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Original Article

Study of the gastrointestinal parasites in the feces of wild and domestic waterfowl with a focus on zoonotic parasites in Ahvaz, Iran

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Abstract

Due to the importance of parasitic infections in birds and the increasing growth of waterfowl breeding, especially goose and duck, this study aimed to investigate gastrointestinal parasites, including worms and protozoa in the feces of some species of wild and domestic waterfowl with a focus on zoonotic parasites. In this study, 100 fecal samples were collected from eight different wild and domestic waterfowl species in Ahvaz, including domestic goose, wild goose, domestic duck, wild duck, swan, pelican, and Ukrainian goose, and to identify gastrointestinal parasites. The samples were evaluated using the Clayton- Lane method and modified Ziehl-Neelsen and Trichrome staining methods. SPSS Statistics 18.0 software was used for statistical analysis. Out of 100 fecal samples, 17 samples (17%) were positive for gastrointestinal parasites. Fourteen samples (14%) were infected with *Eimeria spp.*, and three samples (3%) were infected with *Giardia species*. Among the different species of waterfowl that were positive, pelicans had the highest rate of infection (57.28 %). The highest rate of parasitic infection in waterfowl was related to protozoan infections, and the highest rate of protozoan infection was associated with *Eimeria* (14%). Of the positive cases, only four had clinical symptoms such as lethargy, anorexia, and diarrhea, and the rest of the positive cases were from apparently healthy birds. Considering that most parasitic infections have been detected in apparently healthy birds, it is recommended to observe hygiene and to disinfect cages regularly and also reduce the density of birds. Due to the zoonotic potential of *Giardia* and due to the contamination of some birds in bird shops with this parasite, this issue should be considered by public health officials, bird shops, owners, and breeders of these birds.
Keywords: Waterfowl, Gastrointestinal parasites, Zoonotic diseases, Dropping, Ahvaz

Introduction

The term waterfowl is generally used to refer to those waterbirds of the family *Anatidae* (ducks, geese, pelicans, swans, etc.). These species are found worldwide in a wide variety of aquatic habitats, and are commonly kept in zoological and ornamental collections and are the origin of the breeds kept commercially for agricultural purposes (Tully et al., 2000). Habituated waterfowl, an important poultry division, are claimed to be some pathogens natural reservoirs, and are responsible for the evolution, maintenance, and some diseases

spread (Bi et al., 2016; Dimitrov et al., 2016; Yoon et al., 2014). Most of these wild and domestic reservoirs are important to cause many diseases and can be transmitted from one country to another and from one area to another (Gylfe et al., 2000). Therefore, it is vital to characterize and evaluate potentially pathogenic microbes in flocks of waterfowl to protect public health (Bi et al., 2016; Dimitrov et al., 2016; Yoon et al., 2014). In a farm of backyard poultry, standard hygienic work-outs and constructed health management are not generally applied, and be in touch with wild birds, pets, and farm animals is frequent (Conan et al., 2012; Pohjola et al., 2015). Therefore, such flock's health status is generally poor. In the digestive system of waterfowl, infection with various parasites occurs (Doneley, 2016; Harrison and Lightfoot, 2006; Atkinson et al., 2009; Coles, 2007; Tully et al., 2000; Tully et al., 2009). Parasites such as *Acuaria spp., Amidostomum spp., Hetarakis spp., Hymenolepis spp.* etc., are among the helminthic parasites of the digestive system of waterfowl (Atkinson et al., 2009). Ducks and geese by swallowing certain small aquatic animals, become infected, which are said to serve as the intermediate hosts of parasite. Among different protozoan parasites, *Trichomonas gallinae, Cochlosoma spp., Plasmodium spp., Leucocytozoon spp., Sarcocystis ridleyi, Eimeria spp., Tyzzeria spp., Wenyella spp., Giardia spp., Cryptosporidia spp.,* etc., have significant pathogenic importance (Doneley, 2016; Harrison and Lightfoot, 2006; Coles, 2007). Coccidiosis is in charge for severe losses among geese. It is caused by coccidia, microscopic protozoan parasites that invade the cells of tissues and eventually destroy them. One species of coccidium, *Eimeria truncate*, causes a highly fatal disorder of renal in geese. This coccidiosis type is widespread in the United States and Canada (Tully et al., 2000).

