and prevention strategies (Crozier & Schulte-Hostedde, 2014; Jenkins *et al.*, 2015; Thompson, 2013).

The genetic variability of *T. gondii* in Amazonian countries is very high, with several atypical strains causing severe symptoms known as "Amazonian toxoplasmosis" (Carme *et al.*, 2009). Furthermore, the recent introduction of cats into Amazonian communities, combined with a lack of safe water sources, increases the risk of *T. gondii* transmission (Blaizot *et al.*, 2020). Toxoplasmosis has rarely been addressed from a One Health perspective in the Amazonian region and remains a neglected disease (Aston *et al.*, 2014; Dubey, 2021; Vitaliano *et al.*, 2015).

Our study aimed to describe the risk of *T. gondii* transmission at the human–wildlife–domestic interface in an isolated community that relies on subsistence hunting in a well-preserved forest in the Peruvian Amazon. This study will improve our understanding of toxoplasmosis by integrating epidemiological data from humans, wildlife, and domestic and peri-domestic animals in the same area. The study area, which is remote, isolated, and well preserved, could provide representative insights into *T. gondii* ecology, transmission dynamics, and infection risk in neotropical forests.

Materials and Methods

Study Area

The study was carried out in the Yavari-Mirin basin (04º19'53'' S; 71º57'33'' W; UT5: 00), which is a remote area on the Peru–Brazil border with well-preserved terra firme forests in the Peruvian Amazon. The area is rich in biodiversity with up to 150 mammalians and 27 endangered animal species (Bodmer *et al.*, 2009; Pitman *et al.*, 2003b). The climate is typically equatorial, with annual temperatures ranging from 22 to 36 ºC, relative humidity of 80–100%, and annual rainfall of 1500–3000 mm (Bernárdez-Rodríguez *et al.*, 2021). The only village still occupied in the Yavari-Mirin River basin is Nueva Esperanza (Figure II.1), which is a Yagua indigenous community that had 329 residents and 55 households (with a median of 6 people per household) in 2019.

The main activities of the local community are traditional small-scale agriculture, fishing, logging, and subsistence hunting. Although it is uncommon for residents to travel outside of the village, when they choose to travel, it is usually for short trips to nearby locations, which is primarily motivated by trade-related activities (Bernárdez-Rodríguez *et al.*, 2021). In 2012, a case of human ocular toxoplasmosis was diagnosed in this community (Aston *et al.*, 2014).

Blood Samples from Animals and Humans

Figure III.2 shows the experimental design used, including the chronology of the collection of data and biological samples. Between 2010 and 2020, blood samples were collected from 555 wild mammal individuals belonging to 23 different species, including the orders Primates, Rodentia, Carnivora, Cetartiodactyla, Perissodactyla and Cingulata, as part of a wildlife conservation program, taking advantage of the discarded material from legal subsistence hunting. Hunters impregnated either Protein Saver® or FTA® cards (Scheilcher & Schuell, Dassel, Germany) with blood from the cranial or caudal cava veins.

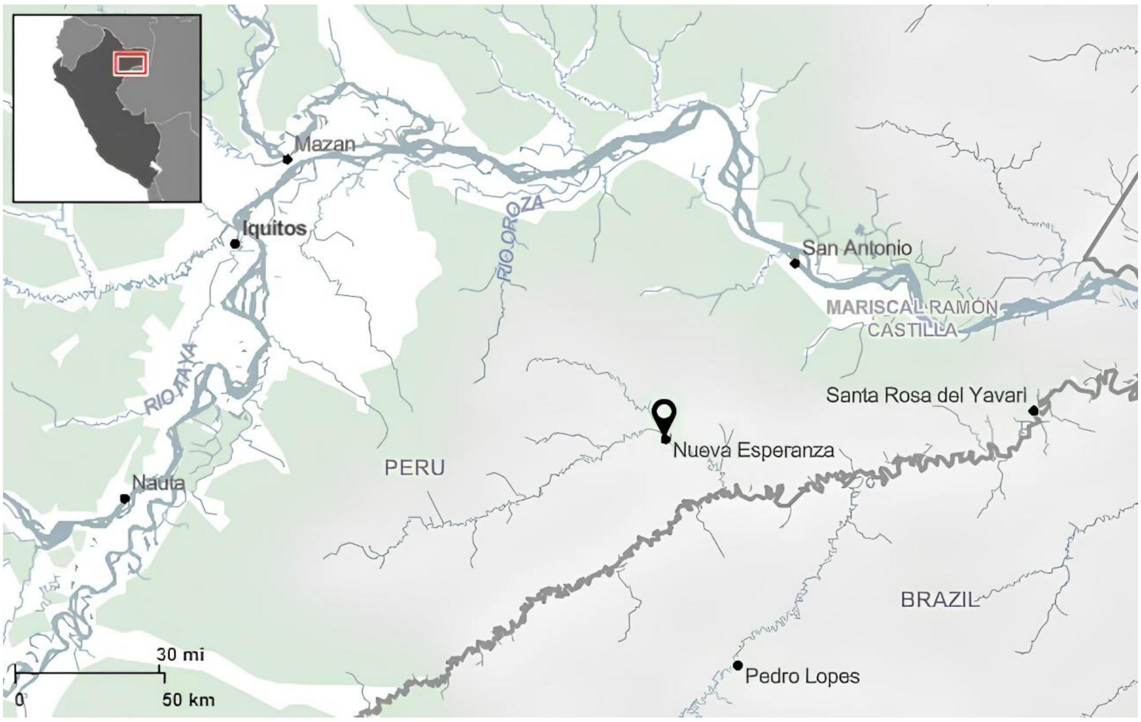

Figure III. 1. Location of the Nueva Esperanza community in the Yavari-Mirin River basin, a remote area on the border between Peru and Brazil, approximately 150 km far from Iquitos, the closest urban center.

This protocol allows for the obtention and maintenance of blood samples from filter paper, which is a low-cost and technically alternative sampling method in areas where it is difficult to sustain an appropriate cold chain (Aysanoa *et al.*, 2017). The sampling was performed in all seasons, and hunters recorded the species, sex, date, and hunting location of each animal. In addition, blood samples from the community's domestic animals (17 dogs and four cats) and small peri-domestic rodents (40 *Rattus rattus* and five *Mus musculus*) were collected in FTA® cards between September 2019 and February 2020. All FTA cards (wildlife, domestic and peri-domestic fauna) were sealed in individual plastic bags with desiccant and stored at room temperature during the stay in Nueva Esperanza (from 15 to 100 days) before being transferred to −70 ∘C for preservation.

In February 2020, clinical examination and whole blood collection were performed by physicians on 132 residents (40.1% of the total population): 81 (61.4%) women and 51 (38.6%) men, aged between 4 and 94, with a median age of 21.0 (10.0–34.0). Serum was extracted from the samples and stored in liquid nitrogen for transport and then stored at −70 ∘C.

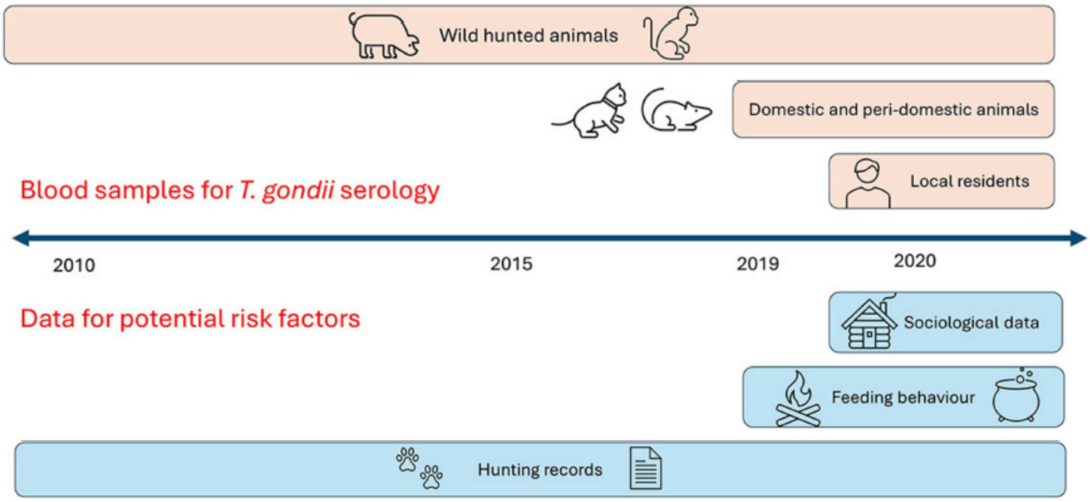

Figure III. 2. Experimental design diagram showing the chronology of biological sample collection and potential risk factor data collection using semi-structured surveys.

Laboratory Procedures

Animal samples were processed by cutting a 132mm^2 area of blood-soaked filter paper and diluting it in 400 µL of sterile phosphate-buffered saline (PBS) (Invitrogen, Barcelona, Spain). Considering that the serum accounts for 40% of the total blood volume (Nobuto, 1963) and the blood concentration on the paper is 40 µL/cm2, a serum dilution of 1:20 was obtained. These samples were vortexed for 20 s and then stored at 4 ∘C for 24 h before being vortexed again for 20 s and frozen at −20 ∘C until analysis. These elutions were tested for antibodies against *T. gondii* using the ID Screen® Toxoplasmosis IgG Indirect Multispecies ELISA kit (IDvet, Montpellier, France).

For human samples, IgG serostatus against *T. gondii* was determined using an in-house enzyme-linked immunosorbent assay (ELISA) validated with the IBL International® kit (Steinberg *et al.*, 2021). IgM levels were measured using a chemiluminescent microparticle immunoassay (CMIA, Alinity Toxo IgM kit—Abbott). IgM antibodies usually indicate recent exposure to T. gondii and first appear 1–2 weeks after infection. IgG antibodies, which indicate past exposure, appear 1 to 3 weeks after IgM, persisting for 12–24 months or even decades, and on occasion are considered lifelong (Joynson & Guy, 2001).

Social Structure, Hunting and Feeding Characteristics as Potential Risk Factors

In February 2020, family-based interviews were conducted to gain insights into the behavior and dietary habits of the community in order to determine significant risk factors associated with *T. gondii* infection. The semi-structured surveys were divided into sections that addressed different people based on their activities within the community and households. As a result, response rates varied depending on the question. Due to the low individual correspondence between serological results and behavioral factors, no association was made between risk factors and *T. gondii* serology.

A) Sociological Data

The semi-structured surveys were conducted on 84 heads of families from 42 different households (76.4% of total households). The survey included 47 (55.9%) women and 37 (44.1%) men with a median age of 32 years old (ranging from 18 to 77). Outdoor activities, particularly those related to hunting (number of hunters per household, frequency, animal handling safety measures, and wild meat handling), and human–animal interaction outside and within households (number and species of animals raised at home, presence of cats) were described (Table A4). Additionally, field observations related to the human–animal interface, the handling of wild meat, and events related to the health of people living in the community were registered.

B) Hunting Registers and Preference of Wild Meat Consumption

Between 2010 and 2020, an annual average of 14.3 hunters recorded all hunted prey (median of 11, ranging from 7 to 33). Hunting registers included the number, species, and sex of hunted animals as well as the day and location of the hunt. This information was used to calculate the yearly count of prey hunted and consumed by residents and estimate the consumption of animals infected with *T. gondii* per household, considering only the seroprevalences found in species with a minimum of five hunting records. Also, 14 local hunters were surveyed on their prey and the wild meat flavor preferences of all recorded hunted species (n = 25), ranking them on a progressive scale from 1 (highest) to 25 (lowest) preference.

C) Feeding Behavior

Two surveys were conducted with adult residents in charge of cooking. The first recorded the daily primary dietary intake of seven randomly selected families (12.7% of all households) over 2.43 ± 1.99 months (from January to April 2019, and September to December 2019), totaling 513 meals. The participants in the second semi-structured survey were 60 adult residents, with 39 (65.0%) women and 21 (35.0%) men, with a median age of 33 years old (ranging from 18 to 73), and the survey included questions about water sources and handling, processing, preservation, and consumption of wild meat (Table A5).

Statistical Analyses

D) Wildlife

Species from all orders with a sample size n > 3 were used to assess the relationship between antibody presence against *T. gondii* using pairwise Fisher's tests comparisons with Bonferroni correction. A generalized linear mixed model (GLMM) was used to explore relationships between *T. gondii* antibody results in wildlife and their biological and ecological variables. The serological result (negative or positive) was used as a response variable. "Habitat" (terrestrial/arboreal) and "diet" (herbivorous/frugivorous/omnivorous/carnivorous) were used as fixed explanatory variables. Given that the variables “habitat”, “weight” and “diet” are associated with the species rather than the individuals, we supplemented the variable “species” as a random effect.

E) Humans

A GLM was used to investigate the effect of age and sex on the serological result for IgG and IgM antibodies against *T. gondii*. The serostatus of the individual (positive/negative) was the response variable, whereas the interaction between their age (in years) and their gender (male/female) were the explanatory variables. Pearson correlation was used to assess whether the mean hunting preference or taste predilection among hunters were correlated with the seroprevalence of the species. For interviews and surveys related to dietary consumption patterns and human behaviors, descriptive statistics were conducted, presenting results as mean ± standard deviation or median and quartile values whenever possible. All data were analyzed using R 4.2.2 (R Core Team, 2023). All significance for hypothesis testing was considered at a Type I error of probability of 0.05.

Results

Wildlife and Domestic/Peri-Domestic Animals

The overall seroprevalence of *T. gondii* infection in wildlife was 30.45% (169/555; 95% CI 26.8–34.4%). Of the 23 wild species evaluated, we detected at least one seropositive individual in 17, including species from all the analyzed orders: Primates, Rodentia, Cetartiodactyla, Perissodactyla, Carnivora, and Cingulata (Table III.1).

Pairwise Fisher tests did not reveal any significant difference in the seroprevalence among the different taxonomic orders ($p > 0.05$). However, the GLMMs revealed that “habitat” was a statistically predictive value for the prevalence of *T. gondii* ($p = 0.0285$, Estimate ± Std. Error: −0.903 ± 0.41, z value: −2.19). Terrestrial animals presented a higher seroprevalences of antibodies against *T. gondii* compared to arboreal species.

Table III. 1. Occurrence of IgG antibodies against *Toxoplasma gondii* among wild mammals hunted in the indigenous community (Peruvian Amazon) between 2010 and 2020.

Order, Family	Species	Tested	Positive (%)	95% CI
O. Carnivora		**22**	**2 (9.1%)**	**2.5–27.8%**
Felidae	*Leopardus pardalis*	1	0 (0.0%)	0.0–79.4%
	Panthera onca	2	0 (0.0%)	0.0–65.8%
Procyonidae	*Nasua nasua*	19	2 (10.5%)	2.9–30.4%
O. Cingulata		**38**	**17 (44.7%)**	**30.2–60.3%**
Dasypodidae	*Dasypus novemcinctus*	38	17 (44.7%)	30.2–60.3%
O. Primates		**155**	**39 (25.2%)**	**19.0–32.5%**
Atelidae	*Alouatta seniculus*	3	1 (33.3%)	6.2–79.2%
	Ateles chamek	20	3 (15.0%)	5.2–36.0%
	Lagothrix l. poeppigii	66	15 (22.7%)	14.3–34.2%
Pitheciidae	*Cacajao clavus*	16	1 (6.25%)	1.1–28.3%
	Plecturocebus cupreus	4	1 (25.0%)	4.6–69.9%
	Pithecia monachus	6	0 (0.0%)	0.0–39.0%
Callitrichidae	*Leontocebus fuscicolis*	1	0 (0.0%)	0.0–79.4%
Cebidae	*Cebus albiforns*	7	3 (42.9%)	15.8–75.0%
	Sapajus macrocephalus	32	15 (46.9%)	30.9–63.6%
O. Rodentia		**148**	**59 (39.9%)**	**32.3–47.9%**
Cuniculidae	*Cuniculus paca*	139	57 (41.0%)	33.2–49.3%
Dasyproctidae	*Dasyprocta fuliginosa*	6	1 (16.7%)	3.0–56.4%
Caviidae	*Galea musteloides*	1	0 (0.0%)	0.0–79.4%
	Hydrochoerus hydrochaeris	1	1 (100%)	20.7–100%
Sciuridae	*Sciurus igniventris*	1	0 (0.0%)	0.0–79.4%
O. Cetartiodactyla		**171**	**48 (28.1%)**	**21.9–35.2%**
Cervidae	*Mazama americana*	51	13 (25.5%)	15.6–38.9%
	Mazama nemorivaga	1	1 (100%)	20.7–100%
Tayassuidae	*Pecari tajacu*	65	18 (27.7%)	18.3–39.6%
	Tayassu pecari	54	16 (29.6%)	19.1–42.8%
O. Perissodactyla		**21**	**4 (19.1%)**	**7.7–40.0%**
Tapiridae	*Tapirus terrestris*	21	4 (19.1%)	7.7–40.0%
Total		**555**	**169 (30.45%)**	**26.8–34.4%**

Domestic animals had seroprevalence rates of 94.1% (16/17, 95% CI 73.2%–98.9%) in dogs and 100% (4/4, 95% CI 51–100%) in cats. In peri-domestic animals, *Rattus sp*. presented seroprevalences of 10.0% (4/40, 95% CI 3.9–23.1%), and all *Mus musculus* were negative (0/5, 95% CI 0–43.5%).

