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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

GASTROINTESTINAL PARASITISM BY HELMINTHS AND PROTOZOA IN WILD BIRDS RESCUED FROM TRAFFICKING IN RIVERSIDE COMMUNITIES ON THE SÃO FRANCISCO RIVER, SERGIPE, BRAZIL

PARASITISMO GASTROINTESTINAL POR HELMINTOS E PROTOZOÁRIOS EM AVES SILVESTRES RESGATADAS DO TRÁFICO EM COMUNIDADES RIBEIRINHAS DO RIO SÃO FRANCISCO, SERGIPE, BRASIL

PARASITISMO GASTROINTESTINAL POR HELMINTOS Y PROTOZOOS EN AVES SILVESTRES RESCATADAS DEL TRÁFICO EN COMUNIDADES RIBERAS DEL RÍO SÃO FRANCISCO, SERGIPE, BRASIL

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ABSTRACT

Illegal wildlife trafficking is a practice that represents a significant threat to biodiversity and public health in many regions of the world. Birds that are victims of trafficking are subjected to conditions of stress and confinement that favor the proliferation of parasites, compromising animal health and well-being. Furthermore, some of these parasites have zoonotic potential, posing an additional risk to human health. Therefore, this study aims to identify the gastrointestinal parasites present in wild birds originating from trafficking in the São Francisco River Basin region, providing important information about the risks to bird health and human health. Fecal samples were collected from wild birds (n=80) belonging to the orders Passeriformes, Columbiformes, and Psitaciformes. The samples were analyzed using two parasitological techniques: Mini – FLOTAC© and centrifugal-sedimentation with Ziehl-Neelsen staining, to identify the presence of gastrointestinal parasites. The results indicate a prevalence of helminths, such as *Capillaria* sp. (1.5%) and Trichostrongylidae (32.8%), and protozoa, such as *Eimeria* sp. (5%), *Isospora* sp. (53,7%), *Cryptosporidium* sp. (83.7) and *Entamoeba* sp. (44.8%). Furthermore, these parasites compromise animal health and well-being, causing clinical signs such as diarrhea, loss of appetite, weight loss, and death. In short, the identification of gastrointestinal parasites in wild birds rescued from trafficking in riverside communities is essential to understanding the risks to bird health and public health in the region.

Keywords: birdlife – helminths – protozoa – zoonosis

RESUMO

O tráfico ilegal de animais silvestres é uma prática que representa uma ameaça significativa para a biodiversidade e para a saúde pública em muitas regiões do mundo. As aves vítimas do tráfico são submetidas a condições de estresse e confinamento que favorecem a proliferação dos parasitos, comprometendo a saúde e o bem-estar animal. Além disso, alguns desses parasitos apresentam potencial zoonótico, sendo um risco adicional para a saúde humana. Diante de exposto, o objetivo desse estudo é relatar o parasitismo gastrointestinal por helmintos e protozoários em aves silvestres resgatadas do tráfico de animais em comunidades ribeirinhas do Rio São Francisco, Sergipe, Brasil, fornecendo importantes informações sobre os riscos à saúde das aves e à saúde humana. Foram coletadas amostras fecais de aves silvestres (n=80) pertencentes às ordens Passeriformes, Columbiformes e Psitaciformes. As amostras foram analisadas a partir de duas técnicas parasitológicas: Mini – FLOTAC© e centrífugo-sedimentação com coloração de Ziehl-Neelsen, para identificar a presença de parasitos gastrointestinais. Os resultados indicam parasitismo por helmintos, como *Capillaria* sp. (1.5%) e Trichostrongylidae (32.8%), e protozoários, como *Eimeria* sp. (5%), *Isospora* sp. (53,7%), *Cryptosporidium* sp. (83.7) e *Entamoeba* sp. (44.8%). Esses parasitos comprometem a saúde e bem-estar animal, ocasionando sinais clínicos como diarreia, inapetência, perda de peso e morte. Em suma, a identificação de parasitos gastrointestinais em aves silvestres resgatadas do tráfico em comunidades ribeirinhas é essencial para compreender os riscos à saúde das aves e à saúde pública na região. Além disso, destaca a necessidade de medidas de controle e prevenção das parasitoses, visando promover a conservação da biodiversidade e a sustentabilidade dos ecossistemas ribeirinhos.

