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Survey on the gastrointestinal parasites in *Passeriformes* and *Psittaciformes* with a focus on zoonotic parasites

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ABSTRACT: Considering the increase in the keeping of pet birds and the importance of gastrointestinal parasitic infection, especially zoonotic parasites, this study was conducted to determine the prevalence of gastrointestinal parasites in different species of *Passeriformes* and *Psittaciformes*. In this study, one hundred and twenty fresh fecal samples were collected from different species of captive pet birds in Ahvaz from Apr 2021 to Aug 2021. The collected samples were analyzed using Clayton-Lane methods, Modified Ziehl-Neelsen, and Trichrome staining. Light microscopic morphometry was used for the identification of helminth eggs and oocysts. The results of this study showed that 37 samples (30.83%) were infected with gastrointestinal parasites. Among the helminthic parasites, three samples (2.5%) were infected with *Hymenolepis spp.*. Among protozoan parasites, 24 samples (20%) were infected with *Eimeria spp.*, three samples (2.5%) were infected with *Isospora spp.*, two samples (1.66%) were infected with *Cryptosporidium spp.*, and five samples (4.16%) were infected with *Giardia spp.*. The results of the present study showed that the highest rate of gastrointestinal parasitic infection was in *Passeriformes* kept in pet shops and the prevalence of gastrointestinal parasitic infection was higher in the zebra finch, common mynah, and canary compared to other pet birds. But the highest rate of zoonotic parasitic infection was in *Psittaciformes*. According to the results, zoonotic parasites are present in pet birds in the Ahvaz area and should be considered by the owners of these pet birds, breeders, veterinarians, and public health organizations.

Keywords: *Passeriformes, Psittaciformes,* gastrointestinal parasites, zoonotic parasites, Ahvaz, Iran.

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INTRODUCTION

Passeriformes and *Psittaciformes* are the orders of birds and some of their species are kept as captive pet birds. With more than 5,300 identified species, *Passeriformes* includes more than half of all bird species. Birds such as the canary, finch, bulbul, mynah, and starling belong to this order (Johansson et al., 2008) . *Psittaciformes* have more than 350 species and about 83 genera. Most parrots are mainly native to the southern hemisphere, but are not exclusively found in tropical regions (Kalmar et al., 2010) . In recent years, the amount of keeping pet birds of different species of *Passeriformes* and *Psittaciformes* has increased (Kalmar et al., 2010; Doneley, 2016; Harrison and Lightfoot, 2006). In the digestive system of *Passeriformes* and *Psittaciformes*, 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 *Ascaridia spp.* , *Capillaria spp.* and *Heterakis spp.* are among the helminthic parasites of the digestive system of *Passeriformes* and *Psittaciformes* (Atkinson et al., 2009). Among different protozoan parasites, *Trichomonas gallinae*, *Giardia spp.*, *Cryptosporidium spp.*, and several species of coccidia have significant pathogenic importance (Doneley, 2016; Harrison and Lightfoot, 2006; Coles, 2007). *Cryptosporidium* 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 of people who have been in direct and close contact with livestock and poultry (Dabirzadeh et al., 2003; Heidari and Gharakhani, 2012). *Cryptosporidium* in the digestive system of the host causes damage to the small intestine epithelium and its dysfunction leads to mild or severe diarrhea and other abdominal symptoms (Ryan et al., 2014; Fayer, 2010). 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 hosts distribution (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).*G.duodenalis* were detected in shore birds such as gull (Ryan and Cacciò, 2013).This shows the importance of zoonotic transmission of *Giardia spp.* between humans and birds (Lasek-Nesselquist et al., 2008; Ryan and Cacciò, 2013). Symptoms of giardiasis vary from asymptomatic to watery diarrhea, nausea, abdominal pain, and weight loss (Lasek-Nesselquist et al., 2008; Hooshyar et al., 2019). The main symptoms of acute giardiasis in humans include diarrhea, flatulence, epigastric cramps, nausea, vomiting, and weight loss (Stark et al., 2009; Hanevik et al., 2014). There is particular concern regarding *Giardia spp.* because the parasite is easily spread between humans and has the potential to cause long-term side effects (Litleskare et al., 2018; Han et al., 2020). Transmission of *Giardia spp.* and *Cryptosporidium spp.* is oral-fecal (Johansson et al., 2008; Fayer and Xiao, 2007; Xiao and Fayer, 2008; Feng and Xiao, 2011; Ludwig and Marques, 2011; Savioli et al., 2006). Infections with *Cryptosporidium spp.* and *Giardia spp.* primarily occur in developing countries because of poverty and lack of access to appropriate resources (Sandoval-Rodríguez et al., 2021; Sreedevi et al., 2015). *Hymenolepiasis* is a potentially zoonotic disease caused by the cestode *Hymenolepis spp.* (Singh et al., 2020; Di Lernia et al., 2004). Mild hymenolepiasis is usually asymptomatic and heavy infection occurs usually due to autoinfection. Heavy infection results in headache, dizziness, anorexia, abdominal pain with diarrhea, nausea, vomiting, pruritus, and weight loss. Allergic reactions, such as urticarial, skin eruption, and phlyctenular conjunctivitis, may also occur (Kim et al., 2014; Sadaf et al., 2013; Sungkar et al., 2017, 2008; Thompson, 2015). Hymenolpiasis is one of the zoonotic diseases and, and requires more surveys because of emerging issues relating to the epidemiology and impact on public health (Radfar et al., 2012). Most of the gastrointestinal parasites have no clinical symptoms or the symptoms are subclinical and the birds suffer from anorexia, weakness and lethargy, emaciation, and weight loss. Since parasitic infections may develop subclinically and 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). Zoonotic parasitic diseases in pet birds are of great importance in terms of public health, society, and economy (Kalmar et al., 2010; Harrison and Lightfoot, 2006). Considering the increase in the keeping of pet birds and the importance of zoonotic parasites and the fact that no study has been conducted on the gastrointestinal parasites of *Passeriformes* and *Psittaciformes* in Ahvaz, this study aimed to investigate the gastrointestinal parasites of *Passeriformes* and *Psittaciformes* with a focus on zoonotic parasites in Ahvaz city.

