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Survey on helminths and protozoa of captive wild and exotic birds from Northeastern Brazil

[*Pesquisa sobre helmintos e protozoários de aves silvestres e exóticas em cativeiro do Nordeste do Brasil*]

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INTRODUCTION

Due to its impressive territorial extension and biodiversity, Brazil has one of the most diversified bird faunas in the world, composed of approximately 1,832 species. The Brazilian avifauna is remarkable for its diversity, richness, vibrant colors, and varied songs (Barros, 2011; Espécies…, 2011).

These characteristics make birds a very popular group kept as pets, in commercial breeding facilities, genetic banks for endangered species, among other purposes. Unfortunately, many of these animals are kept in inadequate conditions, leading to the emergence of diseases caused by gastrointestinal parasites (Almeida *et al.,* 2006; Bezerra *et al.,* 2012).

Enteroparasitosis is a pathology caused by parasites, whose main groups are nematodes, cestodes, and protozoa. These agents can cause infections and diseases, particularly in captive populations, leading to significant losses. The severity of the infection depends on various factors, including the type of management, animal resistance, biotic potential of the pathogens, and hardiness of the birds. In captivity, these animals require a comfortable and stress-free environment, free from predators and diseases, with sufficient space to fly freely and access to high-quality nutrition (Santos *et al*., 2008).

As clinical signs are nonspecific and often absent, the diagnosis of enteroparasitosis is made through coproparasitological analysis, performed periodically. Some of the most used techniques in the analysis of bird fecal material are direct microscopy and flotation technique, which allow for the morphological identification of eggs, cysts, and oocysts of helminths and protozoa (Bowman, 2014). Thus, it is possible to determine the most effective therapeutic protocol to be performed, as well as the associated environmental control (Cubas *et al*., 2014).

However, the lack of knowledge of pet owners, especially regarding the management of these animals and the importance of preventive exams, contributes to the occurrence of these parasitic diseases, which are often diagnosed only during post-mortem examinations. (Marietto-Gonçalves *et al*., 2009; Gomes *et al*., 2009).

Therefore, parasitological studies are of great value in identifying and controlling parasite species capable of producing diseases in wild and exotic birds, as well as generating data that allow us to understand the dynamics of parasitosis and the potential transmission to other animals and humans (Papini *et al*., 2012).

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MATERIAL AND METHODS

The present study was approved by the Authorization System Information on Biodiversity of the Chico Mendes Institute for Biodiversity Conservation (n° 78323-1),

and subsequently authorized by the Animal Ethics Committee of the Federal University of Sergipe (n° 5485210921).

The present study was carried out in collections of private breeders, zoos, and rehabilitation centers in the states of Alagoas (9°33´42´´ S and 36°28´54´´ W) and Sergipe (10°54´40´´ S and 37°04´18´´ W). Both are in the Northeast region of Brazil. The Northeast region is characterized by having the second-largest population in the country, with approximately 56.1 million inhabitants in an area of approximately 1.554.291,6km², with a population density of 36.1 inhabitants/km² (IBGE, 2022).

Pools of fecal samples (n = 182, 111 Sergipe, 71 Alagoas) were collected through spontaneous defecation from 656 captive wild birds (Alagoas n = 229; Sergipe n = 427), belonging to nine avian orders (Columbiformes n = 13, Gruiformes n =1, Passeriformes n = 28, Phoenicrpteriformes n = 1, Psittaciformes n = 68, Piciformes n = 5, Falconiformes, Accipitriformes and Strigiformes [Birds of Prey] n = 66) and 53 species (Table 1). The pools were formed with varying numbers of specimens according to the density of individuals per enclosure. Sampling was done by non-probabilistic convenience, thus obtaining the largest possible number of fecal samples per location.

