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Identification of gastrointestinal parasites in birds from the Moroleón Zoo collection, Guanajuato, Mexico

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Abstract

Background: This work aimed to identify gastrointestinal parasites in birds from the Moroleón Zoo collection, Guanajuato, Mexico.
Methods: The birds studied were inhabitants of the "Ruminant" section without restriction. However, during the autumn season, birds cohabited in the "Safari" (antelope, deer, and ostrich) and "Cameroon goat" sections. The bird collection consisted of 62 specimens, which are medically reviewed on a permanent basis as part of a routine veterinary care program; thus, a total of 33 fecal samples, grouped into the orders Galliformes ($n = 18$), Anseriformes ($n = 13$), and Struthioniformes ($n = 2$), were non-probabilistically obtained. Fecal samples were collected directly from the cloaca and preserved in 70 % alcohol. The samples were analyzed using two fecal parasitoscopic techniques: (1) direct microscopy and (2) zinc sulfate concentration (Faust). Additionally, all samples were subjected to Kinyoun staining.
Results: Overall, parasites were identified in 84.8 % of samples, with most containing 2 or more parasite types. The most frequent genera were *Blastocystis* (66.7 %) and *Cryptosporidium* (27.3 %), the former associated with *Numina meleagridis* (Galliformes; $p = 0.01668$) and the latter associated with Anseriformes ($p = 0.03854$).
Conclusion: Knowledge of parasitic biodiversity in birds is crucial to understanding their transmission potential. Therefore, new studies must be conducted to determine the genotypes of the parasites transmitted between birds and other hosts.

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Keywords: *Blastocystis*; *Cryptosporidium*; birds; zoo; parasite.

Identificación de parásitos gastrointestinales en aves de la colección del Zoológico Moroleón, Guanajuato, México

Resumen

Antecedentes: este trabajo tuvo como objetivo identificar parásitos gastrointestinales en aves de la colección del Zoológico de Moroleón, Guanajuato, México. **Métodos:** las aves estudiadas pertenecían a la sección de rumiantes sin restricciones. Sin embargo, durante el otoño, cohabitaban en las secciones de safari (antílope, venado y avestruz) y en la de cabra de Camerún. La colección constaba de 62 especímenes, que recibían atención veterinaria permanente como parte de un programa de cuidados rutinarios. Se obtuvieron 33 muestras fecales, agrupadas en los órdenes Galliformes (n = 18), Anseriformes (n = 13) y Struthioniformes (n = 2), de manera no probabilística. Las muestras fecales se recolectaron directamente de la cloaca y se conservaron en alcohol al 70 %. Se analizaron mediante dos técnicas parasitoscópicas fecales: (1) microscopía directa y (2) concentración con sulfato de zinc (Faust). Además, todas las muestras se sometieron a la tinción de Kinyoun. **Resultados:** en general, se identificaron parásitos en el 84,8 % de las muestras, la mayoría de ellas con dos o más tipos de parásitos. Los géneros más frecuentes fueron *Blastocystis* (66,7 %) y *Cryptosporidium* (27,3 %); el primero, asociado a *Numina meleagridis* (Galliformes; p = 0,01668), y el segundo, a Anseriformes (p = 0,03854). **Conclusión:** el conocimiento de la biodiversidad parasitaria en las aves es crucial para comprender su potencial de transmisión. Por lo tanto, se deben realizar nuevos estudios para determinar los genotipos de los parásitos transmitidos entre aves y otros huéspedes.

Palabras clave: *Blastocystis*; *Cryptosporidium*; aves; zoológico; parásito.