Cochlosoma spp. is a motile protozoan that is common in waterfowl. It has a direct lifecycle, with transmission due to ingesting infective trophozoites in fecal material and contaminated food and water (Doneley, 2016). *Cryptosporidium spp.* is a protozoan parasite that causes zoonotic disease in humans, birds, and other animals (Dabirzadeh et al., 2003; Fayer and Xiao, 2007; Heidari and Gharakhani, 2012). Zoonotic transmission plays an important role in cryptosporidiosis epidemiology (Xiao and Fayer, 2008). There are many reports of infection by people who have been in direct and close contact with livestock and poultry (Dabirzadeh et al., 2003; Heidari and Gharakhani, 2012). Cryptosporidiosis is an important parasitic disease that causes diarrhea and gastroenteritis in humans and animals worldwide (Fayer, 2010; Lujan and Svärd, 2011). *Giardia* is another protozoan that has a wide geographical and host distribution. It has been reported in most avian species (Doneley, 2016; Feng and Xiao, 2011). The evidence obtained in the last two decades has introduced giardiasis as a zoonotic disease (Lasek-Nesselquist et al., 2008). *Cryptosporidium spp. and Giardia Spp.* Infections, because of poverty and lack of access to appropriate resources, primarily occur in developing countries (Sandoval-Rodríguez et al., 2021; Sreedevi et al., 2015). Most of the gastrointestinal parasites have no clinical symptoms, or the symptoms are subclinical, and the birds suffer from anorexia, weakness, lethargy, emaciation, and weight loss. Since parasitic infections may develop subclinically, weaken the immune system, and increase the bird's susceptibility to other infectious agents, endoparasites of birds should be detected and treated (Doneley, 2016; Harrison and Lightfoot, 2006; Coles, 2007; Tully et al., 2000; Tully et al., 2009; Garcia, 2009). Considering the increase in the keeping of waterfowl, especially goose and duck, and the importance of zoonotic parasites, the current study main objectives were to investigate gastrointestinal parasites, including worms and protozoa, in the feces of some species of wild and domestic waterfowl with a focus on zoonotic parasites, to provide a scientific foundation for the clinical diagnosis and parasitic diseases prevention that have health implications for both waterfowl and humans.

Materials and methods

In this study, 100 fecal samples were collected from eight different wild and domestic waterfowl species, including domestic goose *(Anser anser domesticus)*, wild goose *(Anser anser)*, domestic duck (*Anas platyrhynchos domesticus*), wild duck (*Anas platyrhynchos*), swan (*Cygnus cygnus*), pelican (*Pelecanus onocrotalus*), swan goose (*Anser cygnoides*) and Ukrainian goose *(Anser anser domesticus)* in Ahvaz, the Khuzestan province capital in southwest Iran, were collected from November 2021 to July 2022. To identify gastrointestinal parasites, samples of fecal were obtained from apparently healthy birds from bird shops in Ahvaz city, and from diseased birds referred to the Avian Medicine Department, Ahvaz, Iran. Most diseased birds had nonspecific signs such as lethargy, losing weight, anorexia, vomiting, and diarrhea. Clinical symptoms were written down based on external physical examination and the owner's history. The number and species of birds sampled are shown in Table 1. Sterile paper sheets were placed on the cages floor, and about half an hour later, using sterile wooden spatulas, fresh fecal samples were gathered from the bed of each bird cages and then stored in sterile vials separately and were immediately moved to the laboratory for further processing. Then, two smears were prepared from each sample, one for modified Ziehl-Neelsen staining and the other for Trichrome staining, and after drying, the smears were fixed by pure methanol (Fayer and Xiao, 2007; Feng and Xiao, 2011; Henriksen and Pohlenz, 1981; Zajac et al., 2021, Adam et al., 1971).

Modified Ziehl–Neelsen staining

Modified ZN staining (Kinyoun's modification of acid-fast staining) was done on smears made from fresh samples. The slides were screened under ×100 objectives of a light microscope to identify the *Cryptosporidium spp.* (Henriksen and Pohlenz, 1981; Zajac et al., 2021)

Trichrome staining method

All samples were analyzed by Modified Trichrome for detecting *Giardia species.* The slides were screened under ×100 objectives of a light microscope (Henriksen and Pohlenz, 1981; Zajac et al., 2021)

Also, the fecal samples were examined by the Centrifugal fecal flotation technique (Clayton-Lane) to identify helminthic and protozoan parasites (Adam et al., 1971; Soulsby, 1982; Papini et al., 2012). In the Clayton-Lane method, a saturated solution of sugar (Sheather's solution) and a saturated solution of zinc sulfate were used. In positive samples, for the detection of coccidia oocysts, 2.5% potassium dichromate was used**.**

Sporulation with potassium dichromate

The precipitates were used for coccidian sporulation. Sporulation was performed in a wet chamber at 24–26 °C in 2.5 % potassium dichromate solution (K2Cr2O7) (Soulsby, 1982).