Humans

Overall, 82.6% (109/132, 95% CI 75.2–88.1%) of the Nueva Esperanza residents tested positive to the IgG ELISA, while 6.1% (8/132, 95% CI 3.1–11.5%) tested positive for IgM. All IgM reactive samples also tested positive for IgG. The GLMs indicated that the age of individuals was positively associated with the detection of IgG against *T. gondii* (Estimate ± SE = 0.07 ± 0.03, z value: 2.33, $p = 0.019$; Figure III.3) but not with the detection of IgM ($p > 0.05$). The youngest inhabitant with IgG was 5 years old, the average age with IgG was 26 years (26.2 ± 17.7), and the median was 23 years. For IgM antibodies, the average age was 23.5 years old (23.5 ± 20.9), with a median of 10 years old, ranging from 6 to 60 years.

There was no significant effect of sex nor the interaction between sex and age ($p > 0.05$) on the serology of IgG nor IgM.

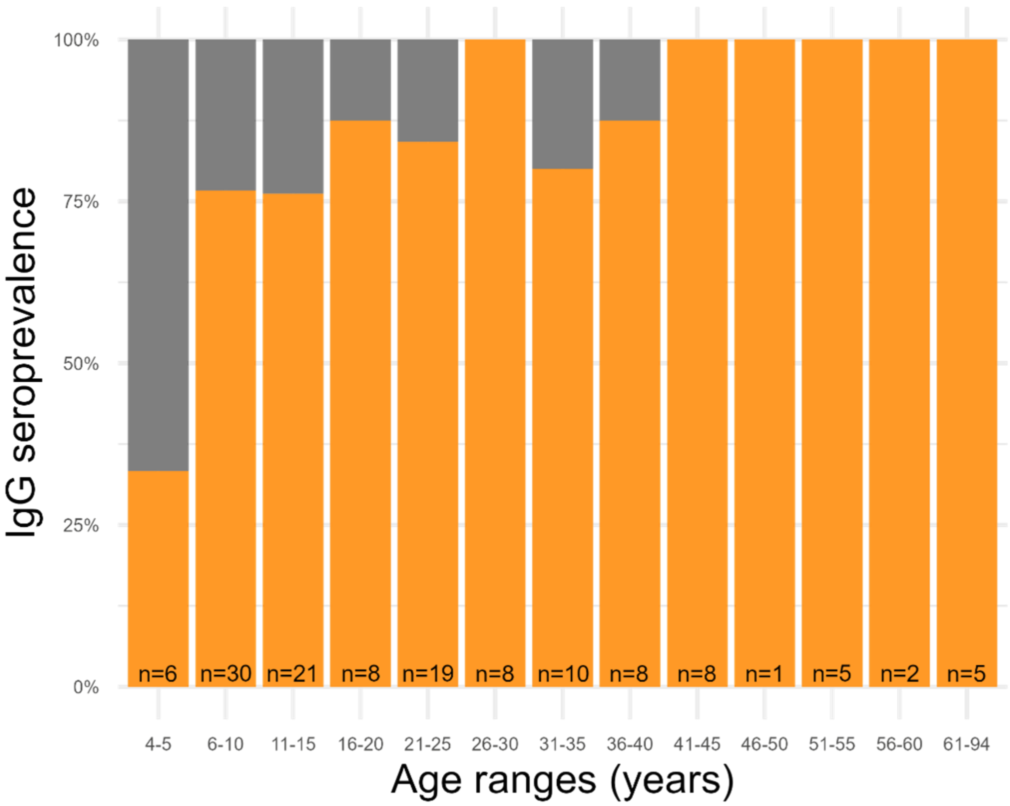

Figure III.3. Distribution by age of IgG seroprevalence in residents of the indigenous community (Peruvian Amazon). Blood samples were collected in February 2020.

Risk Factors

F) Sociological Data

Semi-structured surveys revealed that each household had a median of one hunter per household (range from 0 to 2) with a median of hunting frequency of two days per week (range from 0 to 7). A minority (17.7%, 3/17) of the hunters interviewed used dogs to aid in their hunting. None (0%, 0/17) of the hunters surveyed mentioned employing any safety measures (e.g., wearing protective gloves and clothes when handling the hunted animals). The hunters clean the carcass and remove the viscera either in the field (50.0%, 8/16), at home (6.25%, 1/16), or, depending on the animal's size, in both locations (43.8%, 7/16).

Concerning the interaction with domestic animals, 85.7% (36/42 households) reported having animals at home, including chickens or ducks (81.0%, 34/42 households), dogs (71.4%, 30/42 households), and cats (19.1%, 8/42 households). It was also reported that the first domestic cat was introduced in 2014. Additionally, in 21.4% (9/42) of households, captive wild species as pets were reported, particularly parrots (six households), peccaries (two households) and monkeys (one household). Some households fed their domestic animals with uncooked offal from hunted animals; for instance, dogs and cats (73.8%, 31/42 households) or chickens (59.5%, 25/42 households).

All interviewed heads of families (42/42 households) reported the presence of peridomestic rodents; 88.1% (37/42 households) reported nests and mouse feces in their house, while 73.8% (31/42 households) mentioned food contaminated with mouse feces. Additionally, identifying rodent sounds at night in the kitchen and food storage was very common, and peri-domestic rodents were captured in all the houses where the traps were placed.

G) Hunting Records

From 2010 to 2020, local hunters reported a total of 3204 animals, including 2556 (79.8%) mammals from 24 species, 630 (19.7%) birds from 12 species and 18 (0.56%) reptiles from five species.

Considering the serological results for *T. gondii* from our study and the median of one hunter per household, we calculated the estimated number of infected and annually consumed animals per household in Nueva Esperanza. Of the total number of hunted animals registered, it is estimated that 792.8 infected animals were consumed during this 10-year span (Table A6). Considering at least one hunter per household, these findings correspond to approximately 14 (25.45%) of the 55 families in Nueva Esperanza. Therefore, it can be inferred that around 5.67 infected animals could have been annually consumed per family. The species exhibiting a higher estimated annual consumption of *T. gondii*-infected meat (individuals/household) were *Cuniculus paca* (n = 2.79), *Pecari tajacu* (n = 0.62), *Tayassu pecari* (n = 0.44), *Lagothrix l. poeppiggi* (n = 0.43), *Mazama americana* (n = 0.41), and *Dasypus novemcinctus* (n = 0.26). None of the variables associated with hunting behavior (prey preference and taste) showed significant associations with *T. gondii* seroprevalence.

H) Feeding Behaviors

According to the family dietary daily registers, the main source of animal protein was fish, with presence in 50.9% (261/513) of total meals recorded, followed by wild meat (37.8%; 194/513), chicken (9.9%; 51/513), and canned meat (1.4%; 7/513). All wild animals were hunted by men (100.0%, 108/108), whereas fish were predominantly obtained by women (69.3%, 158/228). On a daily basis, each inhabitant consumed 189 ± 139 g (45.5%) of fish, 158 ± 30 g of wild meat (38.0%), and 69 ± 78 g of chicken (16.5%), including non-consumable elements of the meat such as bones, spines, and scales, among others. When asked how they obtained wild meat, mothers from households reported that 60.0% (128/194) of the consumed animals were hunted by the house hunter, 20.6% (40/194) were gifts from relatives or neighbors, and 13.4% (26/194) of the wild animals were purchased.

According to the interviewed inhabitants, the meat was cooked at high temperature (54.8%, 23/42 households), undercooked (11.9%, 5/42 households), or both ways (19.0%, 8/42 households). Offal was consumed by 60.8% (31/51) of local inhabitants, the heart being the most consumed organ (51.0%, 26/51), followed by the liver (47.1%, 24/51), kidneys (15.7%, 8/51), and lungs (9.8%, 5/51). The most frequent method of preserving meat that was not consumed immediately was keeping it fresh (92.9%, 39/42 households), which was followed by drying/salting (66.7%, 28/42 households) and smoking (54.8%, 23/42 households). On the other hand, the water utilized for cooking is predominantly drawn from rivers (28/34, 82.4% households), with rainwater (18/34, 52.9% households) and stream water (2/34, 5.9% households) accounting for smaller proportions. The disposal of wastewater after cooking is carried out in the household yard (76.5%, 26/34 households), in the river (26.5%, 9/34 households), through rustic drainage systems (8.8%, 3/34 households), or in the forest (2.9%, 1/34 households).

Discussion

Food-borne zoonoses in subsistence-based communities in tropical forests are considered neglected infectious diseases, as evidenced by the scarcity of research studies on this specific wildlife–human interface. Recently, some authors have pointed out the necessity of increasing research efforts into the impact of infectious diseases on public health and wildlife conservation in the Amazon region (Van Vliet *et al.*, 2022). The present study evidences the wide circulation of *T. gondii* in wildlife, domestic/peri-domestic animals, and humans in a remote indigenous community of the Peruvian Amazon with a highly interactive human–wildlife interface.

The prevalence of antibodies found in the studied wildlife varied greatly between Orders and was consistent with previous studies from other Amazon areas (Carme *et al.*, 2002; Furtado *et al.*, 2015) with the exception of the surprisingly low prevalence of infection observed in the Order Carnivora (Furtado *et al.*, 2015; Minervino *et al.*, 2010). This low sample prevalence in carnivores, particularly wild felids, could be due to the small number of animals sampled, as the local population does not consume them. Specifically, the few wild felines sampled in our study were killed due to conflicts with humans in Nueva Esperanza. Our results indicate that wild terrestrial animals are more exposed to *T. gondii* than arboreal mammals. As felines serve as definitive hosts of the parasite and shed oocysts in their feces, terrestrial species are expected to be infected more frequently (Carme *et al.*, 2002). Nonetheless, we also observed high seroprevalences (around 25%) in non-human primates, suggesting that monkeys are also exposed to *T. gondii*. The diets of the primate species studied are primarily frugivorous and supplemented with terrestrial arthropods (de Souza Jesus *et al.*, 2022; 2023). However, non-human primates occasionally access the ground to obtain nutritional supplements from soils, particularly from mineral licks (Blake *et al.*, 2010; Link *et al.*, 2011), where parasites and diseases spread more widely in wildlife due to high animal density, the variety of species, and the accumulation of feces and urine (He *et al.*, 2022; Johnson *et al.*, 2011).

The serological analyses of domestic and peri-domestic animals showed a high *T. gondii* circulation within the community. The very high prevalence observed in dogs and cats is even higher than that reported in rural communities in the Brazilian Amazon (80% in cats, >50% in dogs) (Carme *et al.*, 2002; Cavalcante *et al.*, 2006a; Minervino *et al.*, 2012). Cats are commonly kept outdoors in rural areas, which contributes to *T. gondii* oocyst contamination of the environment (Ferreira *et al.*, 2009; Morais *et al.*, 2021). In addition, the detection of *T. gondii* antibodies in peri-domestic rodents indicates a wider contamination of immediate household surroundings (Ferraroni *et al.*, 1980).

The high seroprevalences observed in wildlife and the clinical case of human ocular toxoplasmosis diagnosed in the community (Aston *et al.*, 2014), both prior to the introduction of cats in the community in 2014, suggest the existence of a sylvatic cycle of the parasite, as previously reported in other regions of Amazonia. However, the emergence of a domestic cat population in the community may have altered the epidemiology of the parasite, making these felines an important player in the maintenance and dissemination of *T. gondii* at the community level, and favoring the establishment of a new domestic cycle of *T.*

gondii, as demonstrated in rural areas of Neotropical regions (Mercier *et al.*, 2011). Therefore, the co-existence of both "wild" and "domestic" cycles may pose an additional risk to human health due to the gene flow between "wild" and "domestic" types of *T. gondii* (Mercier *et al.*, 2011). Thus, future studies are needed to determine the infective types associated with the "wild" and "domestic" cycles of *T. gondii* in the Amazon.

The absence of proper hygienic practices during the handling of wild meat, such as excluding domestic animals from butchery areas, not using personal protective equipment, and not designating surfaces for butchery have been observed in indigenous communities (Tumelty *et al.*, 2023). These practices, combined with cooking practices that involve undercooked or rare meat, as well as the consumption of animal organs, are significant contributors in documented cases of toxoplasmosis in Amazon communities (Blake *et al.*, 2010; Carme, 2001).

The present study confirms this previous research, assessing a high IgG seroprevalence in local residents of the community under study. Our findings are also consistent with previous studies where the rate of IgG seroprevalence increases with age, which is probably due to an increased likelihood of exposure to the parasite over time (Cavalcante *et al.*, 2006a; Vitaliano *et al.*, 2015). We also found that initial infections typically occur at a young age, before the age of five, and that the infection rate in children between six and ten can reach 75%. In contrast, there were no significant statistical differences in *T. gondii* IgG seroprevalence between genders, which is probably due to the equal rates of wild meat consumption in men and women (Blaizot *et al.*, 2020). Moreover, the IgM results and the absence of statistical differences between age groups suggest the existence of recurrent infections in the community. The results obtained from the IgG and IgM tests were not confirmed, so we cannot rule out false positives.

Study IV

A Survey of Hepatitis B Virus and Hepatitis E Virus at the Human–Wildlife Interface in the Peruvian Amazon

Microorganisms 2024, 12, 1868

Introduction

Hepatitis B virus (HBV) and Hepatitis E virus (HEV) are critical health concern worldwide, particularly in low- and medium-income countries (LMIC) (Grimm *et al.*, 2011; Purcell & Emerson, 2008; WHO, 2017). In particular, in rural Amazonia, diverse areas of high endemicity for HBV and HEV have been identified, exacerbated by elevated levels of poverty and limited access to healthcare (Cabezas *et al.*, 2020a; Costa &Kimura, 2012; Vasconcelos *et al.*, 2024).

HBV, a DNA virus from the Family Hepadnaviridae, affects around one-third of the world population (Carey, 2009), and is responsible for approximately one million deaths annually due to cirrhosis, liver failure, hepatocellular carcinoma (Dienstag, 2008), and immune dysfunction linked to the intensification of other viral infections (Zhao *et al.*, 2022). For decades, HBV has been hyperendemic in Amazonian rural populations, with a prevalence of over 50%, resulting in chronic active hepatitis and liver cirrhosis (Braga *et al.*, 2001; 2012; Cabezas & Braga, 2020; Costa & Kimura, 2012; Viana *et al.*, 2005). Transmission in these communities primarily occurs through direct contact with infected bodily fluids—including sexual intercourse and perinatal transmission—getting tattooed and contact with non-indigenous populations [Costa &Kimura, 2012; Ormaeche *et al.*, 2012; Viana *et al.*, 2005).

This virus often exhibits genetic variants that are linked with specific hosts, but some studies indicate the presence of HBV and HBV-like in different wild mammals, suggesting the virus's ability to infect and share hosts within their natural habitats (Bonvicino *et al.*, 20014; Vartanian *et al.*, 2002; Li *et al.*, 2010; Vieira e al., 2015). While HBV transmission from wildlife reservoirs—often linked to bat bites, frequent in communities that regularly interact with wildlife—has been proposed and transmission between species of primates has been suggested (Bonvicino *et al.*, 2014; Cabezas *et al.*, 2006), the transmission in rural communities with frequent contact with wildlife remains understudied (Costa &Kimura, 2012).

HEV, an RNA virus from the Family Hepeviridae, is the main cause of acute viral hepatitis in humans worldwide and is associated with large outbreaks and epidemics in LMIC (Meng, 2009). HEV is an emerging food-borne pathogen, transmitted through the handling and consumption of raw and undercooked infected meat and meat-derived products (Harrison & DiCaprio, 2018). Acute outbreaks have also been reported in rural Amazonian communities due to lack of access to potable water and inadequate sanitation (WHO, 2017).

In South America, rural communities present seroprevalences ranging from 2.1% to 17% (Fernandez *et al.*, 2022; Pisano *et al.*, 2018). However, essential epidemiological aspects related to human zoonotic transmission still remain underexplored (Pisano *et al.*, 2018; Vasconcelos *et al.*, 2024). Domestic pigs and wild boars are considered the main reservoirs of HEV worldwide; however, the virus has also been identified in a wide range of wildlife species (Ahmed & Nasheri, 2023; Moraes *et al.*, 2021). In Amazon rural communities, where animal farming and consumption of processed pork products are uncommon, there is a potential risk of HEV exposure from consuming infected wild meat or water and from direct contact with wild animals during hunting activities (Vasconcelos *et al.*, 2024).

Despite the importance of HBV and HEV in the health of Amazonian rural communities, the functional role of wild mammals in HBV and HEV transmission through complex wildlife–human interactions facilitated by hunting and wild meat manipulation remains underexplored (Cunha *et al.*, 2023; Mirazo *et al.*, 2018; Vieira *et al.*, 2019).

The present study aimed at evaluating HBV and HEV circulation in the human–wildlife interface and identifying risk factors and behaviors using a sociological analysis in an indigenous community that relies on subsistence hunting in a well-conserved and isolated area of the Peruvian Amazon.

Materials and Methods

Study Area

This study was conducted in the Yagua indigenous community of Nueva Esperanza, located in the Yavarí-Mirín River basin (04º19'53'' S; 71º57'33'' W; UT5: 00), a geographically isolated and well-preserved forest along the border between Brazil and Peru in the Peruvian Amazon (Figure IV.1) (Pitman *et al.*, 2003a). This community of 370 people relies on a subsistence economy based on small-scale agriculture, hunting, and fishing (Aston *et al.*, 2014; Mayor *et al.*, 2015). The absence of feral pigs and livestock eliminates the possibility of disease transmission from these domestic sources (Aston *et al.*, 2014).

Hepatitis-related mortality has been observed in other rural communities in the Yavarí River basin for over fifteen years, and since 2001, they have been grappling with a severe HBV outbreak (Nascimento, 2008); 22 individuals died between 2001 and 2004, some with confirmed HBV and others with symptoms of hepatitis (Centro de Trabalho Indigenista, 2004). However, the lack of laboratory confirmation and serological data hinders authorities from addressing this situation (Nascimento, 2008). Immunity against HBV in indigenous communities bordering Brazil and Peru in the Yavarí River basin is notably low due to lack of vaccination programs or significant delays between doses (Nascimento, 2008).

