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Detection and molecular identification of *Giardia* in Mynahs: A case report

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A B S T R A C T

Giardia is a protozoan parasite affecting many animals, including humans, mammals, and birds. The infection of this parasite is related to the digestive system. The three common species of *Giardia* in birds include *G. psittaci*, *G. duodenalis*, and *G. ardeae*. Ornamental birds as pets can transmit this organism to humans. For this reason, investigating the presence of this parasite in ornamental birds is of great importance. In general, the studies done in birds for molecular identification of *Giardia* are limited. Also, *Giardia duodenalis* is usually found in mammals, and its report is rare in birds. This study investigated the presence of this parasite in five mynahs with symptoms. For this purpose, several methods, including radiology, blood tests, wet smear tests, Giemsa stain, and PCR, were used to confirm the presence of *Giardia*. To our knowledge, this is the first report on the presence of *Giardia* parasites in Mynah in Iran.

Keywords: *Giardia duodenalis, Mynah, Zoonotic, Birds, Protozoan parasite*

1 Introduction

Giardia spp. is a parasitic protozoan that affects the digestive system in humans and animals. The prevalence of this parasite is variable in some ornamental birds but is common in wild and captive birds (1). Giardiasis in ornamental birds has been detected mainly in parrots, including cockatiels (*Nymphicus hollandicus*), lovebirds (*Agapornis*) , budgerigars (*Melopsittacus undulates*), and African grey parrots (*Psittacus erithacus*) (2). The three common species of *Giardia* in birds include *G. psittaci*, *G. duodenalis*, and *G. ardeae* (3). *Giardia psittaci* and *G.*

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ardeae are specific to birds and are not zoonotic (4). Giardiasis is a common parasitic disease in parakeets and cockatiels, but infected birds usually do not show any sign, especially adult cockatiels and budgerigars (5). This disease is transmitted through contaminated water, food, soil, and direct contact with the feces of other birds that contain cysts or trophozoites of the parasite (4). This parasite causes a self-limiting disease in humans (*G. duodenalis*) (6). The incidence of this disease in birds ranges from asymptomatic in adults to symptoms such as weight loss, lethargy, anorexia, ruffled feathers, and feather picking with screaming. The diagnosis methods for this disease include observation of clinical symptoms, wet smear from fresh feces, polymerase chain reaction (PCR), and flotation methods (7). Motile trophozoites or cysts can be seen in the wet smear; however, when the fecal sample is not fresh, the probability of observing trophozoites is reduced (8). Nitroimidazoles, such as metronidazole, can be used to treat this disease. Vitamins A and E are also used because *Giardia* decreases vitamins A and B absorption.

2 Case Presentation

Fecal samples were collected from five mynahs with related symptoms who had been referred to the Birds Clinic of the Faculty of Veterinary Medicine, University of Tehran. Samples were collected from the bottom of the cage and, after macroscopic examination, were tested by two direct methods of wet smear and staining and then were stored at -20 ºC in order to perform the PCR using specific primers for the GDH region of *Giardia duodenalis* genome. A whole-body radiograph with the ventrodorsal and lateral positions was taken by a digital radiography system (Kodak Directview CR 850, Japan) from mynahs, and blood samples were taken from the wing vein to evaluate the blood parameters.

Giemsa staining was done as follows (9, 10): Air-dried samples were fixed in methanol for 10 min. After evaporating all methanol, samples were stained with 5% Giemsa stain (diluted in tap water) in a Coplin jar for 20 min, washed in a large beaker filled with tap water until the excess Giemsa stain was removed, air-dried again, and examined under a light microscope (Nikon, Japan).

To conduct the wet smear, a portion of the bird droppings was first dissolved on a slide using a sterile swab with two drops of normal saline solution (0.9% NaCl). Subsequently, several coverslips were placed over the prepared solution. The slides were then examined under light microscopy using 10× and 40× magnification to identify the presence of trophozoites or parasite cysts(9, 10)(9, 10).