Introduction

Research in zoos is crucial for wildlife conservation; similarly, research on gastrointestinal parasites in zoos is important for monitoring animal health and preventing disease transmission within collections. The study of parasites that affect captive animals enables the development of prevention and control strategies to protect the lives of domestic and wild animals (1–3). The study of birds is particularly relevant, as they are an important biological group in ecological systems, serving as sentinels for various diseases, such as avian influenza (2,4), and for both natural and anthropogenic factors (5). In Mexican society, birds are culturally important, even though they are the third-most traded group of animals kept for ornamental and pet purposes (6). There are many types of parasites that can affect birds; clinical signs in birds are often subtle and can easily go unnoticed. However, diagnosis in birds is generally difficult because they do not show symptoms of disease, especially in subclinical infections or when dealing with wild birds (7,8). In the wild, if a bird shows signs of disease, it becomes prey, so in zoos, the most common diagnostic practice is through laboratory tests (8). Given the importance of zoos as biodiversity reservoirs, specialized veterinary oversight is required (9). Parasitic diseases are notable for their impact on bird health (10), and it is important to evaluate their effects in confined environments and among species that do not naturally coexist to identify interactions and the possible adaptability of parasitic agents in their transmission.

In Mexico, some research has been conducted on avian parasitism, although information remains limited for certain contexts, species, or regions. As an example, *Ascaridia galli* (62.7 %), *Capillaria* sp. (46.5 %), *Heterakis* sp. (30.2 %), and *Trichostrongylus tenuis* (19.5%) were detected in backyard poultry from rural villages in the Mexican tropics; as a matter of fact, 42 % of infections were mixed infections, and 58 % were monoparasitism (11). In ducks (*Anatidae*) from Mexican wetlands, an infection frequency of 89.6 % (52/59) has been reported (12). A study conducted on the wild Gould's turkey (*Meleagris gallopavo mexicana*), an important wildlife resource in Mexico's Sierra Madre Occidental, reported 100 % prevalence of *Raillietina tetragona* and *Eimeria* sp., and 59.4 % prevalence of *Heterakis gallinarum* (13). In a study conducted with psittacine birds kept in captivity as companion and ornamental animals in Yucatán, a prevalence of 14.28 % (12/84) for intestinal parasites was estimated. The reported parasites were *Ascaridia* spp. (33.3 %), *Capillaria* spp. (16.6 %) and *Heterakis* spp. (16.6 %), *Hymenolepis* spp. (16.6 %), *Raillietina* spp. (8.3 %), and *Eimeria* spp. (16.6 %) (14). Similarly, research in Brazil and Colombia has documented mixed infections of helminth and protozoan parasites in backyard and ornamental birds (15,16).

Cryptosporidium has been reported in a frequency of 56.6 % (56/99) from wild species and 43.4 % (43/99) from domestic animals (2). Data from North Mexico showed that 49 % of ducks were infected with *Cryptosporidium* (17). These are consistent reports of migratory waterfowl, such as wild geese, ducks, and swans, infected with *Cryptosporidium*, *Cyclospora*, and *Microsporidia*, highlighting birds' role as potential disseminators of these pathogens (18).

According to these authors' knowledge, information on parasitic biodiversity in zoo birds in the southern area of Guanajuato remains scarce. Thus, the birds' parasite study has a dual importance. First, some of these organisms, such as *Cryptosporidium* and *Eimeria,* are pathogens and cause disease in birds. Second, some protozoa, such as *Blastocystis*, behave as commensals, meaning they can be present without causing clinical disease (19). One study reported that the prevalence of *Blastocystis* sp. in birds is approximately 26.4 %, with variations depending on geographic location and species. This estimate is based on reports published in 2024 (47 articles/98 datasets), with the highest prevalence observed in the following groups: guinea fowl (83.3 %), pheasants (50.4 %), peacocks (41 %), exotic birds (34.4 %), chickens (24.9 %), waterfowl (24.3 %), quail (15.8 %), and pigeons (12.8 %) (20). It is worth noting that some subtypes have been associated with pathogenic gastrointestinal disorders in humans (19).

Finally, it is important to highlight the presence of parasitic agents that appear to be part of the microbiome, such as *Balantidium struthionis* in *Struthio camelus*, which may have pathogenic potential in other species due to their genetic similarity to other Balantidium species (21,22). Understanding the biodiversity of gastrointestinal parasites contributes to a better comprehension of avian health, the status of their microbiota, and their susceptibility to pathogens under stress. Furthermore, the study of parasites helps identify factors relevant to public health, veterinary medicine, and wildlife conservation. Therefore, this study aimed to identify gastrointestinal parasites in birds collected at the Moroleón Zoo, Guanajuato, Mexico.