MATERIALS AND METHODS

In this study, 120 fresh fecal samples from different species of captive birds *(Passeriformes* and *Psittaciformes)* in Ahvaz, the capital of Khuzestan province in southwest Iran, were collected from Apr 2021 to Aug 2021. 60 fecal samples were taken from apparently healthy birds from pet shops in Ahvaz city and 60 fecal samples were collected from diseased birds referred to the department of avian medicine, Ahvaz, Iran. Most of the diseased birds had nonspecific signs such as losing weight, anorexia, and lethargy. Clinical symptoms were written down by the owner's history and external physical examination. Out of 120 fecal samples, 70 samples belonged to *Psittaciformes* and were collected from seven different species including cockatiel (*Nymphicus hollandicus*), budgerigar (*Melopsitacus undulates*), African gray parrot (*Psittacus Erithacus*), rose-ringed parakeet (*Psittacula krameria*), lovebird (*Agapornis roseicollis*), Alexandrine parakeet (*Psittacula eupatria*) and monk parakeet (*Myiopsitta monachus*). 50 samples belonged to *Passeriformes* and were collected from four different species including zebra finch (*Taeniopygia guttata)*, common mynah (*Acridotheres tristis*), canary (*Serinus canaria*), and white-eared bulbul (*Pycnonotus leucotis*). The number and species of birds sampled are shown in Table 1. Sterile sheets of paper were placed on the floor of the cages and about half an hour later, using sterile wooden spatulas, fresh fecal samples were gathered from the bed of cages of each bird and then stored in sterile vials separately and were immediately carried to the laboratory for further processing in the laboratory. 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 for identification of the *Cryptosporidium spp.* (Henriksen and Pohlenz, 1981; Zajac et al., 2021)

Trichrome staining method: All samples were analyzed by Modified Trichrome for detecting *Giardia spp.*.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 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).

This study was approved by the Shahid Chamran University of Ahvaz Ethical Commission for Animal Experiments under verification number EE/1400.3.02.9753/scu.ac.ir and EE/1400.3.02.9749/scu.ac.ir.