Polymerase Chain Reaction (PCR) was performed to confirm the presence of *Giardia* molecularly. To extract DNA, fecal samples were taken from the -20 ºC freezer, and a commercial DNA extraction kit (MBST, Iran) was used according to the manufacturer's instructions. All extracted DNA was stored at -20 ºC until further use. In this study, a pair of GDHiF (Forward) and GDHiR (Reverse) primers, which can amplify the genome of glutamate dehydrogenase (GDH) region in *Giardia duodenalis* protozoa (Acc. No: JF918460.1), were synthesized by SinaClon (Tehran, Iran) (Table 1). Amplification conditions were modified as follows. PCR master mix was composed of 1x PCR buffer, 1.5 mM $MgCl_2$ (SinaClon), 200 µM of each of 4 dNTP (Fermentas, Lithuania), 0.5 µM of each primer (F/R), 2.5 U of *Taq DNA polymerase* (SinaClon) and two microliter of template DNA in a final volume of 20 microliter. PCR was performed on a Gradient Mastercycler (Eppendorf, Germany) with the following amplification program: 1 cycle of 94 ºC for 10 min followed by 50 cycles of 35 sec at 94 ºC, 35 sec at 61 ºC and 50 sec at 72 ºC, with a final extension step of 7 min at 72 ºC. In all PCR reaction sets, dH_2O (instead of template DNA) was used as the negative control. The amplified products were detected by gel electrophoresis in 1% agarose gel containing Safe Stain® (SinaClon) in 1 x TAE buffer and then visualized under UV-LED Transilluminator (Fargene, Iran). A 100 bp DNA ladder (Yekta Tajhiz Azma, Iran) was used as a molecular weight marker for the PCR products in gel electrophoresis.

Table 1. Nucleotide sequence of primers for amplification of GDH region

Reactions	Name of primers (Acc. No.)	Nucleotide sequence (5'- 3')	PCR product
PCR for all samples	GDHiF (JF918460.1) GDHiR (JF918460.1)	CAGTACAACTCTGCTCTCGG GTTGTCCTTGCACATCTCC	432 bp

In the radiograph, hepatomegaly, splenomegaly, and gastroenteropathy were observed, which could be due to Mynah's improper diet. Also, the Giardia parasite was involved. The results of the blood test were as follows:

Table 2. Blood panel (g/L)

WBC	Neutrophils	Lymphocytes	Eosinophils	Monocytes
10.00	40.00	55.00	3.00	2.00

In the examination of the wet smear, considering that the test was done about 5 minutes after dropping the waste, we failed to observe the trophozoite and the cyst form of the parasite (Figure 1). However, the cystic form of *Giardia* was confirmed in the Giemsa-stained slide (Figure 2).

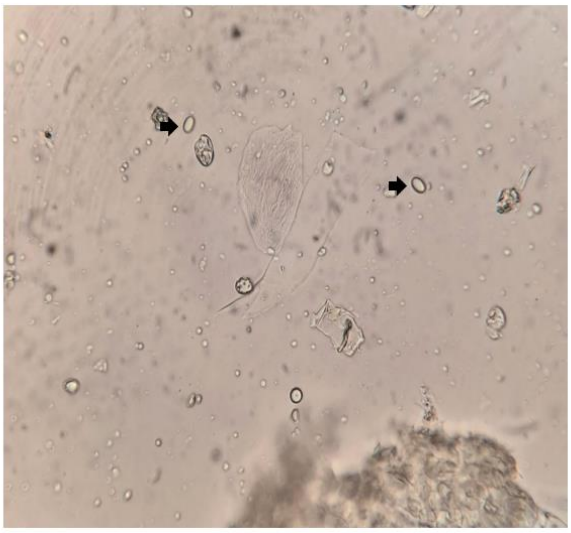

Figure 1. Wet smear of the cystic form of Giardia in Mynah (light microscopy, ×400 magnification)