SPSS Statistics 18.0 software was used for statistical analysis. The data were represented as mean ± SD.

Results

Our results showed that 17 samples (17%) were infected with gastrointestinal parasites. All birds were negative for the helminthic parasites. Among the protozoan parasites, 14 samples (14%) were infected with *Eimeria spp.*, and three samples (3%) were infected with *Giardia spp.* (Figure 1 and Table 1).

Table 1- Collected samples number, the percent of positive samples, and the prevalence of gastrointestinal parasites in waterfowl in Ahvaz, Iran.
*Confidence Interval: CI (95%)

Common name	Number of birds sampled	Positive samples for protozoa (%)	No. of positive for *Eimeria* spp.	Positive samples for *Eimeria* spp.(%)	No. of positive for *Giardia* spp.	Positive samples for *Giardia* spp.(%)	Positive samples for *Cryptosporidium* spp. (%)	Positive samples for helminths (%)
pelican	7	28.57±7.3*	2	28.57±7.3*	0	0	0	0
wild duck	5	20 ±6.1	1	20 ±6.1	0	0	0	0
domestic duck	36	19.44±7.3	5	13.88±12.3	2	5.55±3.5	0	0
domestic goose	22	18.18±11.6	3	13.63±7.6	1	4.54±7.1	0	0
Ukrainian goose	6	16.66 ±8.5	1	16.66 ±8.5	0	0	0	0
wild goose	7	14.28±11.2	1	14.28±11.2	0	0	0	0
swan	13	7.69±8.4	1	7.69±8.4	0	0	0	0
swan goose	4	0	0	0	0	0	0	0
Total	100	17±9.5	14	14±9.5	3	3±0.54	0	0