Materials and Methods

This study was conducted at the Laboratorio de Veterinaria, Facultad Maya de Estudios Agropecuarios, Universidad Autónoma de Chiapas, Mexico. The sample collection was conducted at the Moroleón Zoo in Guanajuato, Mexico. Data analysis was performed at the Laboratorio de Investigación Multidisciplinaria en Parasitología, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Mexico.

Ethical Considerations: Birds at the Moroleón Zoo

This study was approved by the administration and veterinary staff of the Moroleón Zoo, Guanajuato, Mexico. The bird collection consisted of 62 specimens, which were under continuous medical supervision as part of a routine veterinary care program. Samples were collected after eight months without the administration of antiparasitic medications. Biological material was collected during routine veterinary inspections, always in accordance with animal welfare standards and under the supervision of a zoo veterinarian responsible for the birds' handling.

Study Population and Biological Material

The sampled birds were not previously dewormed, although a deworming program is implemented after a biannual diagnostic monitoring. Fecal samples were non-probabilistically collected by opportunity directly from the birds' cloaca using a sterile nylon swab. A total of 33 samples were obtained and were grouped into three orders: Galliformes (n = 18), Anseriformes (n = 13), and Struthioniformes (n = 2). The birds studied were inhabitants of the "Ruminant" section without restriction. However, during the autumn season, birds cohabited in the "Safari" (antelope, deer, and ostrich) and "Cameroon goat" sections. In the "Ruminant" section, silky hen (*Gallus gallus*) and guinea hen (*Numina meleagridis*) roost and move freely within the section. In the "Safari" section, two Anseriformes, peacocks (*Pavo cristatus*) and mute ducks (*Cairina moschata*), as well as one Struthioniform, the ostrich (*Struthio camelus*), coexist freely with buffaloes (*Syncerus caffer*), deer (*Cervus canadensis*), antelopes (*Capra nubiana*), and llamas (*Lama glama*). In the "Cameroon goat" section, Anseriform geese (*Anser anser*) cohabit freely with goats (*Capra aegagrus hircus*). Table 1 shows the distribution of genera from which fecal samples were obtained.

Coprological Techniques

The samples were analyzed following the methodology described in the Practical Manual of Veterinary Parasitology of the Faculty of Veterinary Medicine, University of Extremadura (23). An adaptation of the direct examination technique for fresh feces was performed using preserved samples transported to the laboratory. Subsequently, the enrichment methods described in the manual were applied. All fecal samples were processed by direct microscopy using Lugol's iodine as a counterstain for parasitic forms (23). In addition, all samples were subjected to Kinyoun staining for the detection of *Cryptosporidium* oocysts, following the methods described by Jian and Zhang (24) and Mesfun et al. (25). Each sample was analyzed in triplicate. Parasitic structures were identified using a professional biological microscope (Labomersa, model BA310, Bogotá, Colombia). Eggs, cysts, and oocysts were identified based on their morphological and micrometric characteristics.

Statistical Analysis

The association between birds and parasites was assessed by constructing 2×2 contingency tables, and the analysis of association (chi-square) was performed using the freely available open-source OpenEpi version 3 software for epidemiology (https://www.openepi.com/Menu/OE_Menu.htm) (26).

Results

Overall, gastrointestinal parasites were identified in 28/33 (84.8 %) of the analyzed fecal samples, with most samples harboring multiple parasite types. In order of abundance, fecal samples were obtained from Galliformes (n = 18), Anseriformes (n = 13), and Struthioniformes (n = 2) birds.