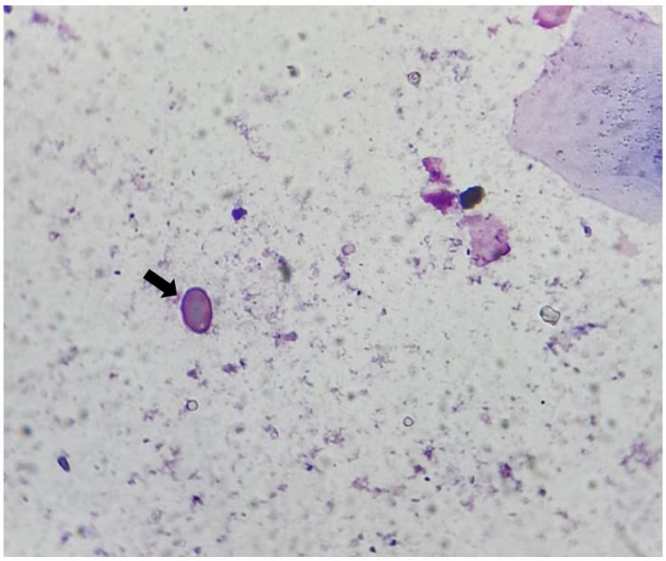

Figure 2. Giemsa staining of the cystic form of Giardia in Mynah (light microscopy, ×1000 magnification)

The size of the amplified fragment of the GDH region after the PCR reaction with the specific primers (Table 1) was 432 bp. A sequenced sample was used as the positive control (Figure 3). After electrophoresis of PCR products, a fragment of 432 bp was observed, confirming the presence of *the Giardia duodenalis* parasite. The wet smear, radiograph, and PCR results showed that all 5 Mynas were infected with *Giardia*.

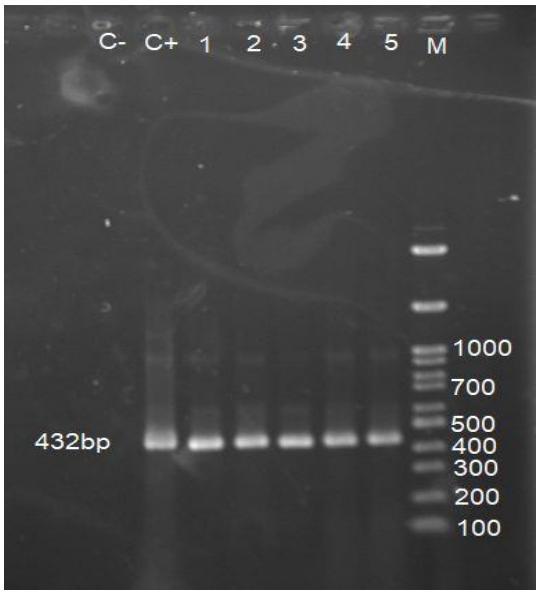

Figure 3. Electrophoresis results of PCR products. C1, negative control; C+, positive control; Lanes 1-5, positive samples; M, 100 bp DNA ladder.

3 Discussion

Giardia is a protozoan parasite that can infect many hosts, including humans, mammals, and birds. *Giardia duodenalis* is one of the common protozoan parasites affecting human intestines (11).