In the Peruvian Amazon, studies show that vaccination records are not well registered and serological tests do not align with reported vaccinations (Cabezas *et al.*, 2020b). In the studied community, the indigenous population does not report any specific vaccination program against HBV, suggesting that the local population has not been appropriately vaccinated.

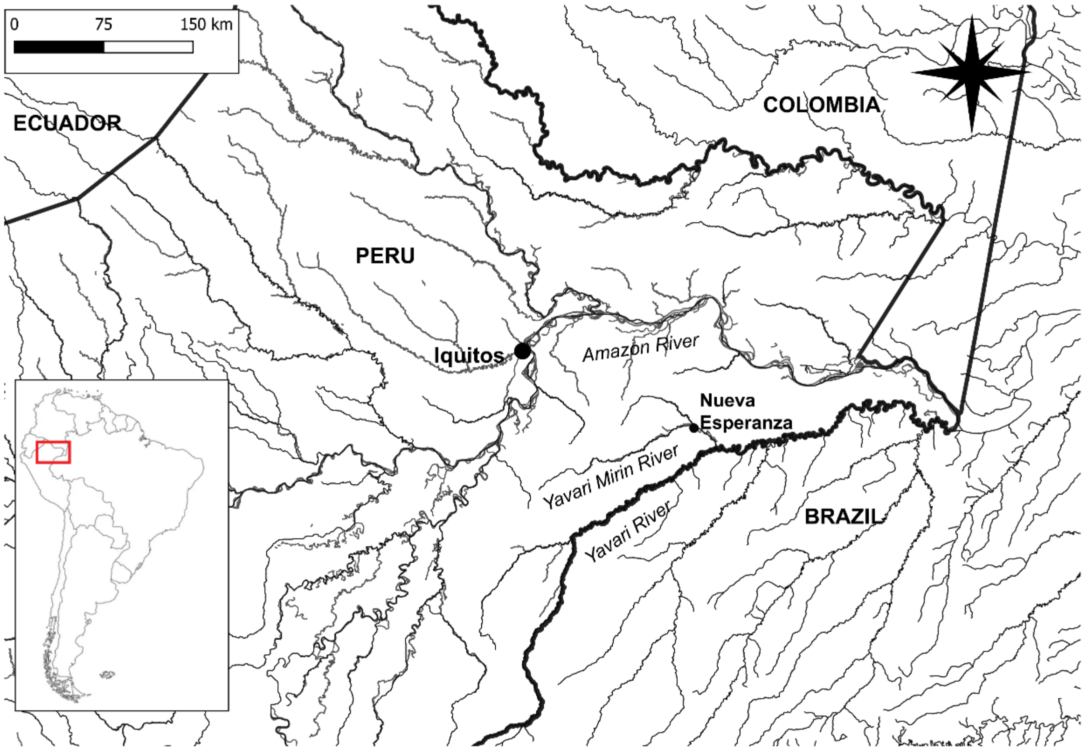

Figure IV.1. Location (red square) of the Nueva Esperanza community in the Yavari-Mirin River basin, a remote area on the border between Peru and Brazil, approximately 150 km far from Iquitos, the closest urban center. There is no accessibility to the study area through roads, only by river.

Blood Sample Collection

The blood sampling of wildlife took advantage of the discarded material from subsistence hunting, allowing for an extensive collection of 431 wild animals consumed by the local inhabitants between 2008 and 2020, including 125 peccaries (62 *Tayassu pecari* and 63 *Pecari tajacu*), 122 pacas (*Cuniculus paca*), 83 brocket deers (74 *Mazama americana* and nine *Mazama nemorivaga*), 66 primates (*25 Lagothrix poeppigii*, 15 *Sapajus macrocephalus*, 6 *Ateles chamek*, 6 *Pithecia monachus*, 5 *Cacajao calvus*, 5 *Cebus albifrons*, 2 *Saimiri macrodon*, 1 *Alouatta seniculus*, and 1 *Plecturocebus cupreus*), and 35 tapirs (*Tapirus terrestris*). In September 2019 and February 2020, a total of 43 peri-domestic rodents (38 rats and 5 mice) were also sampled. Blood samples were collected on either Whatman filter paper n. 3 or FTA® cards (Scheilcher & Schuell, Dassel, Germany), preserved from 15 to

100 days in the community and later transported and stored at −70 ºC, as previously reported (Study III; Morales *et al.*, 2017).

Whole blood samples were also collected from 88 local residents, including 35 men and 53 women (39.8% vs. 60.2%, respectively), aged between 5 and 79 years, with a median age of 25 (10.0–34.0). Serum was extracted from the samples, stored in liquid nitrogen for transport, and then stored at −70 ◦C until laboratory analysis.

Laboratorial Procedures

Blood-soaked filter papers containing the animal samples were processed by cutting a 132 mm2 piece, which was eluted in 400 µL of sterile phosphate-buffered saline (PBS) before vortexing for 20 s. The samples were stored at 4 ◦C for 24 h, then vortexed and frozen at −20 ◦C until analysis. The elutions from wild and peri-domestic animals and the human serum samples were tested for antibodies against HBV core antigens (HBcAbs) using the commercial ELISA kit "Human anti-hepatitis B virus core antibody" (Cusabio, Wuhan, China) (He *et al.*, 2019). HBcAbs are used to detect past or current HBV infections but does not indicate immunity from vaccination (Cusabio, 2024). IgG antibodies against HEV were screened using the "ID Screen® Hepatitis E Indirect Multi-species" kit (IDvet, Montpellier, France) for samples from wild and peri-domestic animals. For human blood samples, analysis for HEV antibodies was performed using the "Human hepatitis E virus antibody (IgG)" kit from Cusabio (Wuhan, China). Both commercial ELISA kits against HBcAb and HEVAb had intra- and inter-assay precision of CV% < 15%.

A recent study using the same samples on Whatman filter paper n. 3 and FTA® cards revealed that only the DNA quantity and quality were adequate for molecular tests, whereas RNA was likely of limited use for viral pathogen research (Li *et al.*, 2024). For this reason, only HBV DNA was extracted from the samples using the IndiMag Pathogen kit (Indical Bioscience, Leipzig, Germany), and the quality and quantity of DNA were determined using the Qubit dsDNA BR Assay Kit (Fisher Scientific, Waltham, MA, USA). A qPCR was conducted to detect HBV DNA using Promega PCR Master Mix, following the manufacturer's instructions (Promega Corporation, Madison, WI, USA). The PCR analyzed the pre-S2/S region using the following primer pair and fluorescent probe purchased from Integrated DNA Technologies (IDT): forward primer (50-GAATCCTCACAATACCGCAGAGT-30), reverse primer (50-GCCAAGACACACGGGTGAT-30), and probe (50-FAM-AAGTCCACCACGAGTCTAGNFQ/ MGB-30) (Ahmed & Nasheri, 2023). A plasmid carrying a human HBV 1.3mergenomewas used as a positive control. Given budget limitations, only 68 samples were analyzed, including all seropositive animals and humans.

Interviews for Risk Assessment

In 2020, we used semi-structured surveys to collect data on habits and activities in the community to identify potential risk factors related to HBV and HEV infections. Interviews were conducted for 84 heads of families (47 (55.9%) women and 37 (44.1%) men, aged 18 to 77) from 42 different households (76.4% of total households). The questions were focused on activities usually related to the transmission of HBV, HEV, and other common bloodborne or foodborne pathogens, such as outdoor activities, especially hunting, but also contact with animals, the presence of domestic animals in households, and meat preparation and processing, as highlighted in previous studies (Bonvicino *et al.*, 2014; Cabezas & Braga 2020; Costa & Kimura, 2012; Douglas *et al.*, 2024; Ormaeche *et al.*, 2012; van Vliet *et al.*, 2022; Vasconcelos *et al.*, 2024; Viana *et al.*, 2005). The semi-structured surveys were divided into sections addressed to people based on their roles and activities within the community (See "Results section" for the detailed questions of the survey). Consequently, the number of respondents varied across different survey questions. By analyzing this information, we aimed to highlight practices that may expose individuals to HBV and HEV, allowing us to better understand the virus transmission and design appropriate prevention strategies.

Statistical Analysis

A Generalized Linear Model (GLM) was employed to analyze the correlation between HBV and HEV antibody presence in wildlife and explanatory factors including species, habitat, and diet. The serological status (negative or positive) was utilized as the response variable, while "Habitat" (terrestrial/arboreal) and "Diet" (herbivorous/frugivorous/omnivorous/ carnivorous) were considered as fixed explanatory variables.

Two GLMs were employed to examine the effect of age (in years) and sex (and their interaction) in relation to the serological results for HBV and HEV in humans. The response variable was the serological result, categorized as positive or negative. Model selection was based on the Akaike Information Criterion (AIC) (Burnham & Anderson, 2002).

GLM was also used to analyze the data collected in surveys on habits and activities in the community, with the aim of identifying potential risk factors related to HBV and HEV seropositivity. The serological status (negative or positive) was utilized as the response variable.

All data analyses were performed using R 4.2.2 (R core Team, 2024), and we considered a Type I error probability of 0.05 for hypothesis testing.

Results

Serological Analysis

HBcAbs were only detected in three wildlife species: *Cuniculus paca* (0.8%; 1/122, 95% CI 0.1–0.5%), *Tayassu pecari* (1.6%; 1/62, 95% CI 0.3–8.6%), and *Mazama americana* (4.1%, 3/74; 95% CI 1.4–11.3%). The rest of the samples from wild animals were negative (Table IV.1). The serology of peri-domestic rodents was also negative, including rats (0/38; 0.0% CI 0.0–9.2%) and mice (0/5; 0.0% CI 0.0–43.5%). No significant association was observed between the HBV seroprevalence and biological or ecological factors ($p > 0.05$).

Table IV.1. Seroprevalence of HBcAbs among wild mammals hunted in Nueva Esperanza community (Peruvian Amazon) between 2008 and 2020.

Order, Family	Species	Tested	Positive (%)	95% CI
O. Primates		**66**	**0 (0.0%)**	**0.0–5.5%**
Atelidae	*Alouatta seniculus*	1	0 (0.0%)	0.0–79.4%
	Ateles chamek	6	0 (0.0%)	0.0–39.0%
	Lagothrix l. poeppigii	25	0 (0.0%)	0.0–13.3%
Pitheciidae	*Cacajao clavus*	5	0 (0.0%)	0.0–43.5%
	Plecturocebus cupreus	1	0 (0.0%)	0.0–79.4%
	Pithecia monachus	6	0 (0.0%)	0.0–39.0%
Callitrichidae	*Saimiri macrodon*	2	0 (0.0%)	0.0–65.8%
Cebidae	*Cebus albiforns*	5	0 (0.0%)	0.0–43.5%
	Sapajus macrocephalus	15	0 (0.0%)	0.0–20.4%
O. Rodentia		**122**	**1 (0.8%)**	**0.1–0.5%**
Cuniculidae	*Cuniculus paca*	122	1 (0.8%)	0.1–0.5%
O. Cetartiodactyla		**208**	**4 (1.92%)**	**0.75–4.8%**
Cervidae	*Mazama americana*	74	3 (4.1%)	1.4–11.3%
	Mazama nemorivaga	9	0 (0.0%)	0.0–29.9%
Tayassuidae	*Pecari tajacu*	63	0 (0.0%)	0.0–5.8%
	Tayassu pecari	62	1 (1.6%)	0.3–8.6%
O. Perissodactyla		**35**	**0 (0.0%)**	**0.0–9.9%**
Tapiridae	*Tapirus terrestris*	35	0 (0.0%)	0.0–9.9%
Total		**431**	**5 (1.16%)**	**0.5–2.7%**

In addition, HBcAbs were detected in 9.1% (8/88; 95% CI 4.7–16.9%) of the human samples. The models with the lowest AICs among all models were considered, specifically those with AIC differences of less than two, which included the models' age (Akaike weight by age = 0.43), sex (Akaike weight by sex = 0.22), and 'Sex + Age' (Akaike weight by Sex + Age = 0.24) (Table IV.2); however, no significant association was observed between the HBV seroprevalence and age or sex ($p > 0.05$).

Table IV.2. Candidate models considered in the study for HBV serology.

Candidate Models	k	AIC	Delta	w
Age	2	54.53	0.00	0.43
Sex	2	55.91	1.38	0.22
Sex + Age	3	55.74	1.21	0.24
Sex * Age	4	57.49	2.96	0.10

Antibodies against HEV were not found in wildlife (0.0%, 0/431, 95% CI 0.0–0.9%) or peri-domestic animals (0.0%, 0/43, 95% CI 0.0–8.2%), but were detected in 17.1% (15/88, 95% CI 10.6–26.4%) of humans. The models with the lowest AICs among all models were considered, specifically those with AIC differences of less than two, which included the models' age (Akaike weight by age = 0.50) and Sex + Age (Akaike weight by Sex + Age = 0.33) (Table IV.3). The frequency of HEV seropositivity in humans increased with age (Estimate = 0.045, Std. Error = 0.018, z value= 2.264, p = 0.0104), but the influence of the sex of individuals was not statistically significant ($p > 0.05$).

Table IV.3. Candidate models considered in the study for HEV serology.

Candidate Models	k	AIC	Delta	w
Age	2	77.17	0.00	0.50
Sex	2	81.48	4.31	0.06
Sex + Age	3	77.99	0.82	0.33
Sex * Age	4	80.08	2.91	0.12

Molecular Analysis

The sixty-eight samples analyzed by conventional PCR to detect HBV DNA included sixty seronegative samples (twenty *Cuniculus paca*, twenty *Mazama americana*, ten *Pecari tajacu*, and ten *Tayassu pecari*) and eight seropositive human samples. All samples resulted negative.

Risk Factors

The semi-structured surveys revealed that all inhabitants consume wild meat, which is cooked with water from unsafe sources (rivers, rain, streams) as they do not have access to potable water. As drinking water, the local population mainly consumes rainwater and previously sedimented river water, but without purification treatment. A significant portion of the community consumes meat with macroscopic lesions, meat prepared at low temperatures, offal, and even animals found dead in the forest. Additionally, domestic (cats, dogs, and chicken) and wild animals frequently come into contact with residents, and bat and mouse bites are common. In terms of safety practices, hunters admitted to not using protective measures when handling hunted animals, with some reporting injuries. Furthermore, a notable portion of the population does not use condoms for reproductive control and/or to prevent the spread of sexually transmitted infections. Table IV.4 summarizes other activities associated with HBV and HEV exposure.

Due to the low individual correspondence between serological results and behavioral factors, no association was made between risk factors and HEV and HBV serology.

Table IV.4. Answers to the semi-structured survey conducted with 84 heads of families from the Nueva Esperanza community in the Peruvian Amazon. The determination of risk factors is based on the literature: HBV and HEV.

Activity	Frequency	HBV Risk [4,9,12–14]	HEV Risk [6,39,40]
Consumption of wild meat	100%, 84/84	No	Yes
Consumption of viscera from wild animals	55.89%, 19/34	No	Yes
Consumption of animals found dead in the forest	15%, 6/40	No	Yes
Use of safety measures when handling hunted animals	0%, 0/17	Yes	Yes
Inspection of lesions in hunted animals	41.17%, 7/17	No	Yes
Consumption of meat with lesions	82.5%, 33/40	No	Yes
Injuries while handling hunted animals	18.75%, 3/16	Yes	Yes
Preparation of meat at low temperatures	47.72%, 21/44	No	Yes

Table 4. *Cont.*

Activity	Frequency	HBV Risk [4,9,12–14]	HEV Risk [6,39,40]
Source of drinking water supply		No	Yes
- Public water system	0%, 0/47		
- River	57.4%, 27/47		
- Rain	87.2%, 41/47		
Drinking water treatment		No	Yes
- No treatment (sedimentation)	76.1%, 35/46		
- Treatment	28.3%, 13/46		
Source of water used for cooking		No	Yes
- Rivers	82.4%, 28/34		
- Rainwater	52.9%, 18/34		
Wastewater disposal after cooking		No	Yes
- Household yard	26/34, 76.5%		
- River	9/34, 26.5%		
- Rustic drainage systems	3/34, 8.8%		
- Forest	1/34, 2.9%		
Use of animal products as medicine	19.35%, 12/62	Yes	Yes
Consumption of non-potable water	100%, 84/84	No	Yes
Presence of domestic animals at home	91.3%, 42/46	Yes	No
- Cats	19%, 8/42		
- Dogs	26.2%, 11/42		
- Chicken	100%, 42/42		
Presence of wild animals at home	25%, 19/76	Yes	Yes
- Birds (*Brotogeris versicolurus*)	73.7%, 14/19		
- Monkeys	5.3%, 1/19		
- Peccaries (*Pecari tajacu*)	15.8%, 3/19		
- Others	5.3%, 1/19		
Presence of mice and rats at home	100%, 84/84	Yes	Yes
Experienced bites from mice and rats	16.7%, 14/84	Yes	No
Presence of bats at home	98.8%, 83/84	Yes	No
Experienced bites from bats	26.2%, 22/84	Yes	No
Has tattoos	26.15%, 17/65	Yes	No
Has had surgeries or transfusions	27.41%, 17/62	Yes	No
Use of condoms	34%, 27/73	Yes	No

Discussion

The present study improves the knowledge of the occurrence of HBV and HEV in humans and coexisting wildlife in the rural Peruvian Amazon. Antibodies against HBV were detected in three wild mammal species and both HEV and HBV antibodies were detected in humans. To our knowledge, this is the first record of antibodies against HBV in free-ranging wildlife in the Amazon region.

