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Gastrointestinal parasites in captive wild birds in Mineiros, Goiás, Brazil

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Summary

Studying parasites in captive wild birds is vital for their health, well-being, biodiversity preservation, species conservation, and safeguarding of both individual birds and ecosystems. It holds significance for public health by identifying potential zoonotic risks. We aimed to describe the occurrence of gastrointestinal parasites in captive wild birds from a Conservation Institute in Brazilian Cerrado biome. Fresh fecal samples were collected from 17 captive wild birds (*Anodorhynchus hyacinthinus*, *Ara ararauna*, *Ara chloropterus*, *Ara macao*, *Megascops choliba*, *Pteroglossus castanotis*, *Ramphastos dicolorus*, *Ramphastos tucanus* and *Strix huhula*) at a Conservation Institution in Mineiros, state of Goiás. The samples were processed for Willis' simple flotation and Hoffman's spontaneous sedimentation examinations to identify parasitic forms of gastrointestinal endoparasites. Macaw aviary birds (*Ar. ararauna*, *Ar. chloropterus* and *Ar. macao*) showed higher positivity, with all six fecal samples positive for helminths or protozoa. In contrast, captive toucans showed only two positive results (*P. castanotis* and *R. dicolorus*). *An. hyacinthinus* showed Ascarididae, Capillarinae and Trematoda eggs; whereas *S. huhula* had Ascarididae eggs. Regular parasitological examinations are essential for the timely detection and treatment of gastrointestinal infections in captive birds, thereby enhancing overall bird management.
Keywords: Avifauna; Conservation; Coproparasitology; Parasitic diseases

Introduction

The escalating challenges of global climate change, a major concern of the 21st century, poses significant threats to biodiversity. These threats are intensified by human activities such as urbanization, industrialization, and agriculture exacerbating the impact on natural habitats and ecosystems worldwide (Dawson *et al*., 2011; Northrup *et al*., 2019). Such changes is uneven across different geographies and ecosystems, with flat biomes like the Cerrado, Brazil's second-largest biome, being particularly vulnerable to rapid alterations, deforestation, wildfires, and habitat fragmentation (Klink & Machado, 2005; Loarie *et al*., 2009; Calaça *et al*., 2010, Liang *et al*., 2023). Amid theses escalating environmental challenges, the Cerrado biome stands as a critical ecological haven for a variety of species. It faces increasing pressures not only to conserve its native vegetation but also restore degraded areas (Strassburg *et al*., 2017).

The conservation of wild animals, including wild birds, holds paramount importance not only for the preservation of biodiversity but also for maintaining ecological balance and ensuring the heath of

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ecosystems worldwide. Wild birds, an indispensable component of the Cerrado's ecology, contribute significantly to ecosystem equilibrium as pollinators, seed dispersers, and pest controllers (ICMBio, 2018; Egerer *et al*., 2018; Del-Claro *et al*., 2019; Pigot *et al.*, 2020; Katumo *et al*., 2022). Their health status also serves as a bioindicator of environment health and potential zoonotic disease risks (Quinn *et al.,* 2018; Egerer *et al.,*2018). However, they face severe threats from habitat destruction and illegal trafficking, which jeopardize their populations and, in turn, the ecosystem's stability (Piancetini *et al.*, 2015; Silva *et al.*, 2015; Sprenger *et al*., 2018; Lacerda *et al.*, 2023).

Such adverse activities exacerbate the spread and transmission of diseases, including gastrointestinal parasitic infections, by disrupting natural habitats and inducing stress, wich compromises the immune systems of theses wild birds (Lacerda *et al*., 2023, Liang *et al*., 2023). These conditions also facilitate the emergence and spread of novel pathogens, heightening the risk of zoonotic diseases that could affect both domestic animals and humans (Allen *et al.,* 2017; Otranto *et al*.,2019; Yasmeen *et al*., 2023; Lorenzo-Rebenaque *et al.*, 2023). The loss of biodiversity, especially instrumental species in pest and disease control, accelerates disease dispersions, creating a feedback loop that threatens global health security (Quinn *et al*., 2017; Lorenzo-Rebenaque *et al.,* 2023; Yasmeen *et al*., 2023).

In response to these challenges, captive breeding programs in conservation institutes and zoos have emerged as critical strategies for preserving wild bird species (Primack & Rodrigues, 2001; Townsed, 2009). These programs are pivotal in maintaining genetic diversity, establishing or enhancing populations, and providing refuge for species at risk of extinction. However, the captive environment, while mitigating some threats, introduces new stressors that predispose theses birds to various health issues, including parasitic diseases (Li *et al*., 2015; Barbosa *et al*., 2020; Lacerda *et al*., 2023).