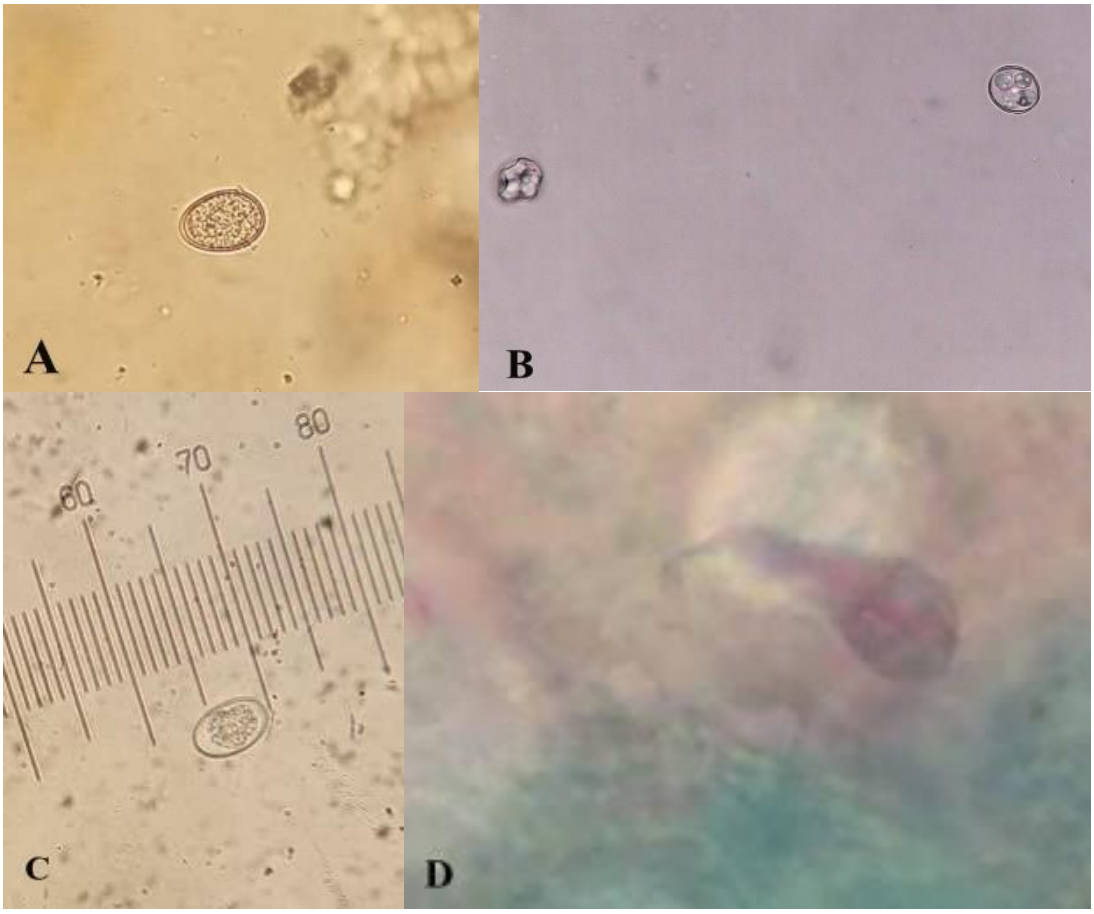

Fig. 1. The gastrointestinal parasites in fecal samples of waterfowl in Ahvaz. A): Non-sporulated oocyst of *Eimeria spp*. (400×), B): Sporulated oocyst of *Eimeria spp*. (400×), C): Non-sporulated oocyst of *Eimeria spp*. (7 µm diameter) (400×), D): *Giardia spp*. trophozoite (1000×).

Waterfowl, pelicans, wild ducks, domestic ducks, domestic geese, Ukrainian geese, wild geese, and swans had the highest rate of protozoan infection, respectively. Among waterfowl, pelicans, wild ducks, Ukrainian geese, wild geese, domestic ducks, domestic geese, and swans had the highest

infection rate with *Eimeria spp.*, respectively. The present study showed that only domestic ducks and domestic geese were infected with *Giardia species*. All four swan geese were negative for the parasitic infection. Mixed parasitic infection was not found in the samples.

Table 2- The birds were sampled by species, scientific name, and health status of the birds sampled.

Common name	Scientific name	Number of birds sampled	No. of Positive birds with clinical symptoms	The clinical symptoms
pelican	*Pelecanus onocrotalus*	23	1	vomiting, loss of appetite, and diarrhea
wild duck	*Anas platyrhynchos*	14	0	-
domestic duck	*Anas platyrhynchos domesticus*	9	2	anorexia, vomiting, loss of appetite, diarrhea, and weakness
domestic goose	*Anser anser domesticus*	4	1	anorexia and diarrhea
Ukrainian goose	*Anser anser domesticus*	23	0	-
wild goose	*Anser anser*	5	0	-
swan	*Cygnus cygnus*	30	0	-
swan goose	*Anser cygnoides*	5	0	-
Total	-	100	4	-

Among the positive birds, four birds had clinical symptoms which referred to the Avian Medicine Department, Ahvaz, Iran, which included two domestic ducks with symptoms such as anorexia, vomiting, loss of appetite, diarrhea, and weakness (positive for *Giardia spp.*), a domestic goose with symptoms such as anorexia and diarrhea (positive for *Giardia spp.*), and a pelican with symptoms such as vomiting, loss of appetite, and diarrhea (positive for *Eimeria spp.*), and the other 13 positive birds were all apparently healthy birds kept in Ahvaz bird shops (Table 2).