Palavras-chave: avifauna **–** helmintos – protozoários – zoonoses

RESUMEN

El tráfico ilegal de animales silvestres es una práctica que representa una amenaza significativa para la biodiversidad y la salud pública en muchas regiones del mundo. Las aves víctimas del tráfico son sometidas a condiciones de estrés y confinamiento que favorecen la proliferación de parásitos, comprometiendo la salud y el bienestar animal. Además, algunos de estos parásitos presentan potencial zoonótico, siendo un riesgo adicional para la salud humana. Ante lo expuesto, el objetivo de este estudio es reportar el parasitismo gastrointestinal por helmintos y protozoarios en aves silvestres rescatadas del tráfico de animales en comunidades ribereñas del Río São Francisco, Sergipe, Brasil, proporcionando información importante sobre los riesgos para la salud de las aves y la salud humana. Se recolectaron muestras fecales de aves silvestres (n=80) pertenecientes a las órdenes Passeriformes, Columbiformes y Psittaciformes. Las muestras fueron analizadas mediante dos técnicas parasitológicas: Mini-FLOTAC© y centrifugación-sedimentación con coloración de Ziehl-Neelsen, para identificar la presencia de parásitos gastrointestinales. Los resultados indican parasitismo por helmintos, como *Capillaria* sp. (1,5%) y Trichostrongylidae (32,8%), y protozoarios, como *Eimeria* sp. (5%), *Isospora* sp. (53,7%), *Cryptosporidium* sp. (83,7) y *Entamoeba* sp. (44,8%). Asimismo, estos parásitos comprometen la salud y el bienestar animal, ocasionando signos clínicos como diarrea, inapetencia, pérdida de peso y muerte. En resumen, la identificación de parásitos gastrointestinales en aves silvestres rescatadas del tráfico en comunidades ribereñas es esencial para comprender los riesgos para la salud de las aves y la salud pública en la región. Además, destaca la necesidad de medidas de control y prevención de parasitosis, con el fin de promover la conservación de la biodiversidad y la sostenibilidad de los ecosistemas ribereños.

Palabras clave: avifauna – helmintos – protozoarios – zoonosis

INTRODUCTION

Brazil stands out as a country that is home to a significant portion of global biodiversity, due to the vast expanses that comprise different biomes (Charity & Ferreira, 2020). However, among the persistent threats to this biodiversity, wildlife trafficking stands out (ICMBio, 2018). The increase in surveillance regarding this practice in Brazil reveals a notable increase in seizures made by environmental agencies, concomitantly highlighting the limitations in effectively combating this illegal activity (Rabelo *et al*., 2015; Miranda & Dos Anjos, 2022).

In this context, birds stand out as the main targets of wildlife trafficking, due to their notable diversity of species, variation in plumage colors, and richness of songs, combined with their wide geographic distribution (Ribeiro & Silva, 2007; RENCTAS, 2014). After being seized and undergoing rehabilitation processes, the success of their return to their natural habitat is linked to several factors, such as the expression of natural behaviors, the genetic characteristics of the rescued species, and the maintenance of health (De Azevedo *et al*., 2016).

The susceptibility of wild birds to parasitism is influenced by several factors, including their species, contacts, and the conditions of the environment in which they live. Furthermore, birds face significant challenges in captivity, where stress becomes an additional catalyst for the development of parasites (Boll *et al*., 2017).

Gastrointestinal parasites are considerable threats, resulting in high rates of morbidity and mortality. Clinical signs associated with these infestations include diarrhea, loss of appetite, weight loss, and anemia, contributing to a negative impact on the health and well-being of birds and, consequently, on the conservation of the species (Reed *et al*., 2012). Furthermore, some of these etiological agents have a zoonotic potential and can also compromise public health, generating social, economic, and environmental impacts (Saegerman *et al*., 2012).