Table 1 shows the type and number of parasites identified by bird species. In Galliformes, infection frequency was 77.8 % (14/18) while in Anseriformes it was 92.3 % (12/13). No statistical association was found between bird type and infection frequency (p = 0.1395), nor were any within-group differences found between peacocks and guinea hen (p = 0.2755) or between ducks and geese (p = 0.1309). According to our data, *Cryptosporidium* infection was 46.15 % (6/13) in Anseriformes, but no association was found between ducks and geese (p = 0.1953). Regarding *Blastocystis,* we did not observe any association between Galliformes and Anseriformes (p = 0.3205). Unexpectedly, we observed that infection frequency was higher in guinea hens than in peacocks (p = 0.006713, OR = 0.00094437–0.9912), suggesting that *Numida meleagris* is a common reservoir of *Blastocystis*. New studies should be conducted to determine whether these birds may be a risk factor in the transmission of the parasite to other hosts. In our study, all ducks (*Cairina moschata*) harbored parasites and had the second-highest prevalence of parasitic forms, at 21.2 % (7/33). The third species with the highest proportion of parasitized individuals was the goose (*Anser anser*), at 12.1 %.

Table 1. Genera of gastrointestinal parasites identified in birds at the Moroleón Zoo

Taxonomy order	Host	Number (%)	Infected (%)	Poly-parasitized	*Blastocystis*	*Cryptosporidium*	*Eimeria*	*Balantidium*	*Reililetina*	*Heterakis*
Galliformes	Silkie hen (*Gallus gallus*)	2 / (6)	2 / (100)	Yes	2	•	•	•	•	1
	Peacock (*Pavo cristatus*)	6 / (18.2)	4 / (66.7)	No	1	1	•	•	1	1
	Guinea hen (*Numina meleagis*)	10 / (30.3)	8 / (80)	Yes	8	1	1	•	•	•
	Total	**18 / (54.5)**	**14 (77.8)**		**11**	**2**	**1**	•	**1**	**2**

Taxonomy order	Host	Number (%)	Infected (%)	Poly-parasitized	*Blastocystis*	*Cryptosporidium*	*Eimeria*	*Balantidium*	*Reililetina*	*Heterakis*
Anseriformes	Domestic duck (*Cairina moschata*)	7 / (21.2)	7 / (100)	Yes	5	4	1	●	●	●
	Graylag goose (*Anser anser*)	6 / (18.2)	5 / (83.3)	Yes	4	2	●	●	1	●
	Total	**13 (39.4)**	**12 / (92.3)**		**9**	**6**	**1**	●	**1**	●
Struthioniformes	Ostrich (*Struthio camelus*)	2 / (6)	2 / (100)	Yes	2	1	●	2	●	●
● No animals tested positive.										