In the non-Amazon areas of Brazil, previous research has shown the occurrence of a virus phylogenetically close to human HBV sequences in domestic swine and wild boars (*Sus scrofa*), horses (*Equus ferus caballus*), domestic dogs (*Canis lupus familiaris*), jaguars (*Panthera onca*), maned wolves (*Chrysocyon brachyurus*), and crab-eating raccoons (*Procyon cancrivorus*) (Vieira *et al.*, 2015; 2019). The presence of HBV antibodies across three distinct wildlife species in our study underscores the complexity of transmission, supporting the hypothesis that HBV or HBV-like viruses can infect different hosts in their natural habitats (Bonvicino *et al.*, 2014; Vieira *et al.*, 2019). However, despite the high precision of the kits used, we cannot rule out the possibility of false negatives. On the other hand, further studies are required to determine which strains and genotypes are circulating in the Amazon and if they are closely related to human HBV, as previously observed in domestic pigs (Vieira *et al.*, 2015), and whether this virus is exchanged between animals and humans.

In the Amazon, human HBV prevalence ranges from 0 to 30% (Monsalve-Castillo *et al.*, 2008; Roman *et al.*, 2010; Russel *et al.*, 2019) and most studies found no clear gender-related nor age-related risk (Cabezas *et al.*, 1999; Craig *et al.*, 1993; Vásquez *et al.*, 1999; Vieira *et al.*, 2015), which is consistent with our results. Our results confirm human exposure to HBV in the Yavarí-Marín River basin, stressing the need for the implementation of an appropriate vaccination program, highlighting the importance of extensive research across the Amazon to determine the prevalence and risk of HBV.

On the other hand, HEV circulation has been widely documented in domestic and wild fauna in South America (Echevarría *et al.*, 2013; Ferreiro *et al.*, 2021; Goncalves de Campos *et al.*, 2018). In our study, all samples from wild and domestic animals were negative for antibodies against HEV. On the contrary, we observed a high seroprevalence of HEV in humans associated with age, as reported by previous studies (Cangin *et al.*, 2019; Fernández *et al.*, 2022;), suggesting continuous and cumulative exposure to the virus over time (Wilhelm *et al.*, 2019). However, RNA analysis, which could have provided more meaningful insights, was not performed due to the highly degraded RNA in our filter paper samples, as reported in a previous study using these same samples and demonstrating the greater degradation of RNA compared to DNA (Li *et al.*, 2024). Our findings suggest that wild meat consumption is not a major factor in HEV transmission; however, this transmission through contact with or consumption of wild animals needs to be further explored, given the absence of interaction with pigs and consumption of pork products in the community. Our results also reinforce the idea that other environmental factors are causing repeated infection processes in humans. Some studies have demonstrated that HEV may be transmitted via the fecal–oral route from contaminated water and large waterborne outbreaks frequently occur, especially in developing countries (Ahmad, *et al.*, 2010; Baez *et al.*, 2017; Takuissu *et al.*, 2022). However, the lack of reports on water-borne HEV outbreaks in the Amazon (Echevarría *et al.*, 2013) evidences that this potential transmission route is not well studied. Further studies are needed to improve knowledge about the source of contamination, its occurrence, and its survival in water.

Due to a low correspondence between serologically analyzed individuals and survey participants, the association between risk factors and serological results could not be analyzed. Despite this limitation, we have identified several common risk factors in Nueva Esperanza that may constitute a risk of HBV and HEV transmission according to the scientific literature (Bonvicino *et al.*, 2014; Cabezas & Braga 2020; Costa & Kimura, 2012; Douglas *et al.*, 2024; Ormaeche *et al.*, 2012; van Vliet *et al.*, 2022; Vasconcelos *et al.*, 2024; Viana *et al.*, 2005). This highlights the need to implement measures to promote safer food handling while also improving sanitation, hygiene, and practices related to close contact with wild animals and the purification of drinking waters. To address the hepatitis burden in the Yavarí River basin, it is critical to target specific risk factors. These results also underscore the need to explore and study cultural practices and animal reservoirs in the Amazon region, which may influence infectious disease pathways (Castro-Arroyave *et al.*, 2023; Manock *et al.*, 2000;).

Study V

Risk Factors for Wildlife-Transmitted Diseases in Communities Engaged in Wildlife Consumption– A Case Study on Neotropical Echinococcosis

Acta tropica (accepted)

Introduction

The transmission of zoonotic diseases from wildlife depends importantly on human contact with wildlife reservoirs, especially through activities related to the obtention, handling and consumption of wild meat, which pose significant risks for both bloodborne and foodborne pathogens (Wolfe *et al.*, 2005; Van Vliet., 2022). Despite the evident risks, information on diseases associated with the handling and consumption of wild meat remains largely deficient. Neotropical echinococcosis (NE) is a clear example of a zoonotic disease associated with the consumption of wild meat (San-Jose *et al.,* 2023). NE is caused by the accidental ingestion of *Echinococcus vogeli* tapeworm eggs through contaminated food or water (D’Alessandro & Rausch, 2008). Although being responsible for 29% of deaths among infected humans (D’Alessandro, 2010), NE is a neglected and underreported disease in Latin America, creating a misleading perception of a low prevalence in the region (San-Jose *et al.,* 2023).

Echinococcus vogeli is a cestode well-adapted to neotropical humid forests, where stable ecosystems and humidity support the parasite's development and egg life cycle (San-Jose *et al.*, 2023). *Echinococcus vogeli* has an indirect life cycle that depends on wild rodents, primarily pacas *(Cuniculus paca)* and agoutis *(Dasyprocta spp.),* as primary intermediate hosts, which develop cysts mainly in the liver and occasionally in the lungs (D’Alessandro & Rausch, 2008; Mayor *et al.*, 2015). The bush dog (*Speothos venaticus*) and the domestic dog, the definitive hosts, can become infected by consuming the infected organs of the intermediate hosts and developing adult tapeworms in their small intestine (D’Alessandro & Rausch, 2008). The cycle closes when the definitive host excrete eggs into the environment that can be consumed by intermediate hosts (Figure V.1).

In the Amazon, pacas are one of the most frequently hunted species and are key for the diet and cultural practices of rural and urban populations (El Bizri *et al.*, 2020; Mayor *et al.*, 2022). Typically, hunters discard the viscera of medium-sized animals, such as pacas, in sites close to the houses where rural inhabitants process wild meat. When these viscera are consumed by domestic dogs, a pathway is created for the parasite to enter and remain within rural communities (San-Jose *et al.*, 2023). In this case, humans can contract the disease by ingesting food or water contaminated with feces from infected domestic dogs, a risk that is particularly common in rural areas with poor hygiene and sanitation practices (Mayor *et al.*, 2015) and can act as intermediate hosts developing cysts in their organs (D'Alessandro & Rausch, 2008; Eckert & Deplazes, 2004; D'Alessandro, 2010).

To our knowledge, only around 200 human cases have been reported in 12 countries in Latin America (D'Alessandro, 2010; San-Jose *et al.*, 2023), and there is no precise map of their presence (Rodrigues-Morales *et al.*, 2015), so its geographical distribution range partially depends on the coexistence of hosts competent to transmit the parasite (das Neves *et al.*, 2017; San-Jose *et al.*, 2023). In addition, human practices related to subsistence hunting and handling of wild meat may be key to facilitating or hindering the transmission of this disease (D'Alessandro & Rausch, 2008; Knapp *et al.*, 2009; Mayor *et al.*, 2015).

Local ecological knowledge (LEK) includes the knowledge and practices of local people regarding ecological relationships that are obtained through extensive personal empirical observations and interactions with local ecosystems (Charnley *et al.*, 2007). Although LEK-based methods have mainly been used to collect information on habitats, extractive uses of biodiversity, human-wildlife conflicts, ecology and species behavior (Joa *et al.*, 2018), they may also be essential to improve knowledge about diseases that affect wildlife. This study aims to identify human behaviors that facilitate or hinder the transmission of *E. vogeli*, using structured surveys, and to generate new geographic insights into the primary risk factors associated with neotropical echinococcosis (NE) transmission.

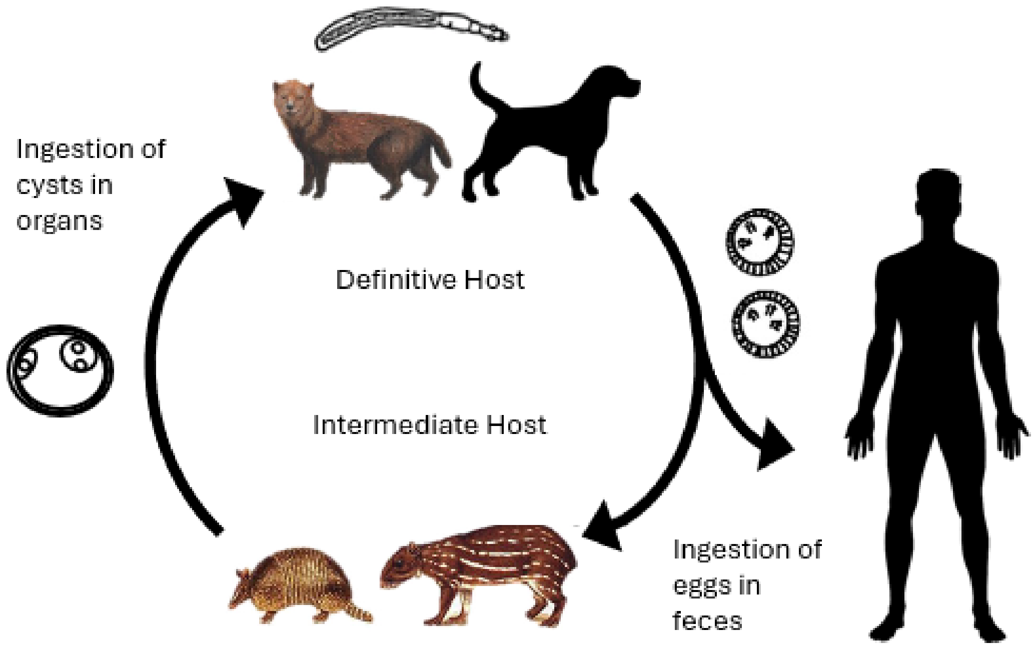

Figure V.1. Epidemiological cycle of *E. vogeli*. This parasite follows an indirect lifecycle, involving intermediate hosts (primarily the *C. paca* or *Dasyprocta spp.,* and occasionally humans) and definitive hosts (domestic dog and *S. venaticus*). Adapted from San-Jose *et al.* (2023).

Materials and methods

Study area

A net-working group was established with fellow researchers developing activities at sites within the territorial range of *E. vogeli* reported by D'Alessandro & Rausch (2008), and included 57 sites, located in Argentina, Bolivia, Brazil and Peru (Table A7). These sampling sites were divided according to their classification either rural community or city, and, in addition, rural communities were classified according to geographic location within or outside the Amazon region. All the cities surveyed were located within the Amazon.

Structured surveys

Since the study took advantage of the presence of active research teams in the surveyed area, a structured questionnaire and survey administration guide were agreed upon for all groups, which were used to train the study's 11 interviewers. Participant inclusion criteria included age (>18 years) and frequent hunting or handling wild animals. Since hunting and consumption of wild meat is a sensitive and often criminalized activity, we reduced personal questions to ensure a safe environment for participants. All interviews were conducted in person in the interviewees' households. To ensure clarity, the questions were translated and adapted to the primary language spoken in each community (Portuguese for Brazil, Spanish for Argentina, Bolivia and Peru).

Structured surveys were conducted to 285 residents across the studied communities. This included 213 (74.7%) participants from rural Amazon communities, 33 (11.6%) from Amazon cities, 39 (13.7%) from non-Amazon rural communities. The participants had a sex ratio of 6.9% women and 93.1% men, and a proportion of 43.2% Indigenous and 56.2% non-Indigenous people.

The questions focused on: a) the observation of *E. vogeli* cysts, presenting photographs of NE cysts from other studies (Figure V.2), b) their perception of the danger of these lesions to human health, c) behaviors, such as handling of viscera and consumption, that could facilitate or hinder the transmission of the parasite, d) presence and potential role of dogs in disease transmission, and e) the perception of ease in accessing health centers, categorized in (easy, mid, difficult and very difficult) (See questions in Table A8). Access to health centers does not affect the risks of transmission but it is essential for NE diagnosis and treatment.

Statistical Analysis

A chi-square test was used to assess differences in response percentages based on participants' locations, especially between rural communities and cities, and also between communities within and outside the Amazon region. Pairwise comparisons were then conducted between these groups, with the Holms's adjustment applied to account for multiple testing.

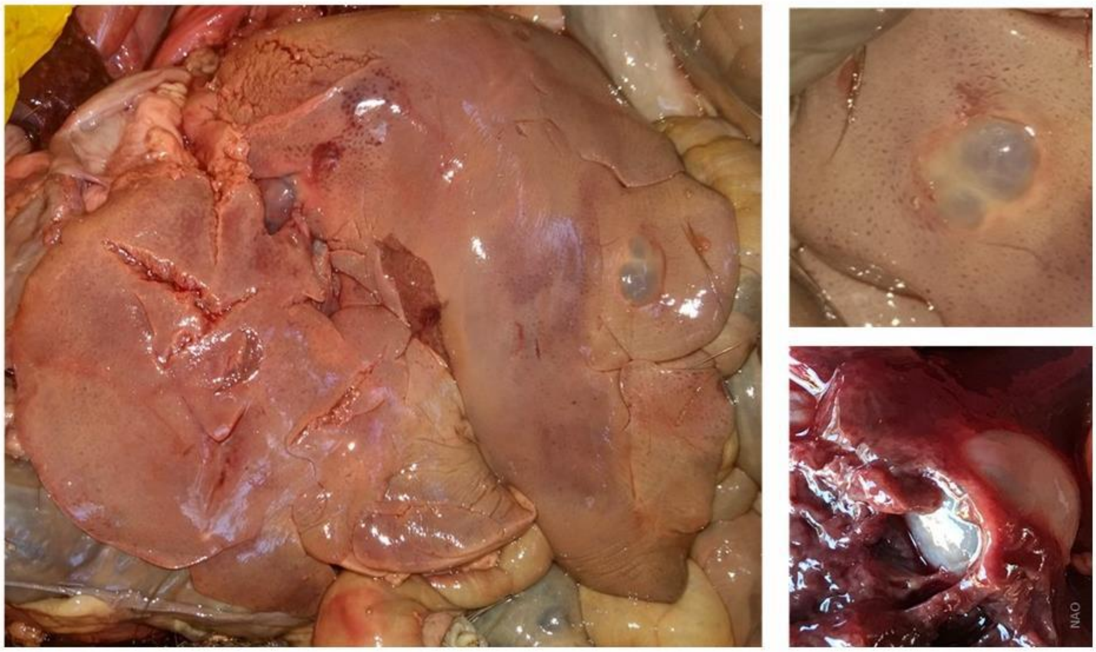

Figure V.2. Photographs of hydatid cysts present in *Cuniculus paca* shown to the 227 surveyed participants. The lesions corresponds to *E. vogeli*, confirmed through histopathological diagnosis.

To examine how rurality and location affected affirmative responses across community types, we fit a Generalized Linear Model (GLM) using a binomial family and logit link. The response was the proportion of affirmative responses within each community type. Later on, we used an ANOVA analysis of deviance to assess the contribution of each factor by comparing changes in deviance. Chi-square tests from this analysis were used to assess the significance of each term.

All data analyses were performed using R 4.2.2 (R team, 2023), and we considered a Type I error probability of 0.05 for hypothesis testing.

Results

The observation of cysts in intermediate hosts offered valuable information into the geographical distribution of NE. Figure V.3 illustrates the habitat suitability of the paca (adapted from San-José *et al.*, 2023), overlaid with the reported extent of NE based on the performed community interviews. A community was classified as having NE presence if at least one respondent reported observing liver lesions in pacas.

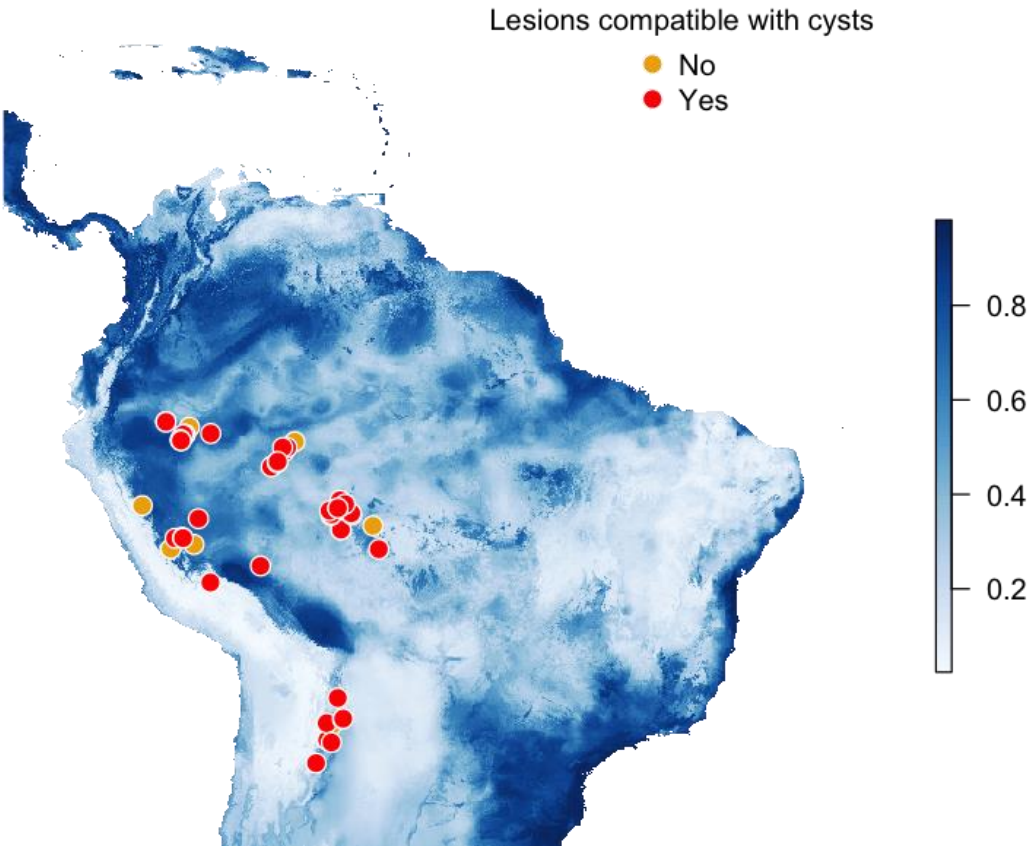

Figure V.3. Survey locations where red points indicate sites where at least one participant reported observing liver lesions in pacas; orange points indicate no such reports. In the background, a *Cuniculus paca* habitat suitability map, adapted from (San-José et al, 2023).