This increased susceptibility to diseases in captive setting underscores the requirement for diligent monitoring and specific treatment protocols. (Carneiro *et al.*, 2011; Cubas *et al.*, 2014; Lacerda *et al*., 2023). Thus, the use of diagnostic tools and regular coproparasitological examinations are indispensable strategies to mitigate the adverse impacts of parasitic diseases (Snak *et al.*, 2014, Lacerda *et al*., 2023). In addition, theses examinations are critical measures for preventing the escalation of dynamics and emerging of new zoonotic threats (Lacerda *et al*., 2023; Egan *et al*., 2023).

Therefore, our study aimed to describe the occurrence of gastrointestinal parasites in captive wild birds in the municipality of Mineiros, in Brazilian Cerrado biome.

Materials and Methods

In 2023, the study was undertaken at the Wildlife Conservation Institute in Mineiros, Brazil, situated within the Cerrado biome. The research encompassed 17 fecal samples, each representing a different captive birds housed at the institute, including *Anodorhynchus hyacinthinus, Ara ararauna, Ara chloropterus, Ara macao, Megascops choliba, Pteroglossus castanotis, Ramphastos dicolorus, Ramphastos tucanus,* and *Strix huhula.* These birds were accommodated in four distinct aviaries, designed to emulate their natural habitats while ensuring their safety and well-being.

Observations during environmental data collection revealed distinct housing arrangements: the first aviary (A1) accommodated psittacids such as *Ar. ararauna*, *Ar. chloropterus*, and *Ar. macao*, totaling six individuals in direct contact; the second (A2) was designated for ramphastids including *P. castanotis*, *R. dicolorus*, and *R. tucanus*, subdivided into three sections to facilitate specific interactions; the third aviary (A3) exclusively housed two *An. hyacinthinus;* and the fourth (A4) was reserved for *M. choliba* and *S. huhula* (Table 1).

Throughout the study period, the birds exhibited no overt signs of parasitic infections or other health concerns, indicating a state of asymptomatic health. A targeted observational protocol was meticulously followed for fecal sample collection, strategically conducted in the early morning to align with the peak defecation period after nocturnal rest. This timing ensured the collection of fresh samples, which were then systematically identified, collected, and attributed to the respective birds by the observers. This careful and precise sample-to-bird attribution was fundamental to maintaining the study's integrity and ensuring the accuracy of the coproparasitological examinations that followed.

Moreover, in an effort to minimize contamination risks, fecal samples were collected directly from the ground, which was preemptively lined with parchment paper beneath the birds' perches. This dual-strategy approach—direct linkage of each sample to its respective bird and the utilization of ground-lining parchment paper—was critical for preserving the integrity of the study. It significantly reduced the chances of sample contamination, thereby enhancing the reliability of the coproparasitological analyses and findings.

Fecal samples were uniformly collected with approximate a weight of 1g to ensure analytical consistency and enable standardized comparisons. Samples were placed in appropriately labeled fecal collection containers and stored at a temperature of 4 °C to ensure their preservation. Concurrently, detailed records were maintained concerning the structural design of the enclosures and the management practices employed at the facility. To ensure the accuracy and reliability of the results, all collected samples were analyzed on the same day of their collection to maintain the validity of the results.

Coproparasitological examination were performed, employing Willis' simple flotation and Hoffman's spontaneous sedimentation techniques (Hoffmann, 1987). In the Willis' technique, 0.5 g of fecal matter was combined with a saturated NaCl solution (specific gravity 1.20), filtered through a four-layer gauze sieve, and then transferred into test tubes to form a convex meniscus at the top. A coverslip was carefully placed on the meniscus and left undis-

Table 1. Distribuition of birds across aviaries and number of cohabitants per animal at the Wildlife Conservation Institute in Mineiros, Brazil.

Species	Aviary	Subsection	Cohabitants
Ara macao	A1	1	5
Ara macao	A1	1	5
Ara macao	A1	1	5
Ara chloropterus	A1	1	5
Ara chloropterus	A1	1	5
Ara ararauna	A1	1	5
Ramphastos tucanus	A2	1	2
Ramphastos tucanus	A2	1	2
Ramphastos tucanus	A2	1	2
Ramphastos dicolorus	A2	2	2
Ramphastos dicolorus	A2	2	2
Ramphastos dicolorus	A2	2	2
Pteroglossus castanotis	A2	3	0
Anodorhynchus hyacinthinus	A3	1	1
Anodorhynchus hyacinthinus	A3	1	1
Megascops choliba	A4	1	1
Strix huhula	A4	1	1

turbed for 5 minutes before being transferred to a slide for microscopic examination. For Hoffman's Spontaneous Sedimentation, 0.5 g of the fecal sample was diluted in water, strained to remove debris, and the resultant liquid was left to settle in a conical glass for 12 hours. Post settling, the supernatant was decanted, and the sediment was prepared for microscopic analysis.