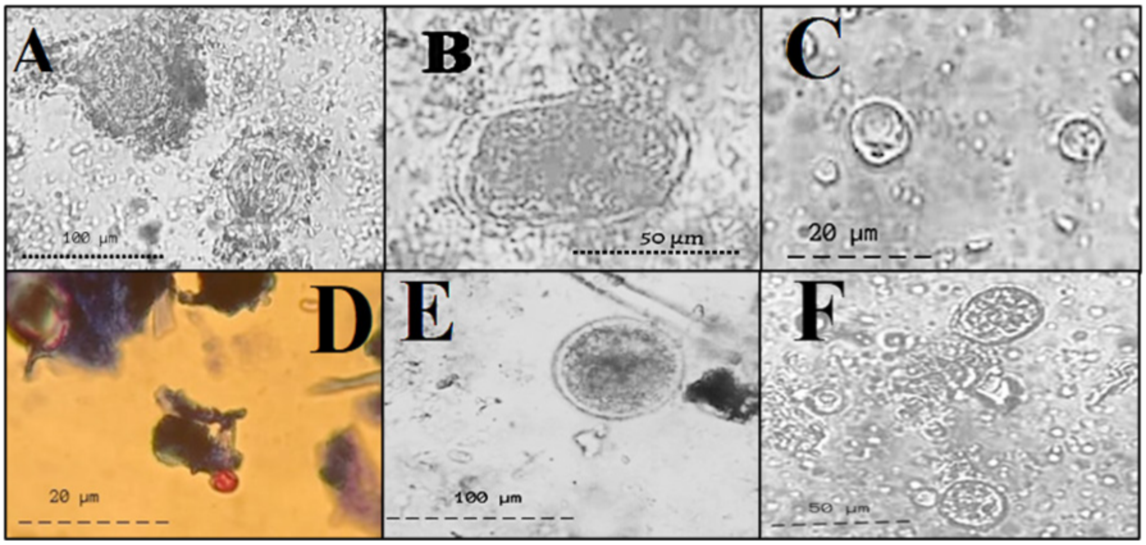
Source: Own elaboration

Four genera of protists were identified: *Blastocystis*, *Cryptosporidium*, *Balantidium*, and *Eimeria* (Fig. 1), as well as two genera of helminths, *Rillietina* (Cestoda) and *Heterakis* (Nematoda). The most frequently detected genus was *Blastocystis*, with a prevalence of 66.7 %. Morphological examination revealed the presence of vacuolar and granular forms of *Blastocystis* (Fig. 1C). *Blastocystis* was most frequently detected in birds belonging to the order Galliformes (11/33). No statistically significant association was found between the presence of *Blastocystis* and *Struthio camelus* (p = 0.2877) or birds of the order Anseriformes (p = 0.6465). *Numida meleagris* (guinea fowl) showed the highest frequency of *Blastocystis* (8/10), representing 24.2 % of the positive samples (p = 0.01668) (27).

Cryptosporidium was the second most frequently identified parasite, with a prevalence of 27.3 %. Its presence was confirmed by Kinyoun staining (Fig. 1D). Birds in the order Anseriformes had the highest prevalence (18.2 %), with a statistically significant association (p = 0.03854). No association was observed with the order Galliformes. The least frequently detected protists were *Balantidium* (6 %; Fig. 1E) and *Eimeria* (6 %; Fig. 1D). *Eimeria* was detected in *Numida meleagris* (Galliformes) and *Cairina moschata* (Anseriformes), whereas *Balantidium* was identified only in ostriches.

Regarding helminths, two genera were identified: *Rillietina* (3 %), a cestode of the family Davaineidae (Fig. 1A), and *Heterakis gallinarum* (3 %), a nematode of the family Heterakidae (Fig. 1B).

Fig. 1. Gastrointestinal parasites in stool samples of birds from *Moroleon* Zoo. A. *Rillietina* eggs (*Cestoda*); B. a *Heterakis* egg (*Nematoda*); C. a *Blastocystis* cyst (*Bigyra*); D. a *Cryptosporidium* oocyst (*Miozoa*); E. a *Balantidium* cyst (*Ciliophora*); F. an *Eimeria* oocyst (*Miozoa*).



Source: Own elaboration

Discussion

The predominance of *Blastocystis* (66.7 %) observed in this study suggests that this protist is widely distributed among captive avian populations and may constitute a common component of the gastrointestinal microbiota. Although *Blastocystis* is frequently considered a commensal organism, it has been associated with opportunistic pathogenicity, particularly in immunocompromised hosts (28). The detection of vacuolar and granular forms is consistent with previous studies, which indicate that these are the most commonly observed morphotypes, especially in preserved samples processed using conventional techniques (29,30).

The high frequency of *Blastocystis* in this study is consistent with reports in both wild and domestic birds, where this protist has been identified as one of the most common commensal or potentially pathogenic organisms in the gastrointestinal tract (31,32). This finding may reflect environmental pollution, particularly when animals are exposed to polluted water or food sources (33). The higher prevalence in Galliformes, especially in *Numida meleagris*, suggests that these birds may play a role in the environmental dispersal of *Blastocystis*. Therefore, further studies are needed to characterize circulating subtypes and to correlate their presence with clinical signs and specific environmental conditions.