Tables V.1 and V.2 show the responses of the surveyed population categorized by location. Liver lesions in pacas were reported by 53% of survey respondents, Amazon location was a significant factor influencing this reporting (P=0.05).

Based on reports from at least one person in each community, NE was observed in 86.5% (32/37) of Amazonian rural communities, 75.0% (6/8) of Amazonian cities, and 75.0% (9/12) of non-Amazonian rural communities. Figure V.3 shows the geographical distribution of NE considering the identification of the disease through surveys.

Of all respondents, 24.9% considered these lesions frequent, the Amazon region was a significant factor, with people more frequently considering the lesions to be common there (P=0.0005), and especially in rural Amazon areas (29.6%) in comparison with rural non-Amazon communities (5.1%) (P=0.0079). Additionally, in rural Amazon communities, respondents reported the observation of lesions in 3.28 (±3.98) pacas annually, compared with 1.80 (±5.49) in non-Amazon rural areas, and 1.44 (±1.63) pacas with lesions in Amazon cities. The percentages of pacas observed with lesions were 13.6% (±15.1), 13.0% (±15.4), and 11.9% (±19.9) pacas, respectively.

Overall, 43.9% of respondents considered these lesions to be dangerous to human health. However, this perception varied significantly across study sites. In Amazonian rural communities, 43.7% of respondents perceived these lesions as dangerous, compared to 69.7% in Amazonian urban communities and 23.1% in non-Amazonian rural communities. Both rurality (P=0.005) and Amazon location (P= 0.004) were important factors influencing this response.

Regardless of the respondent's origin, 92.6% of respondents reported that when they observe these lesions in livers, they usually discard the affected organ, while a smaller proportion bury (3.3%), consume (2.5%), or incinerate them (1.7%).

Figure V.4 shows how they discard the livers that are not normally consumed. In rural Amazon communities, livers were mainly discarded in forests (28.2%), rivers (17.4%) or given to dogs (13.2%). In Amazon cities, they were typically given to dogs (31.25%) or thrown in the trash (21.9%). In non-Amazon rural areas, most affected livers were discarded in the forest (61.5%) or given to dogs (20.5%).

Furthermore, 62.4% of all people surveyed reported owning dogs, significantly higher in rural non-Amazon communities (94.9%) than in rural Amazon communities (53.5%) (P<0.0001). Of the respondents, 53.3% reported that dogs generally consume discarded viscera, with or without lesions. This practice was significantly more frequent in rural communities overall (Amazon (62%) and non-Amazon (43.6%)) compared to Amazonian cities (9.1%; P<0.0001 and P=0.005, respectively). Additionally, only 37.5% of respondents reported that they routinely dewormed their dogs, a practice especially uncommon in rural areas (Amazonian (13.1%) and non-Amazonian (38.5%; P=0.0006)) compared to Amazonian cities (72.7%; P<0.0001 and P=0.0075, respectively). Figure V.5 illustrates and summarizes the ease of *E. vogeli* transmission across natural barriers, based on questions previously mentioned, grouped by respondents' geographic regions.

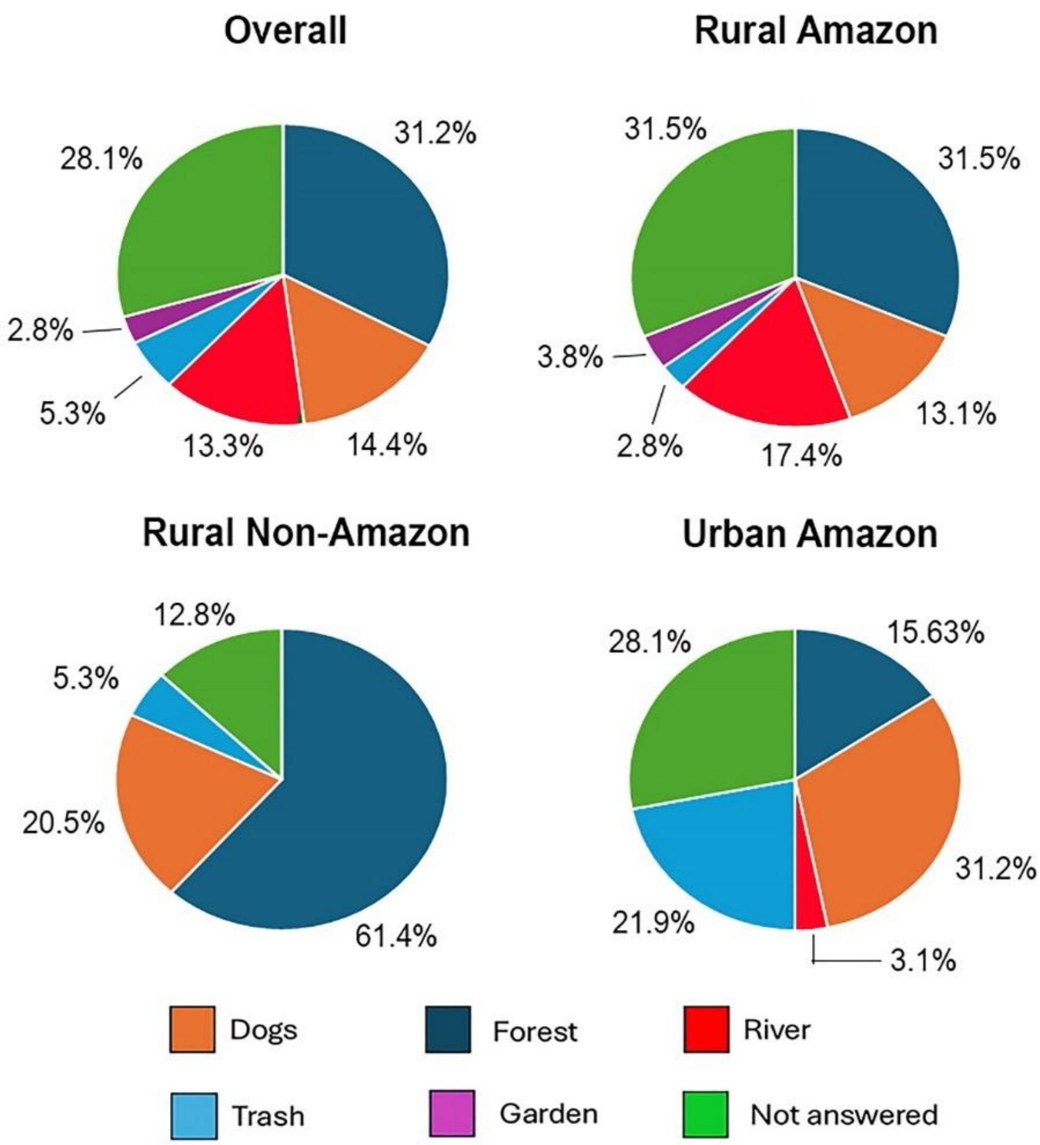

Figure V.4. Detail of the disposal location of the people surveyed when discarding unconsumed livers: general overview and by location-specific responses.

Figure V.5. Funnel plots illustrating the ease of *E. vogeli* transmission across various natural barriers. Wider funnels indicate greater ease of transmission, while narrower funnels highlight bottlenecks that typically prevent spread. The results are categorized by respondents' geographic locations, with Chi-square analysis results integrated to show statistical significance. 'a', 'b' and 'c' values in the same rows indicate significant differences ($p < 0.05$, see Table A7 for further details) (n=285).

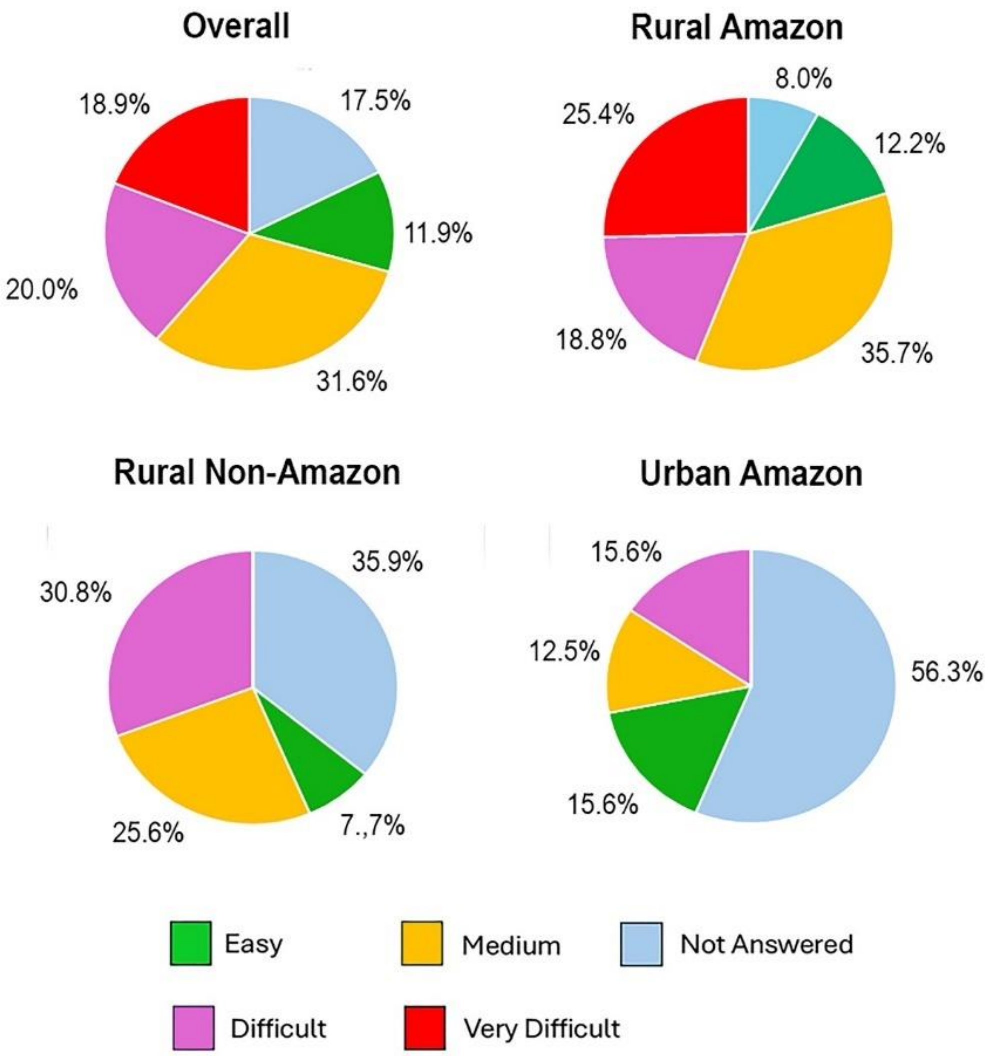

Figure V.6. Detail of the access to health services of the people surveyed: general overview and by location-specific responses.

For 38.9% of the overall population surveyed, accessing health centers was a challenge ("difficult" or "very difficult"), and rurality was a significant factor influencing access to health centers (P=0.008; Figure V.6). In rural Amazon communities, 44.1% reported "difficult" or "very difficult" accessing health services, significantly different compared to 15.2% in Amazon urban areas (P=0.05).

Table V.1. Responses to statements based on key survey questions categorized by location. 'a' or 'b' values in rows with different superscript letters are significantly different ($P < 0.05$).

Statements	Total respondents	Rural Amazon communities	Amazon Cities	Rural non-Amazon communities	p-value	X-squared	df
	n=285	n=213	n=33	n=39			
Statement 1: Reported seeing lesions in pacas	53% (151)	56.8% (121)	45.5% (15)	38.5% (15)	0.0706	5.3028	2
Statement 2: Consider the lesions are frequent	24.9% (71)	29.6% (63) [a]	18.2% (6) [ab]	5.1% (2) [b]	0.0033	11.438	2
Statement 3: Owning dogs	62.4% (178)	53.5% (114) [a]	75.8% (25) [b]	94.9% (37) [c]	0.0014	26.963	2
Statement 4: Confirmed that dogs habitually eat discarded viscera	53.3% (152)	62% (132) [a]	9.1% (3) [b]	43.6% (17) [c]	4.514e-08	33.827	2
Statement 5: Do not deworm their dogs	62.5% (178)	86.9% (185) [a]	27.3% (9) [b]	61.5% (24) [c]	3.398e-14	62.026	2
Statement 6: Present difficulties accessing the local Health Center	38.9% (111)	44.1% (94) [a]	15.2% (5) [b]	30.8% (12) [ab]	0.0034	11.363	2
Statement 7: Perceive these lesions as a danger to human health	43.9% (125)	43.7% (93) [a]	69.7% (23) [b]	23.1% (9) [c]	0.0004	15.791	2

Table V.2. Details of the full model using GLM and ANOVA to verify the influence of location and rurality on key survey questions on the presence of *E. vogeli*.

	GLM						**ANOVA**					
	RegionNoAmaz vs. RegionAmaz			**Cities vs. Rural**			**Region**			**Rurality**		
Statements	**Estimate**	**z-value**	**Pr(>|z|)**	**Estimate**	**z-value**	**Pr(>|z|)**	**Df**	**Deviance**	**Pr(>Chi)**	**Df**	**Deviance**	**Pr(>Chi)**
Statement 1: Reported seeing lesions in pacas	-0.7440	-2.084	0.0372 *	-0.4563	-1.214 +	0.2249	1	3.8342	0.05 *	1	1.4806	0.2237
Statement 2: Consider the lesions are frequent	-2.0503	-2.766	0.00568 **	-0.6366	-1.338	0.18079	1	12.2438	0.0005 ***	1	1.9813	0.1593
Statement 3: Owning dogs	2.7767	3.758	0.000171 ***	0.9984	2.328	0.019900 *	1	26.5633	2.55e-07 ***	1	6.0759	0.0137 *
Statement 4: Confirmed that dogs habitually eat discarded viscera	-0.7462	-2.117	0.03423 *	-2.7909	-4.489	7.16e-06 ***	1	1.720	0.1896	1	35.627	2.389e-09 ***
Statement 5: Do not deworm their dogs	-1.4181	-3.668	0.000244 ***	-2.8690	-6.515	7.25e-11 ***	1	5.116	0.0237 *	1	49.317	2.178e-12 ***
Statement 6: Present difficulties accessing the local Health Center	-0.5751	-1.540	0.12350	-1.4869	-2.946	0.0032 **	1	1.3048	0.2533	1	11.1909	0.0008 ***
Statement 7: Perceive these lesions as a danger to human health	-0.9491	-2.347	0.01893 *	1.0878	2.698	0.007 **	1	8.4176	0.004 **	1	7.8972	0.005 **

Discussion

Neotropical echinococcosis (NE) is a neglected tropical disease most common in communities that rely on wild meat as their primary source of animal protein (San-Jose *et al.*, 2023). However, NE is severely underreported, and its full distribution remains largely unknown (Rodrigues-Morales *et al.*, 2015). In this study, we aimed to understand the individual and cumulated risk behaviors that either promote or hinder the transmission of *E. vogeli* and assess the magnitude of risk across different types of human settlement within the distribution range of NE.

E. vogeli cysts has been widely reported in pacas, domestic dogs, and bush dogs in rural South American communities, and particularly in the Amazon (Mayor *et al.*, 2015; Bosmediano *et al.*, 2023; Bittencourt-Oliveira *et al.*, 2018; das Neves *et al.*, 2017; Soares *et al.*, 2014; Santos *et al.*, 2012; D'Alessandro & Rausch, 2008), where subsistence activities like hunting create close interactions between humans and parasite hosts (San-Jose *et al.*, 2023). However, human cases of NE have also been reported in other regions of Central and South America (WHO, 2001; D'Alessandro & Rausch, 2008). Notably, bush dogs and pacas inhabit a broad range of regions, from southern Mexico to tropical areas of Peru, Bolivia, Paraguay, Argentina, and Brazil (Rodrigues-Morales *et al.*, 2015), and the presence of this parasite has been documented in domestic dogs, and bush dogs in non-Amazon regions (Vizcaychipi *et al.*, 2013). Our findings align with this and show a greater frequency of observation of *E. vogeli* cysts in pacas from rural Amazonian communities, although cysts are also present less frequently beyond the rural Amazon region. The presence of *E. vogeli* cysts in pacas evidences the risk of transmission of the parasite.