Gastrointestinal parasites were identified microscopically using an optical microscope. For the quantification of eggs and oocysts, a Moticam 3.0 camera attached to a Nikon E200 microscope was utilized, with 40x, 100x and 400x of magnifications. The identification of parasite species was conducted following methodologies established by Moravec (1982) and Foreyt (2013).

This study was unable to conduct statistical analyses to determine the prevalence or other measures of parasitological ecology due to constraints in sample size and bird housing conditions.

Ethical Approval and/or Informed Consent

The study was approved by the Animal Use Ethics Committee of the Federal University of Jataí (CEUA/UFJ) under protocol number 011/2021. The authors emphasize that there was no animal manipulation during the study.

Results

Birds from the Macaw aviary showed a higher occurrence of positive results. All six fecal samples obtained from these birds tested positive in at least one of the tests. Among these samples, only one tested positive for protozoan oocysts, whereas the others were parasitized by helminths (Table 2). Flotation examinations identified helminth eggs from the Capillarinae family in three birds (*Ar. ararauna* [n=1], *Ar. chloropterus* [n=1] and *Ar. macao* [n=1]). Additionally, during the spontaneous sedimentation were observed *Entamoeba* spp. oocysts (*Ar. macao* [n=1]) and eggs of Capillarinae (*Ar. ararauna* [n=1], *Ar. chloropterus* [n=2] and *Ar. macao* [n=2]) and *Ascaridia* spp. (*Ar. chloropterus* [n=1] and *Ar. macao* [n=1]) as showed in Figure 1.

In contrast, captive toucans exhibited only two positive results: one for Ascarididae eggs (*P. castanotis* [n=1] in flotation) and the other was unidentified due to the loss of oocyst morphology (*R. dicolorus* [n=1] in sedimentation). Both *An. hyacinthinus* showed the presence of Capillarinae eggs (two in flotation and one in sedimentation), but in one co-infection with Ascarididae eggs (by flotation) was observed and in the other co-infection with Trematode eggs (by sedimentation) was observed. The owl species *M. choliba* was negative for parasites, whereas *S. huhula* was parasitized by Ascarididae eggs observed in flotation.

Discussion

Parasitic infections by nematodes of the genera *Ascaridia* and Capillarinae are substantial concern in both natural and captive bird populations. These parasites are characterized by being mostly monoxenous, which simplifies infection in wild birds and mammals because of their direct life cycles and environmental resistance (Hoque *et al*., 2014; Taylor *et al*., 2017). Effective

Table 2. Coproparasitological examination results of flotation and sedimentation of birds from a Conservation Institution in Mineiros, Goiás in Brazilian Cerrado bioma.

Species	N	Flotation			Sedimentation		
		Positives	Identification	Prevalence	Positives	Identification	Prevalence
Ara macao	3	1	Capillarinae	33.3%	3	*Entamoeba* spp.; Capillarinae; *Ascaridia* spp.	100%
Ara chloropterus	2	1	Capillarinae	50%	2	*Ascaridia* spp.; Capillarinae	100%
Ara ararauna	1	1	Capillarinae	100%	1	Capillarinae	100%
Ramphastos tucanus	3	0	-	0%	0	-	0%
Ramphastos dicolorus	3	0	-	0%	1	NI*	33.3%
Pteroglossus castanotis	1	1	Ascarididae	100%	0	-	0%
Anodorhynchus hyacinthinus	2	2	Capillarinae; Ascarididae	100%	1	Capillarinae; Trematoda	50%
Megascops choliba	1	0	-	0%	0	-	0%
Stix huhula	1	1	Ascarididae	100%	0	-	0%

* Non Identified

management and control of these infections involves adopting measures, such as rigorous sanitation of facilities, administering anthelmintics in water or feed, and protecting feeders and water dispensers from bird fecal contamination (Robertson *et al*., 2016). Periodic elimination of the substrate surface layers, cleaning of cage feces, and implementation of quarantine practices for newly introduced birds are fundamental strategies for prevention and effective control (Godoy, 2007).