Table 1. Number of captive wild bird species that participated in the study

Order	Species	Number of specimens
Columbiformes	*Columba livia* and *Columba talpacoti*	231
Falconiformes, Accipitriformes and Strigiformes (Birds of prey)	*Asio clamator, Asio stygius, Athene cunicularia, Buteogallus aequinoctialis, Buteogallus meridionalis, Buteo albonotatus, Buteo brachyurus, Buteo nitidus, Bubo virginianus, Caracara Plancus, Falco femuralis, Falco sparverius, Falco peregrinus, Geranoaetus melanoleucus, Geranoaetus albicaudatus, Glaucidium brasilianum, Geranospiza caerulescens, Herpetotheres cachinnans, Harpia harpyja, Parabuteo unicinctus, Pulsatrix perspicillata, Rupornis magnirostris, Sarcoramphus papa, Tyto furcata* and *Urubutinga urubutinga*	128
Gruiformes	*Balearica pavonina*	1
Passeriformes	*Cyanocompsa brissonii, Paroaria dominicana, Sporophila albogularis, Sporophila caerulescens, Sporophila nigricollis, Saltatos simlis* and *Zonotrichia capensis*	28
Phoenicopteriformes	*Phoenicopterus chilensis*	5
Piciformes	*Ramphastos toco, Ramphastos dicolorus* and *Ramphastos vitellinus*	9
Psittaciformes	*Ara* sp., *Ara chloropterus, Ara ararauna, Anodorhynchus hyacinthinus, Agapornis* sp., *Amazona aestiva, Amazona ochrocephala, Aratinga jandaya, Aratinga solstitialis, Aratinga auricapillus, Cacatua alba, Ecletus roratus, Eupsittula cactorum, Eupsittula aurea, Guaruba guarouba, Nymphicus hollandicus, Pionus maximiliani* and *Pionites leucogaster*	254

All fecal samples were analyzed using four parasitological diagnostic techniques: direct examination (Hoffmann, 1987); sucrose flotation (Willis, 1921) and Mini-FLOTAC® (Cringoli *et al*., 2013) using flotation solution (FS) #1 (sucrose SG = 1.20) at 1:10 dilution). For the detection of oocyst *Cryptosporidium* sp., the centrifugation-sedimentation technique followed by staining of smears using the Ziehl-Neelsen method was used (Henriksen and Pohlenz, 1981). All methods were performed according to the instructions reported in the original description of each technique.

The parasitic structures identified and observed in fecal samples were photographed and analyzed using the ImageJ software for subsequent identification based on the morphological characteristics provided (Smith, 1995; Taylor *et al*., 2010).

All data were organized in Microsoft Excel (2010) spreadsheets and absolute and relative frequencies (%) were analyzed using InStat software (GraphPad Software, 2000), with a significance level established at $p < 0.05$.

RESULTS

Gastrointestinal parasites were found in 84.07% (153/182; p<0.0001) of the analyzed samples, 56.21% (86/153) of captive birds from the state of Sergipe and 43.79% (67/153) the Alagoas, northeastern of Brazil (p<0.0090). As for the percentage of positivity by parasitological technique, 79.12% (144/182; p=0.0001) were positive in the Mini-FLOTAC®, 43.95% (80/182; p= 0.0202) in the centrifuge-sedimentation with Ziehl-Neelsen staining, 33.51% (61/182; p=0.0001) by direct examination and 31.31% (57/182; p=0.0001) by fluctuation in sucrose.

18 types of parasites were identified (Table 2; Figure 2), grouped as helminths (66.67%; 12/18), protozoa (27.78%; 5/18), and pseudoparasites (5.56%; 1/18) (p<0.0097). In particular, they were classified as Nematoda (83.33%) and Platyhelminthes (16.67%) (Cestoda and Trematoda) (p<0.0001). Monoparasitism (36.60%), biparasitism (28.76%), and polyparasitism (34.64%) were also observed in the birds (p<0.0397).