Iran is one of the countries where *Giardia* is common. Based on research in humans, in a study of 320 individuals, the overall prevalence of *Giardia lamblia* was 20% within 64 cases identified. The male-to-female ratio among those affected was 2:1. Most patients were in the age group of 16 to 20 years, accounting for 20.3% of the cases, and many had a low level of education, representing 31.25% of the participants. The most frequently reported symptom was abdominal pain, which affected 59.26% of men and 40.74% of women. (12). An analysis of 1,008 fecal samples through direct smear examination from Shushtar County from Khuzestan province revealed that 16% of the samples tested positive for pathogenic or non-pathogenic parasites. Among these positive cases, protozoa were detected in 14% of the samples, while helminths were found in 1%. Co-infections involving protozoa and helminths accounted for 0.3% of the cases, and co-infections with two protozoa occurred in 0.7%. The most commonly detected protozoan was *Giardia duodenalis*, identified in 7.7% of the samples, while the most prevalent helminth was *Trichostrongylus* spp., found in 0.5% of the samples (13). Studies on Giardia's prevalence and genetic identification in birds are minimal (14). Despite the abundance of bird species in the Iberian Peninsula, there have been no reports of *Giardia* in wild birds of that region. In a study in the Iberian Peninsula, only 2.1% of the birds tested positive for *Giardia* by performing PCR tests on 42% of the bird species living in that region (1). This value is lower than the prevalence of *Giardia* in wild birds in previous studies, in which the prevalence varied from five to 28% (14-17). *Giardia* species were identified in Barn Owl (*S. aluco*) and *Giardia duodenalis* in the Common Buzzard (*B. buteo*) for the first time (18). By collecting 215 stool samples from birds suspected of *Giardia* spp. infection in Mashhad and preparing wet smears. 60 positive Cockatiels (47.6%), 10 positive cockatiel chicks (43.4%), four positive lovebirds (14.2%), two Green-cheeked parakeets (8.6%), and only one positive African grey parrot (6.6%) were indicated. Among the positive cases, the severity of the infections varied among the birds. Some showed severe, moderate, or mild levels of infection. Upon a second examination, it was found that the birds with mild and moderate contamination were disease-free, while those with severe contamination needed further treatment (19). Raptors are at the top of the food chain; they feed on hunting in nature, and their infection with *Giardia* indicates the presence of this parasite in the environment, as well as their food, which usually are birds and small mammals (18). *Giardia duodenalis* has also been detected in the feces of a white stork (*C. ciconia*) (14). Due to living in water,

waterfowl are more exposed than other birds to water contaminated with the *Giardia* parasite caused by contaminated feces of humans and animals (20). *Giardia duodenalis* is commonly detected in mammals but rarely reported in birds. Mynas (*Acridotheres tristis*) can be host to several types of parasites. According to research, *Toxoplasma gondii*, *Isospora* spp., *Hymenolepis santaniana*, and some haematozoan parasites were diagnosed in Mynah by sampling during the case study (21-23).

To our knowledge, the presence of *Giardia* in Mynah has not been reported in Iran previously. In this case report, we present the presence of *Giardia duodenalis* in five cases of Mynah for the first time.

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None.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

PV, MHT, MN, and MP drafted the manuscript, which SMP critically reviewed and revised. All authors have read and approved the final manuscript and agreed to the published version.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

All ethical principles were considered in this work according to the principles outlined by the ethical committee of the Faculty of Veterinary Medicine, University of Tehran. Informed consent was obtained from the owner for clinical examination, treatment, and publication of this case
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References