Ascaridiasis represents a significant parasitic infection in avian species, notable for its severity and potential lethality, especially among captive wild birds. In this study, Ascaridida was detected in five distinct bird species: *An. hyacinthinus, Ar. chloropterus, Ar. macao, P. castanotis*, and *S. huhula*. Previous research has confirmed the presence of *Ascaridia* spp. in various avian species, including those analyzed in the current study (Martinez *et al*., 1999; Burbano *et al*., 2003; Lekdumrongsak *et al*., 2014; Zhang *et al*., 2018; Yang Fang *et al*., 2018). Futhermore, Silva *et al*. (2022) reported prevalences of 37.5 % in Psittaciforms and 50 % in Strigiforms within a Brazilian zoo, highlighting the widespread distribution and impact of this parasite within avian populations.

The stress experienced by captive birds intrinsically correlates with their susceptibility to severe infections, which can suppress

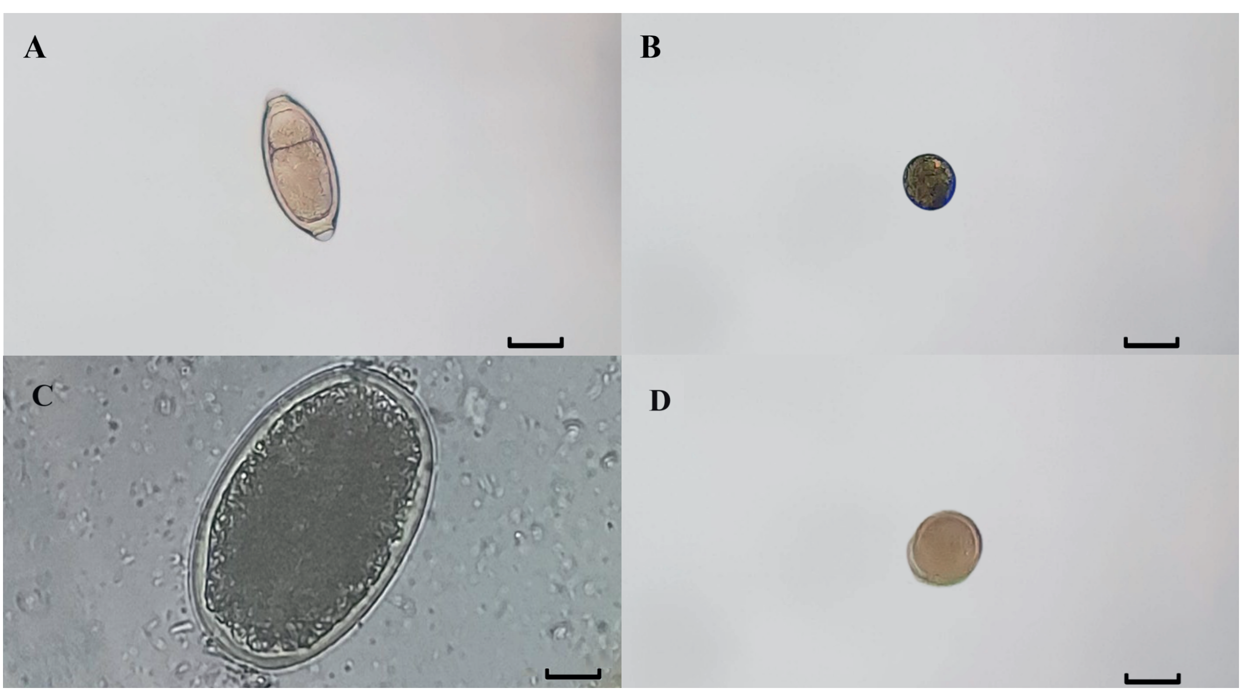

Fig. 1. Light micrograph of coproparasitological examination in wild birds. (A) Capillarinae eggs in *Ara macao*, Scale bar 30µm. (B) *Entamoeba* spp. oocyst in *Ara macao*, Scale bar 15µm. (C) *Ascaridia* spp. egg in *Ara chloropterus*, Scale bar 20µm. (D) Ascarididae egg in *Anodorhynchus hyacinthinus*, Scale bar 40µm.

their immune responses. The severity of these infections depends on the pathogen's virulence, the level of infection, and the host's overall health. Even parasites with low virulence can induce significant clinical diseases in birds, particularly under conditions of immunosuppression or stress (Ayres *et al.*, 2016; Melo *et al.*, 2022). Symptoms of Ascaridiasis, such as weight loss, anorexia, and intestinal obstruction, are prevalent in Psittaciforms (Ritchie *et al*., 1994), underlining the risk to these birds, specially macaws.
Capillarinae infections, while prevalent in this study, affecting six birds, were not observed in ramphastids but were exclusive to psittacids (*An. hyacinthinus* [n=2], *Ar. ararauna* [n=1], *Ar. chloropterus* [n=2] and *Ar. macao* [n=1]). Their direct life cycle (Taylor *et al.*, 2017) and potential for infection via water and food contaminated with eggs (De Santi *et al.*, 2018) suggest that factor such as high population density, , the stress of captivity and the placement of food and water dispensers at ground-level (where contamination are more concentrated) might contribute to the high incidence observed in psittacids.