For laboratory diagnosis, fecal parasitological analysis methods are used, with the Mini – FLOTAC© technique being a prominent option. This technique makes it possible to identify parasite eggs, oocysts, and larvae, and also determine the parasite load (Cringoli *et al*., 2017).

Another method used is centrifugal sedimentation with Ziehl-Neelsen staining, which can be used to diagnose infections by the potentially zoonotic protozoan *Cryptosporidium* sp. (Silva *et al.,* 2016).

Considering the damage caused by gastrointestinal parasites, carrying out new studies becomes essential to contribute to the preservation of threatened species. Given the above, the present study aims to identify gastrointestinal parasites in wild birds rescued from trafficking in riverside communities along the São Francisco River, Sergipe.

MATERIAL AND METHODS

The present work is a retrospective study was carried out in riverside communities with the presence of wild bird victims of trafficking rescued during the 7th stage of the Integrated Preventive Inspection (FPI) in the state of Sergipe (10°54'40" S and 37°04'18" W), Northeast of Brazil. FPI actions took place in 14 municipalities in Sergipe in the São Francisco River Basin: Amparo de São Francisco (10° 9' 58"S and 36° 56' 5" W), Aquidabã (10° 16' 58" S and 37° 1' 12" W), Capela (10° 30' 38" S and 37° 3' 18" W), Graccho Cardoso (10° 13' 42" S and 37° 12' 9' ' W), Japaratuba (10° 35' 43" S and 36° 56' 24" W), Malhada dos Bois (10° 20' 53"S and 36° 55' 23" W), Pirambu (10° 40' 42" S and 36° 52' 25" W), Nossa Senhora da Glória (10° 13' 0" S and 37° 25' 27" W), Monte Alegre (10° 1' 35" S and 37° 33' 31" W), Nossa Senhora de Lourdes (10° 19' 12" S and 36° 34' 44" W), Itabi (10° 7' 11" S and 37° 6' 18" W), Canhoba (10° 8' 15" and 36° 59' 16"W), Feira Nova (10° 16' 4" S and 37° 18' 37" W) and Muribeca (10° 25' 39' S and 36° 57' 45" W).

The experimental design used in this study is classified as cross-sectional with a collection of fecal samples from birds that were victims of trafficking and identification of gastrointestinal parasites present at a single moment in municipalities visited during the 7th stage of FPI in the state of Sergipe.

175 Pools of fecal samples (n=80) were collected through spontaneous defecation from 400
176 wild birds (400/80 = 5 bird/pool), rescued during actions carried out by the Fauna Team in
177 Integrated Preventive Inspection (FPI), during the year 2023, in the state of Sergipe. The
178 specimens belonged to the following three orders: Columbiformes (n = 20), Psittaciformes (n
179 = 50), and Passeriformes (n = 330) (Table 1) (Fig. 1). The *pools* were obtained from specimens
180 of different species, ages and sexes, depending on the number of individuals present in the
181 enclosure. Fecal samples were collected through spontaneous defecation, with only the fecal

Order	**Species**	**Number of specimens**
Columbiformes	*Columbina squammata* (Lesson, 1831)	20
Passeriformes	*Cacicus cela* (Linnaeus, 1758), *Coryphospingus pileatus* (Wied, 1821), *Cyanoloxia brissonii* (Lichtenstein, 1823), *Dolospingus fringilloides* (Pelzeln, 1870), *Euphonia chlorotica* (Linnaeus, 1766), *Gnorimopsar chopi* (Vieillot, 1819), *Icterus jamacaii* (Gmelin, 1788), *Icterus pyrrhopterus* (Vieillot, 1819), *Mimus gilvus* (Vieillot, 1807), *Paroaria coronata* (Miller, 1776), *Piranga flava* (Vieillot, 1822), *Schistochlamys ruficapillus* (Vieillot, 1817) *Genus* Sicalis, *Sicalis flaveola* (Linnaeus, 1766), *Sicalis luteola* (Sparrman, 1789) , *Spinus magellanica* (Vieillot, 1805), Genus Sporophila, *Sporophila albogularis* (Spix, 1825), *Sporophila angolensis* (Linnaeus, 1766), *Sporophila bouvreuil* (Muller, 1776), *Sporophila lineola* (Linnaeus, 1758), *Sporophila nigricolis* (Vieillot, 1823) , *Thraupis sayaca* (Linnaeus, 1766), *Turdus rufiventris* (Linnaeus, 1766), *Volatinia jacarina* (Linnaeus, 1766) and *Zonotrich ia capensis* (Muller, 1776)	330
Psitaciformes	*Alipiopsitta xanthops* (Spix, 1824), *Amazona amazonica* (Linnaeus, 1766), *Aratinga auricapillus* (Kuhl, 1820), *Eupsittula aurea* (Gmelin, 1788) and *Primolius maracana* (Vieillot, 1816)	50