This health risk is not directly related to the consumption of wild meat but is related to the consumption of wild meat offal; thus, wild meat trade chains become potential routes of transmission of the parasite, whenever the viscera, particularly the liver and lungs, are mobilized. In the Amazon, wild mammals are widely sold and consumed in urban areas (El Bizri *et al.*, 2020; Mayor *et al.*, 2022), and *E. vogeli* has already been detected in pacas in Amazonian urban markets (Tantaleán *et al.*, 2012). Thus, as some respondents reported, urban regions are not excluded from the risk of transmission of the disease.

The main human behaviors identified in our study as drivers of disease transmission are related to the handling of paca viscera, and in general all prey hunted, and the handling of dogs, including their access to discarded viscera and deworming. When the lesion is observed in intermediate hosts, the human behavior that most effectively interrupts the parasite transmission cycle is the incineration of the viscera with lesions (Pandey *et al.*, 2020). However, this behavior has been reported as a very rare behavior in our study population. Furthermore, in all communities, the routine behavior of most participants with the viscera, whether or not they observe lesions, is usually to feed them to animals or discard them in the forest. These discarding methods do not block the life cycle of the parasite, but, on the contrary, increase the risk of transmission to other potential hosts and allow recirculation of the parasite in areas closer to human communities. This scenario highlights the need for alternative management of viscera to mitigate transmission.

The introduction of domestic animals into rural communities has likely contributed significantly to the spread of zoonotic disease linked to wild meat practices, and particularly NE. Cats and dogs often prey animals that serve as intermediate hosts for parasites, especially those with complex life cycles, and where they often act as definitive hosts (Han *et al.*, 2021). Therefore, these animals play a pivotal role in the dissemination of parasites in sylvatic environments, harboring nematodes or other parasites in their guts and shedding eggs into the environment through their feces (Winders & Menkin-Smith, 2023; Romig & Wassermann, 2024).

In rural communities, dogs are usually fed using discarded organs and are also rarely treated with effective anti-parasitic medications, contributing to a high risk of parasite transmission due to environmental contamination. The concomitance of these two widespread practices in rural areas increases the risk of dissemination of *E. vogeli*, and other zoonoses linked to wild meat practices, since humans, and particularly children, can become infected through contact with feces from infected dogs (Mayor *et al.*, 2015; Varcasia *et al.*, 2011; Traversa *et al.*, 2014).

Zoonotic transmission is an eco-epidemiological process in which a pathogen must overcome a series of natural barriers to effectively infect a new host species. Traditionally regarded as a singular event, it is increasingly conceived as a complex multilevel process. Lloyd-Smith et al (2017) and Plowright et al (2017) proposed a framework that breaks down zoonotic transmission into distinct subprocesses, each occurring within specific spatial and temporal windows necessary for an effective cross-species transmission. For each pathogen and geographical context, certain subprocesses will act as facilitators while others act as bottlenecks. In this study, we used LEK to break down the transmission of *E. vogeli* into different subprocesses, and illustrated our findings using funnel plots, inspired by Lloyd-Smith's and Plowright's work.

The rural Amazon has a high percentage (34.6%) of population in poverty (Gobernanza Territorial V, 2017), and faces significant barriers to healthcare access, exacerbating health risks in these remote areas (PAHO, 2009; Badanta *et al.*, 2020). Medical facilities are often distant, leaving many communities without timely care. The COVID-19 pandemic particularly affected Indigenous communities in the rural Amazon, where limited Intensive Care Unit availability, overcrowded housing, and limited healthcare systems made it difficult to contain the virus and respond effectively (WHO, 2020; ILO, 2020; Amazon Regional Observatory, 2022; Abizaid *et al.*, 2024). The neglect of these populations in terms of health services and poverty may contribute to the underreporting and limited research on diseases, perpetuating a cycle where both the diseases and the affected communities remain understudied. This was reflected in our study, where most rural inhabitants reported difficulties in accessing medical assistance, increasing a significant diagnostic gap and consequent delay in treatment and severe disease progression to chronic conditions (D'Alessandro, 2010). Furthermore, awareness of the health risks posed by these cysts is lower in rural areas, which complicates the mitigation of the risk behaviors and further delays timely diagnosis and treatment.

Our study faces some limitations that are important to discuss as they may result in bias in our results. Firstly, the identification of NE was based solely on respondents' observation of liver lesions in pacas, and no histopathological or molecular confirmation of *E. vogeli* infection was performed. Despite the lack of a definitive diagnosis, the reported observation of cyst-like lesions in pacas may indicate potential areas of parasite circulation and the risk of transmission of the disease to humans, particularly through the consumption of infected offal by domestic dogs. These findings underscore the ecological and behavioral dimensions of NE transmission, providing a fundamental basis for addressing this neglected disease. While this identification of the lesion was necessarily perceptive— as they are based on the local ecological knowledge —the coincidence within the reported geographic range in the Amazon region (San José *et al.*, 2023) supports the relevance of these observations despite the lack of parasitological confirmation.

Nevertheless, future studies are still needed to confirm the territorial range of the parasite through molecular analysis. Secondly, this study was subject to certain limitations due to its decentralized, community-based design. Even though a consensual structured interview guide and site-specific language adaptations were used to ensure consistency, variability in how different interviewers applied these tools across regions may have introduced some degree of interviewer bias. Additionally, the high proportion of male respondents may have limited the representation of diverse gender perspectives. These limitations reflect the logistical challenges of conducting fieldwork in remote and culturally diverse settings.

5. GENERAL DISCUSSION

Information regarding the epidemiology of infectious diseases in the Amazon region, among both human and wild animal populations, is notably scarce and, in some cases, entirely lacking (Fragoso *et al.,* 2022; Mayor & Bodmer, 2009; WHO, 2020). This scarcity of knowledge had become particularly concerning in the context of the emergence and global spread of infectious diseases in recent decades, a trend largely driven by intensified anthropogenic activities and climate change (Baker *et al.*, 2022; Epstein, 2001). These dynamics underscore the urgent need to enhance our understanding of diseases that threaten Amazonian ecosystems and communities. This thesis aimed to help fill that gap by investigating the presence and circulation of pathogens across wildlife, domestic animals, and humans in Amazonian communities that rely heavily on wild meat.

Although pathogens have been implicated in the collapse of various wildlife species (Smith *et al.*, 2006), infectious diseases have rarely been studied as drivers of population decline of Amazon fauna. This oversight is mainly due to the restricted sampling capacity, the high costs and logistical challenges of fieldwork, limited funding for disease research, and the need for interdisciplinary expertise to ensure ethical and appropriate sampling (Wobeser, 2007). Nevertheless, the long-term fieldwork in the study area for the present thesis, facilitated by collaborations with local hunters, enabled the collection of a robust number of blood samples. Prior studies have shown that serological testing is feasible using samples preserved on filter paper (Aston *et al.*, 2014; Morales *et al.*, 2017), and that DNA can also be successfully preserved under these conditions (Li *et al.*, 2024). These studies support the methodological approach employed in this thesis and reinforce the value of integrating local knowledge with practical field sampling techniques in remote regions.

Diseases with the potential to affect the population dynamics of large WLP herds have been reported in the Amazon region. However, due to limited sample sizes and the absence of longitudinal studies, their true impact remains poorly understood (Martins De Castro *et al.*, 2014; Romero Solorio, 2010) (Study I). This knowledge gap is especially concerning given the ecological importance of peccaries in forest regeneration, nutrient cycling, and biodiversity maintenance (Peres, 2000), and their socioeconomic role as the most hunted and traded wild meat species in the region—critical for both food security and local livelihoods (Mesquita & Barreto, 2015; El Bizri *et al.*, 2020b; Mayor *et al.*, 2022).

To assess the potential role of infectious diseases in the decline of WLP populations, antibodies against multiple swine viruses were tested across a 12-year period and in two regions—one with documented WLP disappearances and one without. Notably, Classical Swine Fever Virus (CSFV) and Aujeszky's Disease Virus (ADV) were detected in both regions (Study II). However, it is unlikely that local disappearance of species is driven by a single factor; rather, it often results from the interaction of multiple pressures, including diseases, overhunting, and migration, which must be considered in conservation strategies (Fragoso *et al.*, 2016; Mesquita & Barreto, 2015).

According to the density-dependent overcompensation theory, high WLP densities may be contributing to these declines (Fragoso *et al.*, 2022). WLP populations' densities range from 3.7 to 25 individuals per square kilometer, while CP densities are typically lower, ranging from 2.8 to 9.8 individuals per square kilometer (Fragoso, 1998; Keuroghlian *et al.*, 2004), creating better conditions for pathogen maintenance or mass mortalities (Fragoso *et al.*, 2022). Therefore, it is plausible that CP did not exhibit a decline due to their smaller herd size, despite evidence of pathogens' circulation.

In the study area with documented WLP disappearances, populations have undergone several boom-and-bust cycles. Before a disappearance in 2000, WLP density peaked at 15 individuals per square kilometer (Fang et al., 2008), while CP biomass was 2–4 times lower (Bodmer *et al.,* 2018). Furthermore, WLP densities, in individuals and even more in terms of biomass, are usually four times lower in the study area without observed disappearances (Perez-Peña *et al.,* 2016), suggesting that population density may be an important contributing factor—but not the sole driver—of these collapses.

Hunters are expected to maximize their net energy gain by prioritizing preys that offer the highest return rates. When high-ranked species become scarce and search times increase, hunters often adapt by broadening their diet to include more abundant but lower-ranked species (Winterhalder, 1981). These alternative preys may be selected not only for their nutritional value, but also for their ease of capture or commercial value (Griffiths *et al.*, 2022).

In the Amazon region, ungulates are typically the primary source of wild meat. Species such as the WLP, CP, red brocket deer (*Mazama americana*), and lowland tapir (*Tapirus terrestris*) are among the most commonly hunted and sold in markets (Bodmer & Pezo, 2001; El Bizri *et al.,* 2020b; Mayor *et al.*, 2022). However, when these ungulates become less available, hunters shift towards other groups such as rodents and primates— species that also serve as zoonotic reservoirs (Mesquita & Barreto, 2015).

This shift in prey selection carries broader health implications. The "empty forest" hypothesis posits that biodiversity loss can significantly increase zoonotic disease risk by disrupting ecological balance and facilitating pathogen spillover (Civitello *et al.*, 2015; Gibb *et al.*, 2020). Hunters in closer contact with species such as rodents and primates—particularly primates, given their phylogenetic proximity to humans—face higher risks of zoonotic disease transmission (Han *et al.*, 2016; Pires & Galetti, 2023).

In the study area, a drastic decline in WLP populations led to a compensatory redirection of hunting towards rodent and primate populations. In this context, this thesis assessed seroprevalence of *Toxoplasma gondii*, Hepatitis E virus (HEV), and Hepatitis B virus (HBV), given their relevance to bloodborne and foodborne transmission pathways, relevant to Amazonian rural communities (Douglas *et al.*, 2024; Vitaliano *et al.*, 2015). Evidence of their presence in both humans and wildlife in the region, along with their potential for severe health outcomes, underscores the need to better understand their dynamics in this unique ecological setting.

Serological findings revealed widespread exposure to *T. gondii* across taxa, including ungulates, rodents, carnivores, and primates, suggesting that direct contact with wildlife, as well as the handling and preparation of wild meat, may play a central role in its transmission (Study III). In the case of HEV and HBV, although they were only sporadically detected in wildlife, human seroprevalence was high, even in communities with minimal contact with pigs or other farm animals (Aston *et al.*, 2014), suggesting that transmission may also occur through alternative routes such as contaminated water or food (Study IV).

In the Amazon, hunting practices are essential for both subsistence and cultural identity, but they also bring people into close and frequent contact with potential sources of zoonotic pathogens. In tropical forests, hunting often involves tools like bows, shotguns, or snares, and requires tracking and processing animals in natural settings where hygiene and protective equipment are typically lacking (Milstein *et al.*, 2020; Van Vliet *et al.*, 2022). This close contact with blood, tissues, and bodily fluids during hunting and butchering increases opportunities for pathogens to cross from animals to humans.

Cooking practices that involve undercooked or raw meat, as well as the consumption of animal organs, are also major contributors to disease transmission in rural societies (Carme, 2021). Limited access to clean water and reliance on untreated natural sources make it difficult to maintain hygiene during meat processing, increasing the risk of food contamination and waterborne diseases. Additionally, the disposal methods for animal remains fail to disrupt the life cycle of parasites, instead facilitating their transmission to new hosts and allowing continued environmental circulation (Study V).

Particularly, the presence of domestic animals in rural Amazonian communities plays a significant role in the transmission dynamics of zoonotic diseases associated with the consumption of offal or wild meat. The introduction of domestic cats, for instance, has likely reshaped the epidemiology of toxoplasmosis by establishing a domestic cycle that complements the one related to the consumption of wild animals (Blaizot *et al.*, 2020) (Study III). Similarly, domestic dogs can contribute to the spread of Echinococcus vogeli and other similar pathogens, when they are fed raw or discarded viscera and are rarely dewormed (Study V).

Domestic animals in rural Amazonian settings are not restricted to indoor spaces and can roam freely through forests, drink from rivers, and even hunt or scavenge wild animals (Winders & Menkin-Smith, 2023; Cook & Karesh, 2011; Guzman *et al.*, 2024). Domestic cats and dogs often consume prey species that serve as intermediate hosts for parasites (Han et al., 2021). Felines, in particular, can act as definitive hosts for certain diseases, making them key contributors to parasite transmission (Romig & Wassermann, 2024). This role is amplified when they are fed raw or undercooked meat. The overlap between domestic and sylvatic cycles not only perpetuates transmission locally by creating new intermediate hosts and increasing environmental contamination near human dwellings but also raises the likelihood of "spill-back" events (Mercier *et al.,* 2011), where pathogens return to wildlife, particularly in the absence of effective animal health management and control measures.

These challenges are further intensified by systemic issues. In the rural Amazon, over a third of the population lives in poverty (Gobernanza Territorial V, 2017), and most communities are far from adequately equipped medical facilities. This contributes to delays or absence in diagnosis or treatment, and to the persistent underreporting of illnesses. In both the Peruvian and Brazilian Amazon, historical neglect, geographic isolation, and limited public health investment have compounded the challenges Indigenous communities face (Balvedi & Ormaza, 2020). These overlapping vulnerabilities not only increase disease burden but also contribute to the invisibility of these populations in scientific research, perpetuating a cycle in which the health status of these populations remain insufficiently addressed (MINSA, 2010).

Due to gaps in information and limited awareness, many individuals in remotes regions like the Amazon do not perceive zoonotic diseases as an immediate or significant threat. This perception is shaped by poverty, inadequate access to healthcare, and a daily reliance on natural resources. As a result, practices such as handling raw meat without protective measures, feeding animal viscera to domestic animals, or consuming undercooked game are considered normal and low-risk, leading to behaviors that heighten exposure to zoonotic pathogens in these settings.

Overall, this thesis provides important insights into pathogen circulation at the human–wildlife interface within Amazonian societies that rely on wild meat. These findings highlight the ecological and epidemiological significance of disease surveillance for both biodiversity conservation and public health. For instance, changes in wildlife populations —such as the disappearances of WLP— can elevate zoonotic risks and affect human health. Moreover, the presence of pathogens across wild, peri-domestic, and domestic animals reveals shared transmission cycles shaped by human behaviors that amplify exposure and disease spread.

The results of this thesis reinforce the value of the One Health framework, highlighting the need for better data and cross-sector collaboration, while emphasizing the importance of involving local communities to develop health strategies that are sustainable, low-cost and culturally appropriate (Franco-Paredes *et al.,* 2007).

6. CONCLUSIONS

- The impact of infectious diseases on the decline of WLP populations has not been evaluated accurately, highlighting the need for research efforts that employ more cost-efficient and culturally friendly sampling methods.
- Peccaries in the Amazon are exposed to viruses that cause disease in pigs, suggesting that infectious diseases may play a role in a multifactorial process causing cyclical WLP disappearances.
- High *T. gondii* infection rates across wild, peri-domestic, and domestic animals suggest an overlap in transmission cycles, with exposure linked to wild meat consumption and domestic animals, especially cats.
- There is evidence of HEV and HBV circulation in humans within the study area. Although the detection of antibodies in animals does not confirm the involvement of wildlife, their potential role should not be dismissed and warrants further investigation alongside other possible contributing factors.
- In South America, the risk of Neotropical Echinococcosis transmission in rural Amazonia is higher than in urban and non-Amazonian rural regions through the wild meat trade chain.
- Traditional hunting, food preparation practices, feeding uncooked offal to domestic animals —especially dogs and cats— and structural issues such as poverty and inadequate sanitation systems increase the risk of zoonotic disease transmission in the rural Amazon.
- Raising community awareness about transmission risks, along with improved management of both wildlife and domestic animals are necessary to mitigate zoonotic disease risk, especially in rural areas that are often overlooked by healthcare systems.

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ANNEXES

Annex 1. Search algorithms used in online databases to retrieve studies on infectious diseases affecting peccaries and domestic swine in the Amazon region.