Table 2. Percentage of positivity by type of gastrointestinal parasite detected in captive wild birds in Brazil

Parasite/Parasitic structure detected	Absolute Frequency (n/N)	Relative Frequency (%)	Prevalence (%)
Amidostomum sp.[E]	1/153	0,65	0,15
Ascarididae [E]	13/153	8,49	17,98
Aspiculuris sp. [E]	2/153	1,30	0,45
Balantidium sp.[C]	5/153	1,96	1,21
Capillaria sp. [E]	12/153	7,84	6,56
Choanotaenia sp. [E]	1/153	0,65	0,15
Coccidian[O]	63/153	41,17	41,92
Cryptosporidium sp. [O]	80/153	52,28	61,89
Echinuria sp.[E]	1/153	0,65	0,15
Entamoeba sp.[C]	71/153	46,40	37,80
Giardia sp.[C,T]	28/153	18,30	10,82
Heterakis sp.[E]	3/153	1,96	9,45
Microsporidium sp. [H]	2/153	1,30	0,30
Platynosomum sp.[E]	1/153	0,65	1,52
Strongyloides sp.[E,L]	30/153	19,60	29,57
Syhacia sp.[E]	2/153	1,30	1,06
Trichostrongylidae[E]	25/153	16,33	27,89
Trichuris sp.[E]	1/153	0,65	0,15

Note: E – Eggs, C – Cyst, O – Oocyst, T – Trophozoite, H – Hypha, L – Larvae.