182 material present on the floor of the cages being collected, after clinical evaluation of the
183 animals. All samples were identified, placed in collection tubes, and refrigerated at 8°C until
184 processing.

185 **Table 1:** Number of bird species and wild animals included in the study.

186

187

188

Figure 1. Wild birds of the Psittaciformes and Columbiformes orders rescued from animal trafficking in riverside communities on the São Francisco River, Sergipe, Brazil (A) Birds of the species *Eupsittula aurea* (B) Specimens of the species *Columbina squammata.*

The samples were analyzed using the Mini – FLOTAC© parasitological diagnostic technique (Cringoli et al., 2013). To research *Cryptosporidium* sp., the centrifugal-sedimentation method with Ziehl – Neelsen staining was used (Henriksen & Pohlenz, 1981).

The data obtained were organized in Microsoft Excel ® (2010) spreadsheets and the absolute frequencies and relative frequencies were analyzed using the InStat software (GraphPad Software, 2000), with a significance level of $p<0.05$.

Ethical Aspects

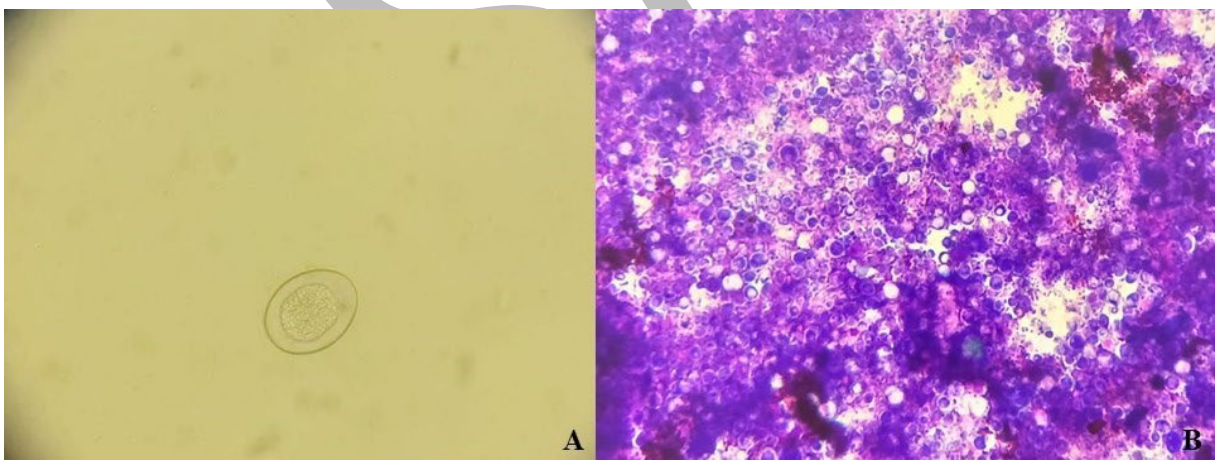
This study was carried out with ethical aspects since secondary data from an action by the Public Ministry of Sergipe (MP) were used, which authorized the analysis and use of the data.