1. Reboredo-Fernandez A, Ares-Mazas E, Caccio SM, Gomez-Couso H. Occurrence of Giardia and Cryptosporidium in wild birds in Galicia (Northwest Spain). Parasitology. 2015;142(7):917-25. [PMID: 25669618] [DOI]
2. Schmidt RE, Struthers JD, Phalen DN. Pathology of pet and aviary birds: John Wiley & Sons; 2024.
3. Ryan U, Cacciò SM. Zoonotic potential of Giardia. International Journal for Parasitology. 2013;43(12-13):943-56. [PMID: 23856595] [DOI]
4. Doneley B. Avian medicine and surgery in practice: companion and aviary birds: CRC Press; 2018.
5. Hosseinian SA. Zoonotic diseases associated with pet birds. Journal of Zoonotic Diseases. 2022;6(3):91-112.
6. McRoberts K, Meloni B, Morgan U, Marano R, Binz N, Erlandsen S, et al. Morphological and molecular characterization of Giardia isolated from the straw-necked ibis (Threskiornis spinicollis) in Western Australia. The Journal of Parasitology. 1996:711-8. [PMID: 8885877] [DOI]
7. Abe N, Makino I, Kojima A. Molecular characterization of Giardia psittaci by multilocus sequence analysis. Infection, Genetics and Evolution. 2012;12(8):1710-6. [PMCID: 22921500] [DOI]
8. Doneley RJ. Bacterial and parasitic diseases of parrots. Veterinary Clinics: Exotic Animal Practice. 2009;12(3):417-32. [PMID: 19732702] [DOI]
9. Campbell T, Bonda M, Morrisey JK, Rupiper DJ, Isaza R. The Gram stain in avian practice. Journal of Avian Medicine and Surgery. 2000;14(3):194-8. [DOI]
10. Lane R. Basic techniques in pet avian clinical pathology. Vet Clin North Am Small Anim Pract. 1991;21(6):1157-79. [PMID: 1767467] [DOI]
11. Dixon BR. Giardia duodenalis in humans and animals–Transmission and disease. Research in Veterinary science. 2021;135:283-9. [PMID: 33066992] [DOI]
12. Taherkhani H, Shariati S, Abdolahi N, Roshandel G. Clinical manifestations of giardiasis in Iran. Journal of Clinical and Diagnostic Research. 2009;3(2):1416-8.
13. Beiromvand M, Panabad E, Rafiei A. Status of intestinal parasitic infections among rural and urban populations, southwestern Iran. Asian Pacific Journal of Tropical Medicine. 2019;12(3):130-6. [DOI]
14. Majewska AC, Graczyk TK, Słodkowicz-Kowalska A, Tamang L, Jędrzejewski S, Zduniak P, et al. The role of free-ranging, captive, and domestic birds of Western Poland in environmental contamination with Cryptosporidium parvum oocysts and Giardia lamblia cysts. Parasitology Research. 2009;104:1093-9. [PMID: 19050920] [DOI]
15. Kuhn RC, Rock CM, Oshima KH. Occurrence of Cryptosporidium and Giardia in wild ducks along the Rio Grande River valley in southern New Mexico. Applied and Environmental Microbiology. 2002;68(1):161-5. [PMID: 11772622] [PMCID: PMC126547] [DOI]
16. Papazahariadou M, Diakou A, Papadopoulos E, Georgopoulou I, Komnenou A, Antoniadou-Sotiriadou K. Parasites of the digestive tract in free-ranging birds in Greece. Journal of Natural History. 2008;42(5-8):381-98. [DOI]
17. Plutzer J, Tomor B. The role of aquatic birds in the environmental dissemination of human pathogenic Giardia duodenalis cysts and Cryptosporidium oocysts in Hungary. Parasitology International. 2009;58(3):227-31. [PMID: 19446039] [DOI]
18. Ryan U. Cryptosporidium in birds, fish and amphibians. Experimental Parasitology. 2010;124(1):113-20. [PMID: 19545515] [DOI]
19. KazemiLifshagerd F, BehrouziNasab O, Koochakzadeh A, Kazemnezhad M. Evaluation of Giardiasis in Companion Birds in Mashhad, Iran. Journal of World's Poultry Science. 2023;2(3):20-3. [DOI]
20. Cacciò SM, Thompson RA, McLauchlin J, Smith HV. Unravelling cryptosporidium and giardia epidemiology. Trends in parasitology. 2005;21(9):430-7. [PMID: 16046184] [DOI]

21. Kumar R, Moudgil AD, Koundal S, Patil RD, Asrani RK. Parasitic enteritis in the free-ranging Common Myna Acridotheres tristis (Aves: Passeriformes: Sturnidae). Journal of Threatened Taxa. 2020;12(15):17168-70. [DOI]
22. Ishtiaq F, Beadell JS, Baker AJ, Rahmani AR, Jhala YV, Fleischer RC. Prevalence and evolutionary relationships of haematozoan parasites in native versus introduced populations of common myna Acridotheres tristis. Proceedings of the Royal Society B: Biological Sciences. 2006;273(1586):587-94. [PMID: 16537130] [PMCID: PMC1560061] [DOI]
23. Mushtaq S, Khan A, Hikal WM, Ali M, Salim M, Alina A, et al. Identifying the hidden parasitic intruders: Molecular insights into the health of common Myna. Microbial Pathogenesis. 2024;196:106985. [PMID: 39343069] [DOI]