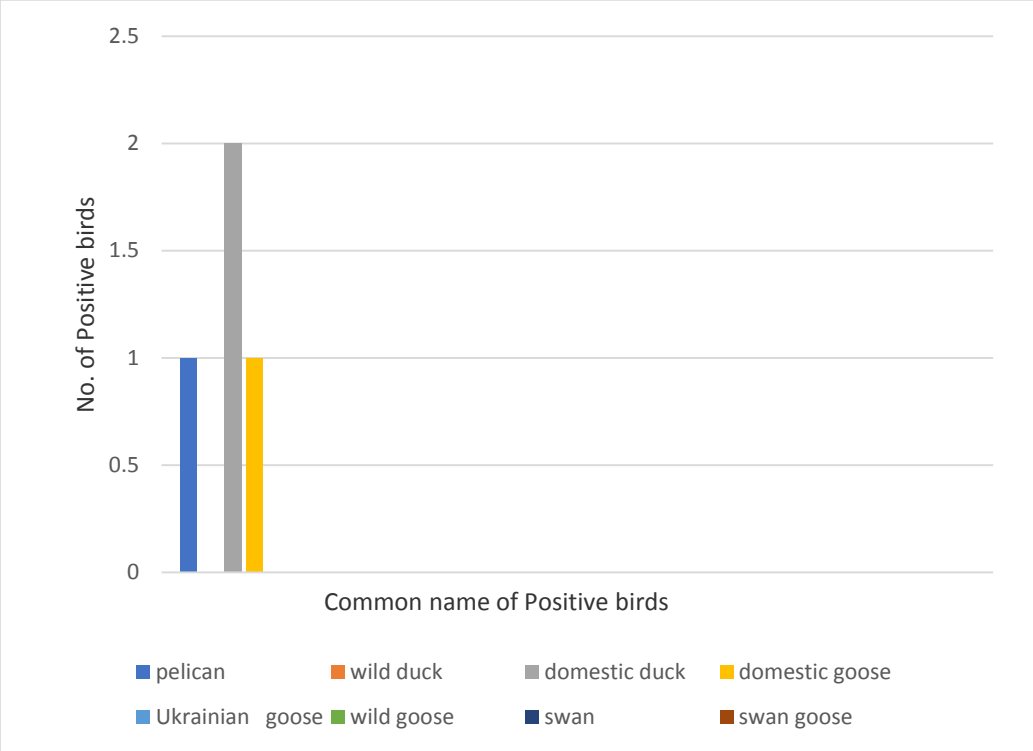

Fig. 2. The number of positive birds based on the common name of the birds sampled.

Discussion

The data of the present study showed the gastrointestinal parasites prevalence in waterfowl in Ahvaz at 17%, and pelicans had the highest infection rate. The zoonotic parasites detected in this study included *Giardia spp., and* only domestic ducks and domestic geese were infected with *Giardia species.* This parasite has a wide host and wide geographical distribution, and it mostly causes diseases in immunosuppressed persons. The findings of this study showed that waterfowl can be reservoirs of zoonotic parasites, and humans can become infected in connection with these birds, and vice versa. Also, this study showed that the highest rate of contamination was related to bird shops, which increases the risk of infection in these centers. Among the positive birds, four birds had clinical symptoms that were referred to the Avian Medicine Department. The other 13 positive birds were all apparently healthy birds kept in Ahvaz bird shops and had no clinical symptoms, and this shows the importance of detection, control, prevention, and treatment of subclinical infections, especially the identification of zoonotic parasites that have a direct impact on the public health.

In a study by Papini et al. in Italy, fecal samples were collected separately from pet and zoo birds from 14 orders and 63 species. All samples were analyzed by the feces flotation method. A total of 35.6% of birds including zoo birds and pet birds were infected with parasites (*Strongyles-Capillarids* (8.9%), *Ascaridia* (6.8%), Strongyles (5.5%), *Porrocaecum* (2.7%), *Porrocaecum-Capillarids* (2%), and *Syngamus-Capillarids* 0.7%) (Papini et al., 2012). These results were in distinction to the current study results of in terms of the type of parasite in the digestive tract. In this study, all birds were negative for helminthic parasites.

In a survey by Larki et al. on gastrointestinal parasites of domestic ducks in Iran, 60.97% of ducks were infected with four protozoan parasites and/or three different nematodes. The identified nematodes were *Capillaria sp.*, (50%), *Subulura* spp. (16.66%), *Echinuria* spp. (33.33%). The protozoan oocysts

were *Cryptosporidium* spp. (50%) and coccidian species (%58.33) and included *Wenionella philiplevinei*, *Tyzerria* spp., and *Isospora. mandari*. Mixed infection with two or more parasites was common. Twenty (80%) had single, four (16%) double, and one (4%) triple infection. In contrast with this study, in the present study, domestic ducks were infected only with two protozoan parasites: *Eimeria spp.*, and *Giardia species.*

About 46% helminths infection rate was reported in the lungs and alimentary tracts of domestic ducks in the Iran several parts that comprised *Tetrameres fissispina, Capillaria obsignata, C. anatis, C. contorta* (Eslami et al., 1985). 70.5% of the total parasitic infection rate in the green-winged teal gastrointestinal tracts (*Anas crecca*) in Fereydunkenar in Mazandaran Province, northern Iran, belonged to *Contracaecum spp., cestoda, Diorchis stefanskii, Hypoderaeum conoideum*, and *Notocotylus attenuates* (Youssefi et al., 2014). Moreover, the infections prevalence with observation of internal organs of *Aythya nyroca* collected from central Iraq was 77.8%, including *Leucocytozoon spp., Plasmodium sp., Amidostomoides acutum, Epomidiostomum uncinatum* and *Diploposthe laevis* (Mohammad, 2015). Infection rate of helminths parasite in domestic ducks in Gilan Province was 50%, which consisted of *Railletina tetragona, Heterakis gallinarum*, and *Capillaria* (Shemshadi et al., 2016).