Capillarinae have cosmopolitan distribution and can infect the gastrointestinal tract, liver, respiratory system, and subcutaneous tissue of various vertebrate groups. Capillariasis is a prevalent parasitic disease among various bird groups, including raptors, psittacines, galliforms, columbiforms, and passerines (Yabsley, 2009). In Northeast Brazil, the prevalence of Capillarinae was recorded at 16 % (Sousa *et al*., 2018). Through, fecal analyss of captive wild birds, *Capillaria* sp. Were detected at significant rates at ttwo locations in the State of Pernambuco: the Chaparral Scientific-Cultural Breeding Center (31.4 %) and at the Dois Irmãos Park (76.4 %) (Freitas *et al.,* 2002). According to Santos *et al*. (2011), the routine practice of maintaining birds in cages or enclosures without adequate sanitation in captive environments facilitates reinfection, despite routine antiparasitic treatments.

The impact of trematode infections extends beyond individual health, influencing the broader ecological dynamics within avian communities. The complex life cycles of these parasites, involving intermediate hosts, often aquatic species, link them intrinsically to environmental health and water quality (Taylor *et al*., 2017; Sousa *et al*., 2018).

Despite their significance, trematodes are less frequently documented in wild birds, particularly those with herbivorous and frugivorous diets. This lower prevalence, noted as approximately 3 % in wild birds from Northeast Brazil, is attributed to the complex life cycle of trematodes, which involves multiple hosts (Sousa *et al*., 2018). However, the reliance solely on coproparasitological examinations often fails to identify many species within this group due to the morphological similarities of their eggs.

The health implications of trematode infections in birds are profound, leading to conditions such as reduced nutrient absorption, impaired body condition, and decreased reproductive capabilities (De Santi *et al.,* 2018). Chronic infections may result in significant morbidity or even mortality, further exacerbated by secondary infections or environmental stresses (Tomás *et al*., 2017). The potential for zoonotic transmission of certain trematode species also presents significant public health concerns, necessitating vigilant monitoring and preventive strategies in both wildlife management and public health domains (Sprenger *et al*., 2018). In our knowledge, this is the firs description of trematode in fecal samples of *An. hyacinthinus*.

The presence of *Entamoeba* spp. in wild birds remains unexplored area in scientific research. However, similar to helminths, protozoan infections generally occur because of ingestion of the infective form, which may be present in food and water (De Santi *et al*., 2018). It is essential to note that parasitism by *Entamoeba* spp. and other protozoa in wild birds is closely linked to stress factors associated with captivity (Farret *et al.*, 2010). Additionally, these protozoa are of public health concern because, although the pathogenicity of these agents in birds is not fully understood, they have zoonotic potential (Farret *et al.*, 2010). Thus, they can infect humans working with birds, such as veterinarians, biologists, caretakers, and traders (Marietto-Gonçalvez *et al*., 2009; Freitas *et al.*, 2002). A deeper understanding of the life cycle, transmission pathways, and factors contributing to *Entamoeba* spp. infection in wild birds is crucial not only for bird health, but also for protecting human health. Future investigations in this area are fundamental to identify prevention and control strategies for these infections in captive environments, thereby reducing the risks for professionals working with these birds and their owners.

Moreover, it is significant to note that it is common in Brazilian culture to keep these bird species in homes, potentially facilitating the transmission of parasitic diseases through close interactions between the birds and residents. Consequently, ongoing research on Brazil's avian populations is essential, considering the ecological importance of these species and their cultural significance (Sousa *et al*., 2018).

Conclusion

In conclusion, this study underscores the significant occurrence of gastrointestinal parasites, such as *Ascaridia* spp. and Capillarinae, in captive wild birds. The findings highlight the critical role of environmental factors, particularly the type of water dispensers, in influencing parasitic infection rates. The observed correlation between ground-level drinkers and increased parasitism provides valuable insights for avian management practices. Consequently, the study emphasizes the importance of continuous health monitoring and regular parasitological examinations in captive wild bird populations. Implementing such practices can lead to timely identification and treatment of infections, ultimately enhancing the overall well-being of these birds. Additionally, the results advocate for ongoing research to deepen our understanding of infection dynamics and the interaction between parasites and their avian hosts in captivity. Such knowledge is essential for refining management strategies and ensuring the health and welfare of captive wild birds.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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