In Mexico, *Blastocystis* has been reported with a frequency of 5 % (6/124) in fecal samples from wild mammals (34), indicating that its presence extends beyond avian hosts. Despite its frequent detection in birds, its pathogenic role remains under debate (27,35). However, the increasing prevalence of *Blastocystis* in humans has raised concerns regarding its potential pathogenicity and zoonotic implications in the region (36).

Cryptosporidium was the second-most prevalent parasite in this study (27.3 %), underscoring its importance as a gastrointestinal pathogen in birds. The association observed with Anseriformes may be explained by their close interaction with aquatic environments, which facilitates oocyst survival and transmission. However, our findings differ from reports in China, where lower prevalences have been documented in free-range chickens (1.3 %), ducks (7.3 %), and geese (1.4 %) (37). Conversely, higher prevalences have been reported in African ducks (42 %) (38).

In Mexico, *Cryptosporidium* has been mainly reported in cattle and other domestic animals, with prevalences ranging from 49 % to 83 %, particularly in dairy-producing regions such as the Mexican highlands (39). Additionally, studies conducted in wild birds have reported high prevalences, such as 64 % in sparrows from the northern Mexican plateau (40) and 73 % in green-backed parakeets (*Aratinga holochlora*) from the Aragón Zoo (41). These findings highlight the variability in prevalence across host species, environmental conditions, and geographic locations. Further research is needed to determine the epidemiological role of Anseriformes in Mexico, particularly considering that many species are migratory and may facilitate pathogen dissemination through interaction with domestic and wild birds (42).

The low frequency of *Balantidium* and *Eimeria* (6 % each) suggests limited circulation within the studied population. Previous studies have reported *Balantidium coli* in ostrich farms in Brazil and Colombia, with a prevalence of 33 % (43) and infection frequencies ranging from 15 % to 80 % in ostriches from Argentina (44). Additionally, *Balantidium* has been reported in rheas (*Rhea americana*), which are morphologically similar to ostriches (45).

The presence of *Eimeria* in this study contrasts with findings from zoological collections in Brazil, where oocysts were detected in only one wild bird and were absent in captive birds (46). Similarly, in Guatemala, a prevalence of 8.1 % (3/37) was reported in peacocks (*Pavo cristatus*) (47). Other studies have reported higher prevalences (19.6 %) across multiple avian orders, including Galliformes, in zoo populations (45), suggesting that environmental and management conditions may influence infection dynamics.

The detection of *Rillietina* and *Heterakis gallinarum* at low prevalence (3 %) is consistent with previous reports describing these parasites as common but often subclinical infections in birds. *Heterakis gallinarum* typically infects the cecum of Galliform birds and generally causes mild or asymptomatic infections (30). The presence of *Rillietina* in birds such as geese and peacocks reflects its wide distribution in tropical environments, likely associated with the ingestion of arthropods acting as intermediate hosts (47).

Finally, in this study, two ostriches (*Struthio camelus*) were analyzed and housed in enclosures with peacocks, ducks, and guinea fowl. Interestingly, *Balantidium* was detected only in these individuals, suggesting a possible host association. However, due to the limited sample size, further studies are required to confirm this relationship. To the best of our knowledge, this represents the first evidence of *Balantidium* in ostriches from a zoo collection in Mexico. Nevertheless, this parasite has previously been reported in zoological and production systems in Latin America (43).

Overall, these findings highlight the diversity of gastrointestinal parasites in captive birds and emphasize the importance of continuous parasitological surveillance to better understand host–parasite interactions, environmental transmission dynamics, and potential risks to animal and public health.

Conclusion

This study determined the presence of six genera of gastrointestinal parasites, two helminths and four protists; the most common being *Blastocystis* associated with the guinea hen. This is also the first report of *Balantidium* in ostriches in Mexico. Further studies should be conducted to determine the risk of transmission of the parasites studied here to other hosts, including other animals in the collection and their handlers. The results of our study are important and useful from a veterinary health perspective, to maintain the well-being of the animals in the collection, and from a human health perspective, to identify hosts that act as reservoirs for parasites that can cause zoonotic problems.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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