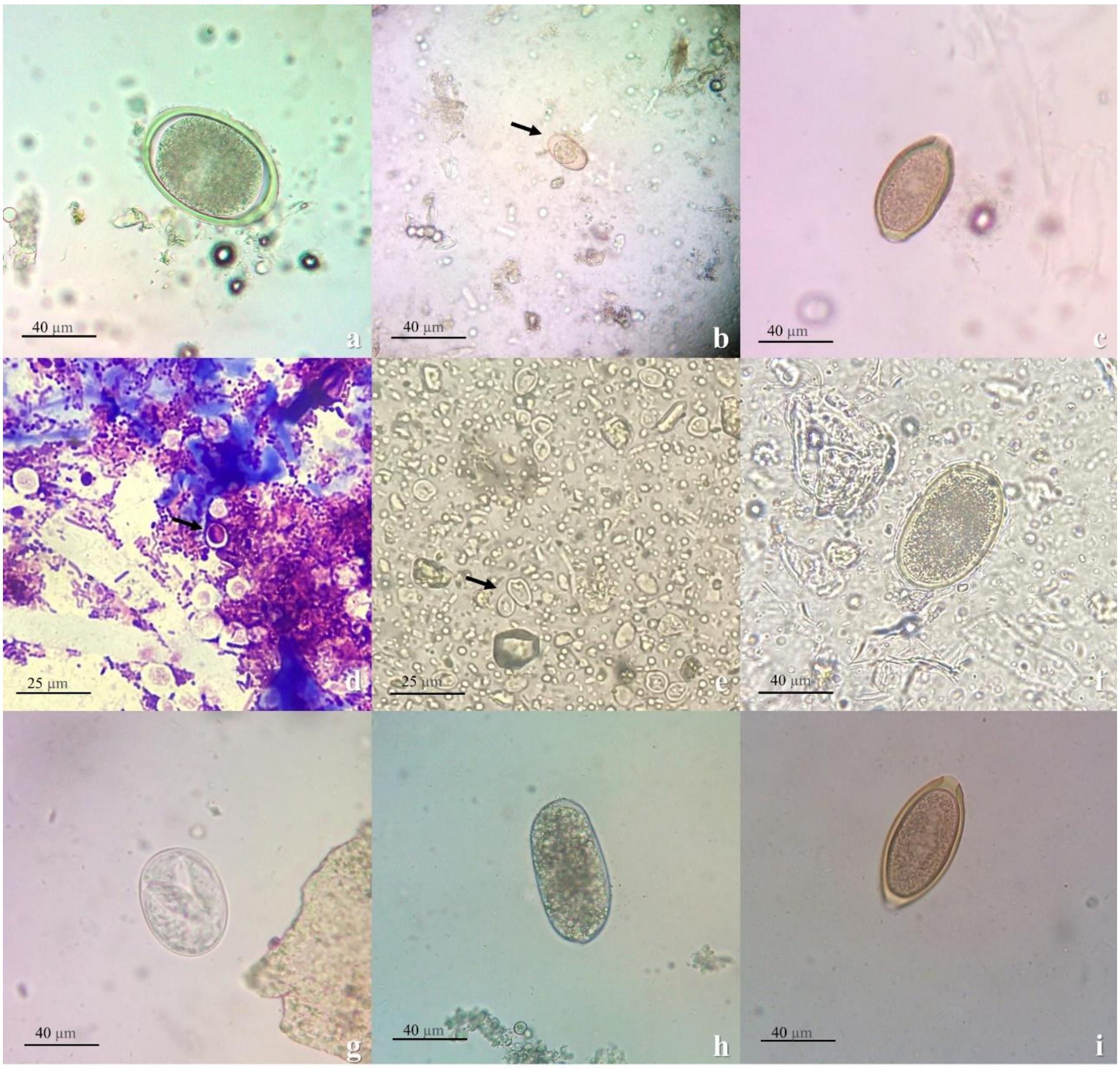

Figure 1. Gastrointestinal parasites detected in fecal samples of wild and exotic birds from Alagoas and Sergipe. a) *Ascaridia* sp. egg; b) Non sporulated coccidia oocyst; c) *Capillaria* sp. egg; d) *Cryptosporidium* sp. oocysts; e) *Giardia* sp. cysts; f) *Heterakis* sp. egg; g) Larvated *Strongyloides* sp. egg; h) Trichostrongylidae egg; and i) *Trichuris* sp. egg. Source: Personal archive (2022).

The most frequent enteroparasites was Coccidian (41.17%; p=0.0001), *Cryptosporidium* sp. (52.28%; p= 0.008), *Entamoeba* sp. (46.40%; p= 0.0480), *Giardia* sp. (18.33%; p= 0.0001), *Strongyloides* sp. (19.60%; p= 0.0001) and Trichostrongylidae (16.33%; p= 0.0001).

Regarding the origin of the animals, 9 (50,00%) types of parasites were detected in birds from the Wild Animal Screening Center (p = 0.4063). In the establishments classified as Wildlife Maintenance Centers, 17 (94,44%) types of gastrointestinal parasites were identified (p= 0.0001). In commercial breeding facilities, birds infected with 10 (55,56%) types of gastrointestinal endoparasites were diagnosed (p= 0.0705).

In Gruiformes birds (*Balearica pavonina*) parasitism exclusively by *Cryptosporidium* sp. (100%; p<0.0001). All fecal samples (n=5; 100%) of Phoenicopteriformes (*Phoenicopterus chilensis*) were positive for *Capillaria* sp. eggs, *Cryptosporidium* sp. oocysts, and *Entamoeba* sp. and *Giardia* sp. cysts (p<0.0035). In Piciformes species, parasitism by *Amidostomum* sp.

(60,00%), *Choanotaenia* sp. (40,00%), Coccidian (20,00%), *Cryptosporidium* sp. (20,00%), *Entamoeba* sp. (20,00%), *Giardia* sp. (20,00%) and *Heterakis* sp. (20,00%) ($p<0.0080$).

In columbiform birds (*Columba livia* and *C. talpacoti*) eggs of Ascarididae (38,46%), *Capillaria* sp. (53,84%), *Heterakis* sp. (23,07%), *Platynosomum* sp. (7,69%) and Trichostrongylidae (46,15%), oocysts of non-sporulating coccidia (84,61%) and *Cryptosporidium* sp. (61,53%), and cysts of *Entamoeba* sp. (15,38%) and cysts and trophozoites of *Giardia* sp (30,76%) (p= 0.5000) were identified in the fecal samples. In Passeriformes seven types of gastrointestinal endoparasites were detected: Ascarididae (7,14%), oocysts of non-sporulating coccidia (57,14%), *Cryptosporidium* sp. (46,42%), *Entamoeba* sp. (75,00%), *Giardia* sp. (17,85%), *Strongyloides* sp. (3,57%) and Trichostrongylidae (10,71%) ($p<0.0249$).

In birds of prey (Falconiformes, Accipitriformes and Strigiformes) were identified 17 (94,44%; p= 0.0004) types of gastrointestinal parasites and/or pseudoparasites: Ascarididae (9,09%), *Amidostomum* sp. (1,51%), *Aspiculuris* sp. (3,03%), *Balantidium* sp. (6,06%), *Capillaria* sp. (9,09%), *Choanotaenia* sp. (3,03%), oocysts of non-sporulating coccidia (46,96%), *Cryptosporidium* sp.(16,66%), *Echinuria* sp. (3,03%), *Entamoeba* sp. (27,27%), *Giardia* sp. (27,27%); *Heterakis* sp. (1,51%), *Microsporidium* sp. (1,51%), *Strongyloides* sp. (6,06%), *Syphacia* sp. (1,51%), *Trichostrongylidae* (6,06%) and *Trichuris* sp. (1,51%) ($p<0.0001$).