208 **RESULTS**

209 Overall, 83.7% (67/80) of the samples analyzed were positive for gastrointestinal
210 parasites, regardless of the diagnostic technique. Monoparasitism was observed in 59.7%
211 (n=40), while co-infections were identified in 40.3% (n=27) of the animals. Among the positive
212 samples, 4.5% were in birds of the order Columbiformes, 10.4% in Psitaciformes and 85.1% in
213 Passeriformes.

214 In the birds rescued during the inspection action, six types of gastrointestinal
215 endoparasites were identified, belonging to the phyla Nematoda and Protozoa. Protozoa were
216 the most frequent, with emphasis on oocysts of *Isospora* sp. (53.7%), *Eimeria* sp. (5%),
217 *Cryptosporidium* sp. (83.7%), in addition to *Entamoeba* sp. (44.8%). Regarding the phylum
218 Nematoda, nematode eggs from the family Trichostrongylidae (32.8%) and the genus
219 *Capillaria* (1.5%) were detected in the samples (Fig. 2).

220

221 

222 **Figure 2.** Egg and oocysts of gastrointestinal parasites identified in fecal samples from birds
223 rescued from trafficking in riverside communities on the São Francisco River (A) Nematode
224 egg from the Trichostrongylidae Family and (B) Oocysts of the protozoan *Cryptosporidium* sp.

225

226 In passerine birds, the following gastrointestinal endoparasites were diagnosed: oocysts
227 of *Isospora* sp. (65.1%), *Cryptosporidium* sp. (86.36%), *Entamoeba* sp. (39.4%) and

Trichostrongylidae eggs (28.8%). In Columbiformes birds (*Columbina squammata*), two types of gastrointestinal parasites were identified: *Eimeria* sp. (75%) and *Cryptosporidium* sp. (25%). Finally, five types of enteroparasites were detected in Psittaciformes: *Capillaria* sp. (5.9%), *Eimeria* sp. (36.8%), *Cryptosporidium* sp. (63.2%), *Entamoeba* sp. (57.3%) and Trichostrongylidae (19.1%).

Regarding the clinical aspects of birds infected and/or parasitized by helminths and protozoa, different clinical signs were observed, with emphasis on apathy, diarrhea, inappetence, cachexia, prostration, and ruffled feathers.

DISCUSSION

Parasitic diseases represent one of the most common and significant health issues affecting wild birds. In captivity, they are exposed to handling conditions that make them susceptible to gastrointestinal parasites (Boll *et al.,* 2017). These conditions include stress, overcrowding, poor hygiene, malnutrition, and space constraints (Sprenger *et al*., 2018).

Furthermore, it is important to highlight that gastrointestinal parasites can occur asymptomatically. However, when there is a high parasite load, affected animals show signs such as apathy, diarrhea, ruffled feathers, prostration, loss of appetite, and cachexia (Cubas *et al*., 2014). These changes were observed in birds evaluated in this study. Other clinical signs resulting from infection with gastrointestinal parasites include intussusception, intestinal obstruction, and, in more severe cases, death (Papini *et al*., 2012; Lima *et al*., 2017).

Regarding the protozoan *Cryptosporidium* sp., a positivity of 83.75% (67/80) was identified in samples from the three orders studied. Passeriformes presented a positivity rate equal to 86.36% (57/66), a higher result than that of Psittaciformes, whose positivity was 63.23% (8/9), and Columbiformes, which was equal to 25% (1/4). Cryptosporidiosis has been reported in several species of wild and captive birds around the world (Ryan, 2010). In general,

253 the infection is asymptomatic, but in immunosuppressed individuals, it causes enterocolitis,
254 diarrhea, and death. Considering that cryptosporidiosis is a zoonotic disease, it is necessary to
255 adopt preventive measures to avoid human and animal infection (Snak *et al*., 2015).

256 The coccidia *Isospora* sp. was detected exclusively in the fecal samples of the
257 Passeriformes evaluated, with positivity equal to 65.1% (43/66). According to Knight *et al.*
258 (2018), *Isospora* sp. is the coccidia that has the highest prevalence in birds of the order
259 Passeriformes, which reinforces the data obtained in this study. The clinical manifestations of
260 isosporiasis in Passeriformes include weight loss, malabsorption, poor digestion, and even
261 death, signs observed in the birds treated (Giacomo *et al.,* 1997).