Pubmed and Scopus search:

- ((peccar* OR swine OR pig OR pigs) AND (amazon* OR (brazil AND (acre OR amapá OR amazonas OR pará OR rondônia OR roraima OR tocantins OR "Mato Grosso" OR maranhão OR Belem)) OR (perú AND (amazonas OR loreto OR ucayali OR "Madre de Dios" OR Iquitos OR Pucallpa)) OR (colombia AND (amazonas OR caquetá OR guainía OR guaviare OR putumayo OR vaupés OR meta OR vichada)) OR (venezuela AND (amazonas OR bolívar OR "Delta Amacuro")) OR (ecuador AND (sucumbíos OR orellana OR napo OR pastaza OR morona OR zamora)) OR (bolivia AND (pando OR beni OR cochabamba OR "Santa Cruz" OR "La Paz")) OR suriname OR guyana) AND (epidemiolog* OR pathogen* OR detection OR infectious OR disease* OR molecular OR serolog* OR parasite* OR bacter* OR virus OR viral))

Google Scholar search:

- (peccaries OR peccary) AND amazon AND (epidemiology OR pathogens).
- (peccaries OR peccary) AND amazon AND (Virus OR bacteria OR parasite).
- (peccaries OR peccary) AND (acre OR amapá OR amazonas OR pará OR rondônia OR roraima OR tocantins OR "Mato Grosso" OR maranhão OR Belem OR loreto OR ucayali OR "Madre de Dios" OR Iquitos OR Pucallpa) AND pathogens.
- (peccaries OR peccary) AND (caquetá OR guainía OR guaviare OR putumayo OR vaupés OR meta OR Vichada OR bolívar OR "Delta Amacuro" OR sucumbíos OR orellana OR napo OR pastaza OR morona OR zamora AND pathogens
- ("peccaries" OR "peccary") AND (pando OR beni OR cochabamba OR "Santa Cruz" OR "La Paz" OR suriname OR guyana) AND pathogens

- (swine OR pigs) AND amazon (epidemiology OR pathogens)
- (swine OR pigs) AND amazon AND (Virus OR bacteria OR parasite)
- (swine OR pigs) AND (acre OR amapá OR amazonas OR pará OR rondônia OR roraima OR tocantins OR "Mato Grosso" OR maranhão OR Belem OR loreto OR ucayali OR "Madre de Dios" OR Iquitos OR Pucallpa) AND pathogens
- (swine OR pigs) AND (caquetá OR guainía OR guaviare OR putumayo OR vaupés OR meta OR Vichada OR bolívar OR "Delta Amacuro" OR sucumbíos OR orellana OR napo OR pastaza OR morona OR zamora AND pathogens
- (swine OR pigs) AND (pando OR beni OR cochabamba OR "Santa Cruz" OR "La Paz" OR suriname OR guyana) AND pathogens

- (huangana OR sajino OR pecari) AND amazonia AND (patogenos OR enfermedad)
- Cerdos AND Amazonia AND (patógenos OR enfermedad)
- (cateto OR queixada OR pecari) AND amazonia AND (patogeno OR doença)
- (suino OR porco) AND Amazonia) AND (patogeno OR doença)

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Table A1. Occurrence of pathogens reported in the studies included in the systematic quantitative literature review on infectious diseases affecting suids in the Amazon region.

Pathogen	Occurence	Location	Host	Ref.
African swine fever virus	Seroprevalence: 0%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
***Anaplasma* spp.**	Prevalence: 100% Seroprevalence: 0%	Mato Grosso (BR), Pará (BR) and Santa Cruz (BOL)	CP and WLP	Karesh *et al.* (1998); Sousa Soares (2013); Sousa Soares, Marcili , *et al.* (2017, 1)
Ancylostomatidae	Prevalence: 0-33.3%	Amazonas (BR) and Madre de Dios (PE)	CP and WLP	Carlos *et al.* (2008); Queiroga Gonçalves (2014)
***Ascaris* spp.**	Prevalence: 0-100%	Amazonas (BR), Beni (BOL) and Madre de Dios (PE)	CP and WLP	Carlos *et al.* (2008); Limachi-Quiñajo *et al.* (2014); Queiroga Gonçalves (2014)
Aujeszky disease virus	Seroprevalence: 0- 100%	Caqueta (COL), Madre de Dios (PE), Pará (BR) and Santa Cruz (BOL)	CP, WLP and pigs	Arbeláez *et al.* (1999;) Karesh *et al.* (1998); Mayor *et al.* (2006); Romero Solorio (2010; Romero Solorio, Neto, *et al.* (2010)
***Babesia* spp.**	Prevalence: 0- 11.1%	Mato Grosso (BR) and Pará (BR)	CP and WLP	De Barros Silva *et al.* (2021); Sousa Soares (2013); Sousa Soares, Marcili, , *et al.* (2017)
***Bacillus* spp.**	Prevalence: 1.7%	Pará (BR)	CP	Pinheiro Bartha *et al.* (2020)
Bartonella bacilliformis	Prevalence: 100% (in ticks)	Madre de Dios (PE)	CP	Del Valle-Mendoza *et al.*, (2018)
***Blastocystis* spp.**	Prevalence: 0%	Amazonas (BR)	CP and WLP	Queiroga Gonçalves (2014)
Bordetella bronchiseptica	Prevalence: 0%	Mato Grosso (BR) and Pará (BR)	CP and WLP	Martins Gomes de Castro *et al.* (2014)
***Borrelia* spp.**	Prevalence: 0%	Mato Grosso (BR) and Pará (BR)	CP and WLP	Sousa Soares, (2013); Sousa Soares, Marcili, *et al.* (2017-1)
***Brucella* spp.**	Prevalence: 0% Seroprevalence: 0- 58.3%	Madre de Dios (PE), Pará (BR), Rodonia (BR) and Santa Cruz (BOL)	CP and WLP	Gatto Brito *et al.* (2017); Karesh *et al.* (1998); Mayor *et al.* (2006); Romero Solorio (2010); Romero Solorio, Neto, *et al.* (2010)
Capillaria hepatica	Prevalence: 100%	Amazonas (BR) and Mato Grosso (BR)	CP and WLP	Pereira Soares *et al.* (2011); Queiroga Gonçalves (2014)

Classical swine fever virus	Seroprevalence: 0%	Madre de Dios (PE) and Santa Cruz (BOL)	WLP	Karesh *et al.* (1998); Romero Solorio (2010); Romero Solorio, Neto, *et al.* (2010)
Cooperia punctata	Prevalence: 100%	Tocantins (BR)	CP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016)
Corynebacterium	Prevalence: 2.2%	Pará (BR)	CP	Pinheiro Bartha *et al.* (2020)
***Coxiella* spp.**	Prevalence: 0%	Mato Grosso (BR) and Pará (BR)	CP and WLP	Sousa Soares (2013)
Cruzia brasiliensis	Prevalence: 100%	Tocantins (BR)	CP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016)
***Cytauxzoon* spp.**	Prevalence: 0%	Mato Grosso (BR) and Pará (BR)	CP and WLP	Sousa Soares, Marcili, *et al.* (2017-2); De Barros Silva *et al.* (2021)
***Ehrlichia* spp.**	Prevalence: 22.2- 100%	Beni (BOL), Mato Grosso (BR) and Pará (BR)	CP and WLP	Limachi-Quiñajo *et al.* (2014); Sousa Soares (2013); Sousa Soares, Marcili , *et al.* (2017-1)
Encephalomyocarditis virus	Seroprevalence: 0%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
Entamoeba histolytica	Prevalence: 0%	Amazonas (BR)	CP and WLP	Queiroga Gonçalves (2014)
***Enterococcus* spp.**	Prevalence: 1.7%	Pará (BR)	CP	Pinheiro Bartha *et al.* (2020)
Erysipelothrix rhusiopathiae	Seroprevalence: 0%	Pará (BR)	CP	Mayor *et al.* (2006)
Escherichia coli	Prevalence: 7.8-68.7%	Mato Grosso (BR) and Pará (BR)	CP, WLP and pigs	Prado Martins *et al.* (2013); Tavares Dias *et al.* (2014)
Eucyathostomum dentatum	Prevalence: 100%	Beni (BOL) and Tocantins (BR)	CP and WLP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016); Limachi-Quiñajo *et al.* (2014)
Foot and Mouth virus	Seroprevalence: 0%	Pará (BR) and Santa Cruz (BOL)	CP and WLP	Karesh *et al.* (1998); Mayor *et al.* (2006)
Gastroenteritis parasites	Seroprevalence: 0%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
Giardia Lamblia	Prevalence: 0%	Amazonas (BR)	CP and WLP	Queiroga Gonçalves (2014)
Hemophilus parasuis	Seroprevalence: 0%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
Hepatitis E virus	Prevalence: 8-32% Seroprevalence: 9.9 - 81.2%	Mato Grosso (BR) and Pará (BR)	Pigs	Da Costa Lana *et al.* (2014); Gonçalves de Campos *et al.* (2018); Guimarães *et al.* (2005); Lopes dos Santos *et al.*

				(2009); Souza De Souza *et al.* (2012)
***Hepatozoon* spp.**	Prevalence: 0-44.9%	Mato Grosso (BR) and Pará (BR)	CP and WLP	De Azevedo Gomes *et al.* (2018); De Barros Silva *et al.* (2021); Sousa Soares (2013); Sousa Soares, Marcili, a, *et al.* (2017-2)
Lawsonia intracellularis	Prevalence: 16.7-16.87%	Mato Grosso (BR)	Pigs	Alberton *et al.* (2011); Moraes *et al.* (2014); Nascimento Chiriboga *et al.* (1999)
***Leishmania* spp.**	Prevalence: 0-7.5% (ticks)	Madre de Dios (PE), Mato Grosso (BR) and Pará (BR)	CP and WLP	Rojas-Jaimes *et al.* (2017); Rojas Jaimes (2017); Sousa Soares (2013)
***Leptospira* spp.**	Prevalence: 9.8-26.7% Seroprevalence: 9.8- 100%	Acre (BR), Iquitos (PE), Madre de Dios (PE), Pará (BR), Santa Cruz (BOL) and Ucayali (PE)	CP, WLP and pigs	Almeida Gomes *et al.* (2022); Celis Vásquez (2015); Díaz Ortiz (2019); Galvez *et al.* (2006); Inagaki de Albuquerque *et al.* (2014); Jori *et al.* (2009); Karesh *et al.* (1998); Mayor *et al.* (2006); Mendoza Becerra (2004); Mendoza *et al.* (2007); Romero Solorio (2010); Romero Solorio, Neto, *et al.* (2010); Tavares Dias *et al.* (2014)
Mayaro virus	Seroprevalence: 0%	Petit South (GUY)	CP	Thoisy *et al.* (2003)
***Micrococcus* spp.**	Prevalence: 33.7%	Pará (BR)	CP	Pinheiro Bartha *et al.* (2020)
Monienzia beneden	Prevalence: 100%	Beni (BOL)	CP and WLP	Limachi-Quiñajo *et al.* (2014)
Monodontus aguiari	Prevalence: 100%	Beni (BOL) and Tocantins (BR)	CP and WLP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016); Limachi-Quiñajo *et al.* (2014)
Monodontus semicircularis	Prevalence: 100%	Tocantins (BR)	CP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016)
Mycobacterium avium	Seroprevalence: 0%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)

Mycobacterium tuberculosis	Seroprevalence: 0%	Pará (BR)	CP	Mayor *et al.* (2006)
Mycoplasma hyopneumoniae	Prevalence: 15.4-19.6%	Mato Grosso (BR) and Pará (BR)	CP, WLP and pigs	Dutra *et al.* (2013); Martins Gomes de Castro *et al.* (2014)
Mycoplasma hyorhinus	Seroprevalence: 5.9%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
Mycoplasma kahanei	Prevalence: 50%	Pará (BR)	CP	Moraes *et al.* (2014)
Mycoplasma suis	Prevalence: 25-82.3%	Maranhao (BR)	Pigs	Dos Santos Martins *et al.* (2019)
Neospora caninum	Seroprevalence: 4.9. 27.81%	Madre de Dios (PE), Mato Grosso (BR) and Pará (BR)	WLP and pigs	Gomes Lopes *et al.* (2018); Kawatake Minetto *et al.* (2019); Machado Lopes *et al.* (2021)
Orbivirus	Seroprevalence: 0-29.2%	Madre de Dios (PE) and Santa Cruz (BOL)	WLP	Cárdenas Pecho (2012); Karesh *et al.* (1998); Rivera *et al.* (2013)
Parabronema pecariae	Prevalence: 100%	Tocantins (BR)	CP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016)
***Paragonimus* spp.**	Prevalence: 12.1%	Madre de Dios (PE)	WLP	Carlos *et al.* (2008)
Pasteurella multocida	Prevalence: 15.4-20.5%	Mato Grosso (BR) and Pará (BR)	CP, WLP and pigs	Dutra *et al.* (2013); Martins Gomes de Castro *et al.* (2014)
Physocephalus sexalatus	Prevalence: 100%	Tocantins (BR)	CP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016)
Picobirnavirus	Prevalence: 39.1%	Parás (BR)	Pigs	Nunes Chagas *et al.* (2021)
***Pneumocystis* spp.**	Prevalence: 46.2%	Mato Grosso (BR)	Pigs	Cavallini Sanches *et al.* (2007)
Porcine circovirus 2	Prevalence: 58.21- 92.3% Seroprevalence: 0-100%	Mato Grosso (BR) and Pará (BR)	CP, WLP and pigs	Dutra *et al.* (2013); Martins Gomes de Castro *et al.* (2014); Mayor *et al.* (2006); Oliveira Filho *et al.* (2018)
Porcine parvovirus	Prevalence and seroprevalence: 0%	Mato Grosso (BR) and Pará (BR) and Santa Cruz (BOL)	CP and WLP	Karesh *et al.* (1998); Martins Gomes de Castro *et al.* (2014); Mayor *et al.* (2006)
Porcine respiratory and reproductive syndrome virus	Seroprevalence: 0%	Pará (BR)	CP	Mayor *et al.* (2006)

***Rickettsia* spp.**	Prevalence: 0-100%	Mato Grosso (BR) and Pará (BR)	CP and WLP	Sousa Soares (2013); Sousa Soares *et al.* (2015)
Rinderpest	Seroprevalence: 0%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
Rotavirus	Prevalence: 22% Seroprevalence: 9.9%	Pará (BR)	Pigs	Camargo *et al.* (2012); de Cássio Veloso de Barros *et al.* (2018); Neves *et al.* (2018)
***Salmonella* spp.**	Seroprevalence: 0-16.6%	Mato Grosso (BR) and Pará (BR)	CP and pigs	Da Silva *et al.* (2009); Mayor *et al.* (2006)
San Miguel sea lion virus	Seroprevalence: 55%	Santa Cruz (BOL)	WLP	Karesh *et al.* (1998)
Sapovirus	Prevalence: 12.4 %	Pará (BR)	Pigs	Das Merces Hernandez *et al.* (2014)
Spiculopteragia tayassui	Prevalence: 100%	Tocantins (BR)	CP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016)
Spirurida	Prevalence: 6,10%	Madre de Dios (PE)	WLP	Carlos *et al.* (2008)
***Staphylococcus* spp.**	Prevalence: 19-30.2%	Pará (BR)	CP and WLP	Pinheiro Bartha *et al.* (2020); Tavares Dias *et al.* (2014)
Stichorchis giganteus	Prevalence: 100%	Beni (BOL)	CP and WLP	Limachi-Quiñajo *et al.* (2014)
***Streptococcus* spp.**	Prevalence: 12.5- 30,2% Seroprevalence: 76.5%	Mato Grosso (BR), Pará (BR) and Santa Cruz (BOL)	CP, WLP and pigs	Karesh *et al.* (1998); Pinheiro Bartha *et al.* (2020); Silva de Faria *et al.* (2010); Tavares Dias *et al.* (2014); Tinasi de Oliveira *et al.* (2018)
Swine influenza	Seroprevalence: 0%	Pará (BR) and Santa Cruz (BOL)	CP and WLP	Karesh *et al.* (1998); Mayor *et al.* (2006)
Taenia solium	Seroprevalence: 12- 27.1%	Amazonas (PE) and Madre de Dios (PE)	Pigs	Carhuallanqui *et al.* (2010); Chino Cusi (2020); Rojas Rivera (2017, 2021)
Texicospirura turki	Prevalence: 100%	Beni (BOL) and Tocantins (BR)	CP and WLP	Alves Pereira-Junior (2014); Alves Pereira-Junior *et al.* (2016); Limachi-Quiñajo *et al.* (2014)
***Theileria* spp.**	Prevalence: 0%	Mato Grosso (BR) and Pará (BR)	CP and WLP	De Barros Silva *et al.* (2021); Sousa Soares, Marcili, *et al.* (2017-2)
Toxocara alienata	Prevalence: 100%	Iquitos (PE)	CP	Gomez-Puerta (2015)

Toxoplasma gondii	Prevalence: 7.14-100% Seroprevalence: 11.3- 100%	Iquitos (PE), Madre de Dios (PE), Maranhao (BR), Mato Grosso (BR), Pará (BR), Petit South (GUY) and Rodonia (BR)	CP, WLP and pigs	Aston *et al*. (2014); Carme *et al*. (2002); Cavalcante *et al*. (2006); Freitas *et al*. (2009); Kawatake Minetto *et al*. (2019); Machado Lopes *et al*. (2021); Minervino *et al*. (2010); Romero Solorio (2010); Romero Solorio, Gennari, *et al*. (2010); Saab Muraro *et al*. (2010); Silva *et al*. (2021); Valente Miranda de Aguirra (2020); Vitaliano *et al*. (2015)
Trichostrongylus	Prevalence: 40%	Amazonas (BR)	CP	Marques Dantas (2009)
Trichuris trichiura	Prevalence: 0%	Amazonas (BR)	CP and WLP	Queiroga Gonçalves (2014)
***Trypanosoma* spp.**	Prevalence: 0-50%	Iquitos (PE), Mato Grosso (BR), Moyobamba (PE) and Pará (BR)	CP, WLP and pigs	Gómez Puerta (2007); Morales *et al*. (2017); Sousa Soares (2013); Valente *et al*. (1998)
Vesicular exanthema virus	Seroprevalence: 60%	Santa Cruz (BOL)	WLP	Karesh *et al*. (1998)
Vesicular stomatitis virus	Seroprevalence: 0- 53.4%	Iquitos (PE), Madre de Dios (PE), Pucallpa (PE) and Santa Cruz (BOL)	CP and WLP	Carruitero H. *et al*. (2013); Karesh *et al*. (1998); Wilson *et al*. (2005)
***Wolbachia* spp.**	Prevalence: 0%	Mato Grosso (BR) and Pará (BR)	CP and WLP	Sousa Soares, Marcili, *et al*. (2017-1)

WLP: White lipped peccaries, CP: Collared peccaries, BOL: Bolivia, BR: Brasil, COL: Colombia, GUY: French Guyana, PE: Perú.