12 (66,67%; p=0.0001) types of parasites were identified in the fecal samples of Psittaciformes: Ascarididae (10,29%), *Balantidium* sp. (10,29%), *Capillaria* sp. (5,88%), oocysts of non-sporulating coccidia (36,76%), *Cryptosporidium* sp. (63,23%), *Entamoeba* sp. (57,35%), *Giardia* sp. (23,68%), *Heterakis* sp. (1,47%), *Microsporidium* sp. (2,94%), *Strongyloides* sp. (1,47%), *Syphacia* sp. (2,94%) and Trichostrongylidae (19,11%) ($p<0.0001$).

DISCUSSION

In this study, the main groups of gastrointestinal parasites that are naturally infecting exotic and wild birds kept in captivity in the states of Alagoas and Sergipe, in the Northeast region of Brazil, are described.

The positivity percentage (84.07%) for gastrointestinal parasites in birds was higher than observed in studies conducted in Rio Grande do Sul (Marques *et al*., 2019), Paraná (Snak *et al*., 2014; Sprenger *et al*. 2018), Paraíba (Batista *et al*., 2021), São Paulo (Marietto-Gonçalves *et al*., 2009), and Sergipe (Santos *et al*., 2022). This fact may be related to the number of birds and samples evaluated, the diversity of species participating in the study, as well as the lack of sanitary management and quarantine observed in some locations. In addition to the use of different parasitological techniques, which together can increase the effectiveness in the diagnosis of gastrointestinal parasites.

Lima *et al*. (2016) also highlight that the combined use of different coproparasitological techniques can increase sensitivity and specificity in the diagnosis of gastrointestinal parasites in birds, and that the methods of fecal sample collection and preservation can influence the interpretation of results.

In exotic and wild birds from Alagoas and Sergipe, eighteen types of gastrointestinal endoparasites were detected and identified, which were classified into Nematodes, Platyhelminths, and Protozoa, with the latter being the group with the highest prevalence in the evaluated samples. In studies conducted in the Northeast region, helminths (*Ascaridia* sp., *Ascaridia hermaphrodita*, *Capillaria* sp., *Heterakis* sp., *Strongyloides* sp. superfamilies Strongyloidae and Spiruroidea) and protozoa (*Entamoeba coli*) have been reported in species of Cracidae, Galliformes, and Psittaciformes birds (Sousa *et al*., 2018).

The presence of gastrointestinal nematodes (e.g. Ascarididae, Capillarinae, and Trichostrongylidae) has been reported in various groups of exotic and wild birds kept in captivity throughout Brazil (Marietto-Gonçalves *et al*., 2009; Snak *et al*., 2014; Lima *et al*., 2016; Batista *et al*., 2021).

Despite the diagnosis of several types of gastrointestinal parasites, the protozoan *Cryptosporidium* spp. was the most frequent endoparasite, with a positivity rate of 52.28% (80/153), in addition to being the first detection reported in species such as *Harpia harpyja* and *Balearica pavonina* in Brazil. This parasite has a cosmopolitan distribution, with an infection rate in captive wild birds ranging from 0.41 to 100% (Wang *et al*., 2021). Although four species of *Cryptosporidium* spp. that exclusively infect birds have been described (*C. meleagridis*, *C. baileyi*, *C. galli*, and *C. ornithophilus*), this protozoan is of great importance to public health (Wang *et al*., 2021).