262 Another coccidia, *Eimeria* sp., was identified in Columbiformes and Psittaciformes
263 birds, with positivity equal to 75.0% (3/4) and 11.1% (1/9), respectively. This genus of
264 protozoan is commonly found in Columbiformes, including the species evaluated in this study,
265 *Columbina talpacoti* (Jamriska & Modry, 2012). It is worth highlighting that, in general,
266 coccidiosis causes clinical signs such as diarrhea, apathy, progressive weight loss, ruffled
267 feathers, lethargy, feces stuck to the cloaca, dehydration, weakness, and death, especially in
268 young birds, manifestations commonly observed in the animals evaluated. in this study
269 (Marietto – Gonçalves *et al*., 2009; Barreto, 2014).

270 The protozoan *Entamoeba* sp. was identified in the orders Passeriformes and
271 Psitaciformes, with positivity of 39.4% (26/66) and 57.3% (4/9), respectively. Studies that
272 identified gastrointestinal parasites in the same orders of birds corroborate these findings (Sousa
273 *et al*., 2018; Souza *et al*., 2019; Prazeres Júnior *et al*., 2024). According to Graczyk *et al.* (2008),
274 the high frequency of *Entamoeba* sp. in birds, indicates a high degree of anthropization in the
275 environment. Therefore, the presence of this protozoan in fecal samples from birds that are
276 victims of trafficking constitutes yet another problem with this illegal activity.

277 The nematode *Capillaria* sp. was identified in fecal samples from a specimen of
278 *Amazona amazonica* (5.8%). Santos *et al.* (2022) described the presence of the parasite in this
279 species in a study also carried out in the state of Sergipe. The clinical signs of capillariasis are
280 hemorrhagic diarrhea, anorexia, loss of appetite, progressive weight loss, and death (Gomez *et*
281 *al.,* 1993).

282 Trichostrongylidae eggs were identified in the Passeriformes and Psittaciformes
283 samples, with positivity of 28.79% (19/66) and 19.11% (2/9), respectively. The data corroborate
284 the study carried out by Lima *et al*. (2017), who identified this parasite in fecal samples from
285 captive birds in the state of Sergipe. Trichostrongylidae is one of the most common
286 gastrointestinal parasites in birds living in captivity, mainly immunosuppressed Columbiformes
287 and Psittaciformes (Freitas *et al.*, 2002).

288 In the present study, the centrifugal-sedimentation method with Ziehl – Neelsen staining
289 showed a higher level of positivity (16.2%) compared to the Mini – FLOTAC© technique
290 (12.5%). The centrifuge-sedimentation method is easy to perform, quick, low cost, and has
291 good sensitivity, being the most used method in Brazil for the diagnosis of cryptosporidiosis
292 (Ortolani, 2000).

293 Wild animals can be carriers and reservoirs of parasitic diseases, affecting the health of
294 natural and domestic ecosystems (Freitas *et al*., 2002). Some gastrointestinal parasites
295 identified in the seized birds evaluated in this study have zoonotic potential, such as
296 *Cryptosporidium* sp., which in immunosuppressed individuals can cause death. It is crucial to
297 carry out parasitological studies to identify and control parasites that can affect birds, preventing
298 their spread to other species, including humans (Papini *et al*., 2012).

299 Therefore, the identification of gastrointestinal parasites in wild birds rescued from
300 riverside communities on the São Francisco River in the state of Sergipe, Brazil is relevant to
301 understanding the impacts of illegal bird trafficking on the health of poultry populations and

the public health of these communities. By identifying the parasites present in birds, especially those with zoonotic potential, such as nematodes and protozoa, the risks to human health can be assessed. Furthermore, the study can contribute to the development of conservation and environmental education strategies in riverside regions, protecting local biodiversity and promoting awareness about the negative impacts of wildlife trafficking.

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