We did not detect any cestode and trematode eggs; this may be because of the habitats lack and trematodes and cestodes intermediate hosts optimal climatic conditions. The surveillance in Tanzania with tropical areas and in Bangladesh with a subtropical monsoon climate did not report any trematodes and cestodes in adult ducks (Muhairwa et al., 2007; Farias et al., 1986) while intestinal surveillance found 42.3% of ducks in Florida (with humid subtropical and tropical climate) suffered endoparasitic infections that showed more than 15% trematodes and 8.9% cestodes (Kinsella et al., 1972).

In the present study, no infection with the helminthic parasite was observed. This may be due to the lack of favorable climatic conditions and the lack of suitable habitats for the intermediate hosts.

In this study, the rate of infection with protozoan parasites was 17%, of which, the rate of infection with *Eimeria spp.* was 14%, and the rate of infection with *Giardia spp.* was 3%.

In the present study, all birds were negative for cryptosporidiosis, but it reported that 17% and 16.6% of wild waterfowl in the lagoons of Mazandaran and Gilan Provinces of Iran were infected with *Cryptosporidium spp.* (Shemshadi et al., 2014; Shemshadi et al., 2016).

It seems that the reason for the difference between the present study results and other research includes the following: the difference in sample size, the geographical area, the presence of intermediate hosts in the investigated area, bird-keeping conditions such as long-term captivity, high density, and the nests' hygiene status. In this study, most of the birds that were positive for parasites had no clinical symptoms and were from bird shops. Among the positive birds, only four birds had clinical symptoms that were referred to the Department of Avian Medicine, which included two domestic ducks, a domestic goose, and a pelican, and the other 13 positive birds were all apparently healthy birds kept in Ahvaz bird's shops, and this shows the importance of detection, control, prevention, and treatment of subclinical infections, especially the identification of zoonotic parasites that have a direct impact on the public health.

In the current study, the parasitic infections prevalence was detected lower than in other literature in Iran and most other countries. Ahvaz (latitude: 31.32°N, Longitude: 48.66°E) has a desert climate with long, very hot summers and mild, short winters. This province was considered to be a semi-arid province of Iran. Therefore, this climatic condition is not suitable for the survival and spread of helminth parasite eggs. Moreover, climate and season can be affected by the frequency and accessibility of intermediate hosts to domestic ducks (Hoque et al., 2014). The present

study gastrointestinal parasites, which involved wild and domestic waterfowl, are usually common parasites infecting domestic chickens when they are kept in the same place. Due to the same food and water use, parasitic infections can be transmitted in some birds living together.

Conclusion

As wild and domestic waterfowl are scavenger animals that ingest wide environmentally contaminated food, they are easily involved in parasites various species. In the present study, the examination of fecal samples of wild and domestic waterfowl in Ahvaz showed that most of the positive samples were from apparently healthy birds without clinical symptoms. Most of the positive samples were reported from bird shops with dense maintenance conditions, which indicates that gastrointestinal parasites can exist without the emergence of clinical symptoms and cause the spread of infection in the nests, and thus create a risk for immunosuppressed birds. This issue shows the importance of periodic monitoring of all bird shops. Considering that the bird shops in Ahvaz City were contaminated, it is recommended to observe hygiene and regularly disinfect the bed of the cages. Also, the results of this study showed that infection with the zoonotic parasite (*Giardia spp.*) is present in wild and domestic waterfowl in the Ahvaz area and should be considered by the owners of these birds, breeders, veterinarians, and public health organizations. According to the presence of this zoonotic parasite in the Ahvaz area and because of the sensitivity of immunosuppressed people to them, people with immune deficiencies such as AIDS, rheumatism, diabetes, hepatitis B, etc. should not be in contact with waterfowl in the Ahvaz area.

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Conflict of interest statement

The authors confirm that there is no conflict of interest.

Ethical approval

This study was approved by the Shahid Chamran University of Ahvaz Ethical Commission for Animal Experiments under verification number EE/1401.2.24.117855/scu.ac.ir.

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