In our study, *Cryptosporidium* spp. was detected in 43.95% (80/182) of fecal samples using the centrifugation-sedimentation technique followed by Ziehl-Neelsen staining (Henriksen and Pohlenz, 1981). However, it is important to highlight that molecular techniques have also been used in studies with birds (Sevá *et al*., 2011; Ferrari *et al*., 2018; Oliveira *et al*., 2017). Despite the scarcity of studies on *Cryptosporidium* spp. infection in wild birds from the Northeast region of Brazil, the high prevalence obtained in this study underscores the need for further research on the identification and genotyping of *Cryptosporidium* sp., given its importance for the conservation of the local avifauna (Sréter and Varga, 2000; Qi *et al*., 2011; Wang *et al*., 2021).

Cysts of *Entamoeba* sp. were also identified parasitizing some bird species in our study. However, Souza *et al*. (2019) highlight that the main taxa affected are Apodiformes, Coraciiformes, Galbuliformes, and Passeriformes. Although it is known that birds usually do not develop clinical signs and their pathogenicity is still uncertain, studies demonstrate that contact with humans is related to the occurrence of cases in wild and exotic birds (Marietto-Gonçalves *et al*., 2008).

Ruffled feathers, prostration, and diarrheic feces have been observed in birds infected by Coccidia and *Giardia* sp., which are considered nonspecific clinical signs (Cunha, 2013; Fernandes *et al*., 2014). Although *G. duodenalis* is known to have a wide range of hosts, parasitism by *G. psittaci* and *G. ardeae* exclusively (species-specific) has been reported in Psittaciformes and Eurypygimorphae (Erlandsen *et al*., 1991; Plutzer and Tomor, 2009).

The study data correlate the gastrointestinal parasitic fauna of wild and exotic birds according to the location of the individuals' maintenance, establishing a direct relationship between the environment and parasitic prevalence, highlighting different parasitic taxa (Nematodes, Platyhelminths, and Protozoa) responsible for causing gastrointestinal diseases in animals, as well as the possible risk for humans who have direct and/or indirect contact with these birds in captivity. The importance of knowledge of the parasite-host relationship by the veterinarian and technical team is evident.

CONCLUSION

The wild and exotic bird species kept in captivity in Northeastern region of Brazil have a high positivity rate (84.07%) for gastrointestinal parasites and are being infected by different taxa of gastrointestinal endoparasites (Nematodes, Platyhelminths, and Protozoa). These parasites can cause gastrointestinal diseases in birds and represent a possible risk to the health of humans who have direct or indirect contact with these animals in captivity.

RESUMO

O Brasil possui mais de 1.800 espécies de aves catalogadas. Quando mantidos em cativeiro, esses animais podem ser susceptíveis a vários tipos de patógenos, inclusive como parasitos gastrointestinais. No entanto, a falta de informações sobre os principais tipos de parasitos que infectam esses animais e as relações parasito-hospedeiro acabam sendo fatores limitantes na manutenção da saúde dessas aves. O objetivo deste estudo foi descrever o levantamento de helmintos e protozoários de aves exóticas e silvestres cativas da região Nordeste do Brasil. Para isso, amostras fecais (n = 182 pools*) foram coletadas de 656 aves em cativeiro, pertencentes a 9 ordens e a 53 espécies. As análises parasitológicas foram realizadas por técnicas de Mini-FLOTAC®, flotação em sacarose, exame direto e coloração de*

Ziehl-Neelsen, além da sedimentação por centrifugação. Todos os dados foram analisados usando o software *InStat, com um nível de significância definido em P<0,05. Parasitos gastrointestinais foram encontrados em 84,07% (153/182; P<0,0001) das amostras analisadas. Foram identificados 18 tipos de parasitos, sendo os enteroparasitos o mais frequentes: Cryptosporidium* sp. *(52,28%; P= 0,008), coccídeos não esporulados (41,17%; P= 0,0001), Entamoeba* sp. *(46,40%; P= 0,0480), Giardia* sp. *(18,33%; P= 0,0001), Strongyloides* sp. *(19,60%; P= 0,0001) e Trichostrongylidae (16,33%; P= 0,0001). As aves de rapina (n=17, 94,44%; P= 0,0004) e os psitaciformes (n=12, 66,67%; P=0,0001) apresentaram os maiores percentuais de infecção por endoparasitos gastrointestinais.*

Palavras-chave: parasitos gastrointestinais, helmintos, protozoários, animais silvestres

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