Table A2. Diagnostic methods and techniques, including frequency of the methodology in the studies included in the systematic review (not mutually exclusive).

Category	Technique	Frequency	Total
Molecular biology	Polymerase chain reaction (PCR)	20	42
	Reverse transcription PCR (RT-PCR)	2	
	Quantitative PCR (qPCR)	2	
	Quantitative RT-PCR (qRT-PCR)	3	
	Nested PCR	1	
	Nested RT-PCR	2	
	PCR HRM (High resolution melt analysis)	1	
	Polyacrylamide gel electrophoresis (PAGE)	2	
	Sequencing	9	
Serology	Buffered Acidified Plate Antigen (BAPA)	1	39
	Enzyme-linked immunoassay (ELISA)	7	
	Direct agglutination test	2	
	Electroinmuno Transferencia Blot (EITB)	2	
	Western Blot	1	
	Agar Gel Immunodiffusion Assay (AGID)	1	
	Enzyme immunoassay (EIA)	1	
	Immunoblotting test (IB)	2	
	Modified agglutination test (MAT)	11	
	Indirectimmunofluorescent antibody test (IFAT)	6	
	Viral neutralization tests (VNTs)	2	
	Immunochromatography	1	
	Rapid antigen test (RAT)	1	
	Complement fixation test (CFT)	1	

	Mantoux tuberculin skin test (TST)	1	
Culture Isolation Microscopy	Direct microscopy	11	29
	Morphometric identification	2	
	Inmunomarking	1	
	Histopathology	5	
	Inmunochemistry	4	
	Culture	2	
	Biochemistry	3	
	Antibiotic sensitivity	1	

Table A3. Details of publication format of the 77 reviewed studies

Journals	Number of Publications
Acta Scientiae Veterinariae	3
Acta Tropica	1
Animal Reproduction Science	1
Archives of Virology	1
Arquivo Brasileiro de Medicina Veterinária e Zootecnia	1
Biomedica	1
BMC Research Notes	1
Brazilian Journal of Development	1
Brazilian Journal of Microbiology	1
Canadian Journal of Microbiology	1
Ciencia Rural	2
Comparative Immunology, Microbiology and Infectious Diseases	2
EMBAPRA Journal	1
Emerging Infectious Diseases	2
Entomological Society of America	2
Experimental and Applied Acarology	1
International Journal for Parasitology: Parasites and Wildlife	3
International Journal of Sciences	1
International Journal of Current Microbiology and Applied Sciences	1
Journal of Parasitology	2
Journal of Wildlife Diseases	1
Journal of Zoo and Wildlife Medicine	2
Meat Science	1
Medical Mycology	1
Neotropical Helminthology	2
Pesquisa Veterinária Brasileira	1
PloS one	1
Research in Veterinary Science	2
Research Square	1
Revista Brasileira de Parasitologia Veterinária	6
Revista de Investigaciones Veterinarias del Perú	4
Revista de la Facultad de Medicina Veterinaria y de Zootecnia (UNAL)	1
Revista de Medicina Veterinaria	1
Revista Pan-Amazônica de Saúde	2
Revista peruana de biología	1
Scientific Reports	1
Ticks and Tick-borne Diseases	1
The American Journal of Tropical Medicine and Hygiene	1
The Veterinary Journal	1
Tropical Animal Health and Production	1
Vector Borne and Zoonotic Diseases	1
Universities	

	Number of Thesis
Escola Nacional de Saúde Pública Sergio Arouca (Master's Thesis)	1
Universidade Federal Rural da Amazonia (Doctoral Thesis)	1
Universidad Nacional Hermilio Valdizan (Master's Thesis)	1
Universitat de Barcelona (Doctoral Thesis)	1
Universidade de Sao Paulo (Master's Thesis and Doctoral Thesis)	2
Universidad Nacional Mayor de San Marcos (Undergraduate Thesis)	1
Universidad Peruana Cayetano Heredia (Master's Thesis)	1
Universidad Nacional Amazónica de Madre de Dios (Undergraduate Thesis)	1
UTC Cotopaxi Ecuador (Undergraduate Thesis)	1
Universidade Federal de Amazonas (Master's Thesis)	1

Conferences	**Number of presentatios**
XI CIMFAUNA	2
IV Conferencia Nacional sobre Defensa Agropecuaria	1
AAZV AND AAWV JOINT CONFERENCE 1998	1

Table A4. Semi-structured survey conducted with 84 heads of families, documenting hunting activities and human-animal interactions, in Nueva Esperanza community in the Peruvian Amazon.

RISK FACTORS (SOCIOLOGICAL DATA)		
1	Are you a hunter?	( ) Yes ( ) No
2	How many days a week do you hunt?	Number: ____
3	Do you use any hunting equipment? (including dogs)	( ) Yes ( ) No Which: ______
4	Do you use any safety measures when handling hunted animals?	( ) Yes ( ) No Which: ________
5	Have you noticed any injuries when handling the animals?	( ) Yes ( ) No
6	Do you wash your hands after handling the hunted animals?	( ) Yes ( ) No
7	Do you check if the hunted animals have any injuries?	( ) Yes ( ) No
8	Where do you remove the viscera of the hunted animals?	Place: ___________
9	Where do you dispose of the viscera of the hunted animals?	Place: ___________
10	Do you raise animals at home?	( ) Yes ( ) No
	Cats	( ) Yes ( ) No
	Dogs	( ) Yes ( ) No
	Chickens/ Ducks	( ) Yes ( ) No
	Pigs	( ) Yes ( ) No
	Do you deworm domestic animals?	( ) Yes ( ) No
	Do you deworm vaccine animals?	( ) Yes ( ) No
	Do you have wild animals at home?	( ) Yes ( ) No Which: ________
11	Have any animals died at home recently?	( ) Yes ( ) No
12	Do dogs eat waste organs or meat?	( ) Yes ( ) No
13	Do chickens eat waste organs or meat?	( ) Yes ( ) No
14	Are there any sick animals at home?	( ) Yes ( ) No
15	Presence of mice at home	Place: _________
16	Have you been bitten by mice at home?	( ) Yes ( ) No

17	Have you seen mouse nests or droppings at home?	( ) Yes ( ) No
18	Have you seen food with mouse droppings at home?	( ) Yes ( ) No
19	Presence of bats at home	( ) Yes ( ) No
20	Have you been bitten by bats at home?	( ) Yes ( ) No
21	Do bats sleep at home?	( ) Yes ( ) No
22	Have you seen bat droppings at home?	( ) Yes ( ) No

Table A5. Semi-structured survey conducted with 60 adult residents, exploring practices related to handling, processing, preservation and consumption of wild meat, in Nueva Esperanza community in the Peruvian Amazon.

FEEDING BEHAVIOR		
1	How many days a week do you eat wild game meat?	Number: _____
2	How do you preserve meat that is not consumed?	Fresh ( ) Smoked ( ) Dried/Salted ( )
3	Where do you prepare the meat that is consumed daily?	Home ( ) Outdoors ( )
4	How is the meat prepared in your home?	( )Cooked at low temperature ( )Rarely cooked ( )Dehydrated with salt (jerky) ( )Cooked at high temperature
5	Do you consume meat with injuries?	( ) Yes ( ) No
6	Do you consume animal viscera ? Which ones?	( )Liver ( )Lung ( )Kidney ( )Heart ( )Fetus ( )Others_________
7	Do you usually eat the viscera of animals that have injuries?	( )Yes, whole( ) No ( ) I remove the injuries and consume the healthy part
8	Do you consume animals that are already dead?	( ) Yes ( ) No
9	What water do you use for cooking?	( )Rainwater( )Stream water ( )River water( )Treated water ( )Other
10	Where do you dispose of wastewater?	Location:_______

Table A6. Estimation of *Toxoplasma gondii* infection in wild mammals hunted and recorded in the indigenous community Nueva Esperanza community (Peruvian Amazon) between 2010 and 2020, considering serology found in the present study.

Species	***T. gondii* seropositivity (%)**	**Number of individuals hunted and registered**	**Estimated number of infected and consumed individuals**
Cuniculus paca	57/139 (41.0%)	952	390.3
Pecari tajacu	18/65 (27.7%)	316	87.5
Tayassu pecari	16/54 (29.6%)	207	61.3
Lagothrix l. poeppiggi	15/66 (22.7%)	268	60.8
Mazama americana	13/51 (25.5%)	227	57.9
Dasypus novemcinctus	17/38 (44.7%)	83	37.1
Sapajus macrocephalus	15/32 (46.9%)	66	30.95
Ateles chamek	3/20 (15.0%)	98	14.7
Tapirus terrestris	4/21 (19.1%)	74	14.1
Cebus albiforns	3/7 (42.9%)	24	10.3
Nasua nasua	2/19 (10.5%)	86	9.0
Dasyprocta fuliginosa	1/6 (16.7%)	34	5.7
Mazama nemorivaga	1/1 (100%)	5	5.0
Plecturocebus cupreus	1/4 (25.0%)	11	2.75
Alouatta seniculus	1/3 (33.3%)	7	2.3
Cacajao clavus	1/16 (6.25%)	33	2.1
Hydrochoerus hydrochaeris	1/1 (100%)	1	1.0
Sciurus cf. igniventris	0/1 (0.0%)	2	0.0
Galea musteloides	0/1 (0.0%)	2	0.0
Leontocebus (Saguinus) fuscicolis	0/1 (0.0%)	28	0.0
Pithecia monachus	0/6 (0.0%)	18	0.0
Total		2556	792.8

Table A7. Geographic and Regional Data on Study Communities

Comunity	Rurality	Region type	Region	River	Country	Geographic coordinates
20 de Enero	Rural	Amazon	Loreto	Nauta	Perú	-4.654240,-73.852129
28 de Enero	Rural	Amazon	Loreto	Itaya	Perú	-4.271305, -73.592023
28 de julio	Rural	Amazon	Loreto	Tigre	Perú	-3.477337, -74.803206
Alto Verde	Rural	Non-Amazon	Noroeste	Santa María	Argentina	-23.344611, -64.686167
Arequipa	Rural	Amazon	Loreto	Nauta	Perú	-4.623944,-73.900838
Atalaya	Urban	Amazon	Ucayali	Ucayali	Perú	-10.732060, - 73.758054
Atalaya	Rural	Amazon	Ucayali	Ucayali	Perú	-10.708335, - 73.790459
Bandeadero	Rural	Non-Amazon	Noroeste	Negrito	Argentina	-23.344611, -64.686186
Buenos Aires	Rural	Amazon	Loreto	Nauta	Perú	-4.656055,-73.849936
Buritis	Urban	Amazon	Rondônia	Candeias	Brazil	-10.212672, -63.828715
Buritis	Rural	Amazon	Rondônia	Candeias	Brazil	-10.212672, -63.828715
Cacoal	Rural	Amazon	Rondônia	Ji-paraná	Brazil	-11.432126, -61.454791
Candeias do Jamari	Urban	Amazon	Rondônia	Candeias	Brazil	-8.788630, -63.698098
CCNN Puerto Rico	Rural	Non-Amazon	Cusco	-	Perú	-13.510850, -72.009472
Conceicao	Rural	Amazon	Medio Juruá	Juruá	Brazil	-6.205592, -68.093589
Cujubim Grande	Rural	Amazon	Rondônia	Madeira	Brazil	-8.587534, -63.722451
Dulce Alegría	Rural	Amazon	Ucayali	Juruá	Perú	-9.765600, -72.680750
Gamitana	Rural	Amazon	Madre de Dios	Socomayo	Perú	-12.476972, -68.869546
Iquitos	Rural	Amazon	Loreto		Perú	-3.823285, -73.326589
Itapuã do Oeste	Rural	Amazon	Rondônia	Jamari	Brazil	-9.286983,-63.145061
Itapuã do Oeste	Urban	Amazon	Rondônia	Jamari	Brazil	-9.189018, -63.185383
Jaci-paraná	Rural	Amazon	Rondônia	Madeira	Brazil	-9.3289443, -64.452400
Jaci-paraná	Urban	Amazon	Rondônia	Madeira	Brazil	-9.254109, -64.408389
La Goma	Rural	Non-Amazon	Tarija		Bolivia	-22.467745, -64.434484
La Morada	Rural	Non-Amazon	Huanuco		Perú	-8.716793, -76.280836
Lago Serrado	Rural	Amazon	Medio Juruá	Juruá	Brazil	-4.725939, -66.720333

Lipeo	Rural	Non-Amazon	Noroeste	Lipeo	Argentina	-22.288839, -64.717730
Luz del Oriente	Rural	Amazon	Loreto	Itaya	Perú	-4.282489, -73.634408
Macueta	Rural	Non-Amazon	Salta	San Martín	Argentina	-22.038748, -63.733834
Maldonadillo	Rural	Amazon	Ucayali	Ucayali	Perú	-10.743716, -73.721408
Melitón Carbajal	Rural	Amazon	Loreto	Itaya	Perú	-4.266267, -73.597317
Nova Uniao	Rural	Amazon	Medio Juruá	Juruá	Brazil	-5.373288, -67.404785
Nueva Esperanza	Rural	Amazon	Loreto	Yavarí-Mirín	Perú	-4.2164145, -71.987413
Nueva Villa Belén	Rural	Amazon	Loreto	Itaya	Perú	-4.280400, -73.717236
Ñacamiri	Rural	Non-Amazon	Sur		Bolivia	-20.719106, -64.028503
Oventeni	Rural	Amazon	Ucayali	Ucayali	Perú	-10.752480, -74.221580
Porto Velho	Urbano	Amazon	Rondônia	Madeira	Brazil	-8.767131, -63.842983
Porto Velho	Rural	Amazon	Rondônia	Madeira	Brazil	-8.385308, -63.890508
Projeto de Assentamento Aliança	Rural	Amazon	Rondônia	Madeira	Brazil	-8.623089, -63.567907
Projeto de Assentamento Joana D'Arc	Rural	Amazon	Rondônia	Madeira	Brazil	-9.026858, -64.541475
Puerto Breu	Rural	Amazon	Ucayali	Juruá	Perú	-9.530803, -72.758697
Quiriru	Rural	Amazon	Medio Juruá	Juruá	Brazil	-6.292404, -68.198547
Raimondi	Urbana	Amazon	Ucayali	Vilcanota	Perú	-10.727423, -73.754393
Riacho Seco	Rural	Non-Amazon	Noroeste	-	Argentina	-23.494480, -64.426135
Rio Branco	Rural	Amazon	Acre	Purús	Brazil	-9.975838, -61.820081
RN Pucacuro Intuto, Loreto	Rural	Amazon	Loreto	Tigre	Perú	-3.491550,-74.792453
Roque	Rural	Amazon	Medio Juruá	Juruá	Brazil	-5.086949, -67.205319
San Martin de Pangoa	Rural	Non-Amazon	Junin	-	Perú	-11-405943, -74.493653
Saquena Bagazán	Rural	Amazon	Ucayali	Ucayali	Perú	-4.593439,-73.879974
Sepahua	Rural	Amazon	Ucayali	Ucayali	Perú	-11.137892, -73.044533
Serranía Mojotoro	Rural	Non-Amazon	Noroeste	-	Argentina	-24.783672, -65.386073
Vila Nova de Teotônio	Rural	Amazon	Rondônia	Madeira	Brazil	-8.870674, -64.017572
Vila Nova de Teotônio	Urbano	Amazon	Rondônia	Madeira	Brazil	-8.870674, -64.017573

Vila Ramalho	Rural	Amazon	Medio Juruá	Juruá	Brazil	-5.089894, -67.482428
Xerua	Rural	Amazon	Medio Juruá	Juruá	Brazil	-6.033484, -67.822106
Xibauazinho	Rural	Amazon	Medio Juruá	Juruá	Brazil	-5.965950, -67.788181
Yacuiba	Rural	Non-Amazon	Tarija	Gran Chaco	Bolivia	-22.009922, -63.691357

Table A8. Semi-structured survey conducted with 285 Residents on *E. vogeli* awareness, risk perception, and related behaviors

Settlement name:	_______________
Rural/Urban:	_______________
Region or Department:	_______________
Country:	_______________
Question 1: Have you seen lesions similar to those in the photo on pacas?	
Yes/No	
Question 2: Do you think these lesions are dangerous?	
Yes/No	
Question 3: Do you think they are very common in pacas?	
Yes/No	
Question 4: Out of 10 animals, how many animals can you see them in?	
Number: ____	
Question 5: How many infected livers do you find per year?	
Number: ____	
Question 6: What do you do with livers that have these lesions?	
Discard/Consume/Bury/Incinerate/Remove injury and consume/Other	
Question 7: Where do you usually dispose of unconsumed livers?	
Forest/River/Dogs/Trash/Garden/Other	
Question 8: Do you have dogs in your house?	
Yes/No	
Question 9: Do dogs eat discarded viscera?	
Yes/No	
Question 10: Do you deworm your dogs?	
Yes/No	
Question 11: Access to health center is:	
Very difficult/Hard/Medium/Easy	