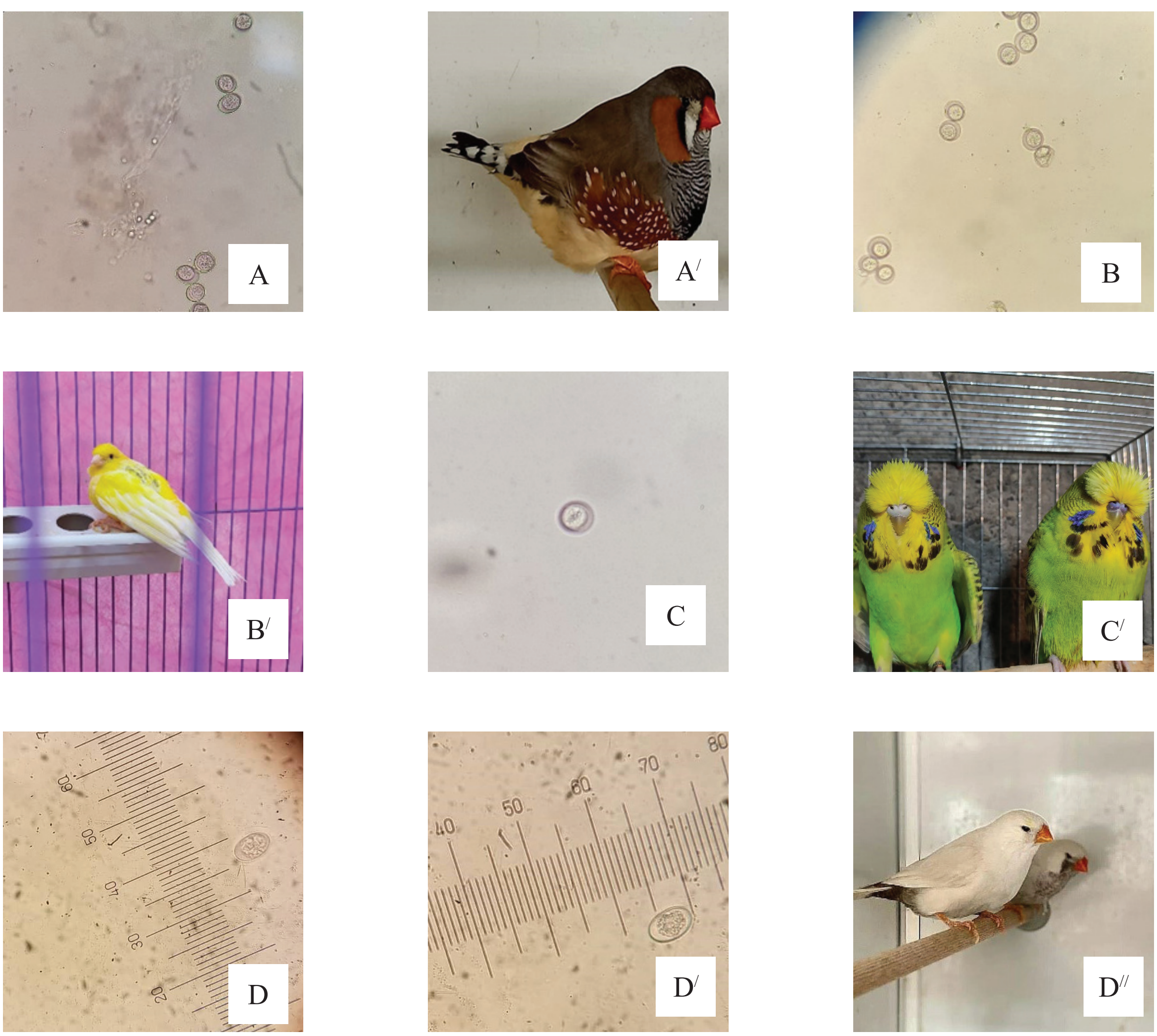
RESULTS

The results of this study showed that 37 samples (30.83%) were infected with gastrointestinal parasites. Among the helminthic parasites, three samples (2.5%) were infected with *Hymenolepis spp.*, and among the protozoan parasites, 24 samples (20%) were infected with *Eimeria spp.*, three samples (2.5%) were infected with *Isospora spp.*, two samples (1.66%) were infected with *Cryptosporidium oocysts* and five samples (4.16%) were infected with *Giardia spp.* (Figure 1 and Table 1).

Table 1: The number of collected samples, the percent of positive samples, and the prevalence of gastrointestinal parasites in *Passeriformes* and *Psittaciformes* in Ahvaz, Iran.

Common name	Number of birds sampled	Percent of positive samples (%)	Positive samples for *Eimeria* spp. (%)	Positive samples for *Isospora* spp. (%)	Positive samples for *Cryptosporidium* spp. (%)	Positive samples for *Girdia* spp. (%)	Positive samples for protozoa (%)	Positive samples for *Hymenolepis* spp. (%)	Positive samples for helminthes (%)
Zebra Finch	23	60.86±19.6*	47.82±8.5	8.7 ±1.2	0	0	56.52±9.3	4.34±0.82	4.34±0.82
Common mynah	14	57.14 ±8.2	50±9.2	0	0	0	50±9.2	7.14 ±0.97	7.14±0.97
Canary	9	55.5 ±9.7	55.5±9.7	0	0	0	55.5± 9.7	0	0
White-eared bulbul	4	25±7.2	0	25 ±7.2	0	0	25±7.2	0	0
Budgerigar	23	21.73±6.3	4.34±0.98	0	0	17.39±4.1	21.74±6.3	0	0
African gray parrot	5	20±5.9	0	0	20 ±5.9	0	20± 5.9	0	0
Cockatiel	30	10±1.4	0	0	3.33±0.88	3.33±0.12	6.66±0.44	3.33±0.14	3.33± 0.14
Rose-ringed parakeet	5	0	0	0	0	0	0	0	0
Lovebird	4	0	0	0	0	0	0	0	0
Alexanderine parakeet	2	0	0	0	0	0	0	0	0
Monk parakeet	1	0	0	0	0	0	0	0	0
Total	120	30.83±7.5	20±6.1	2.5±0.83	1.66±0.55	4.16±0.97	28.33±9.3	2.5±0.22	2.5 ±0.22

* confidence interval (95%)



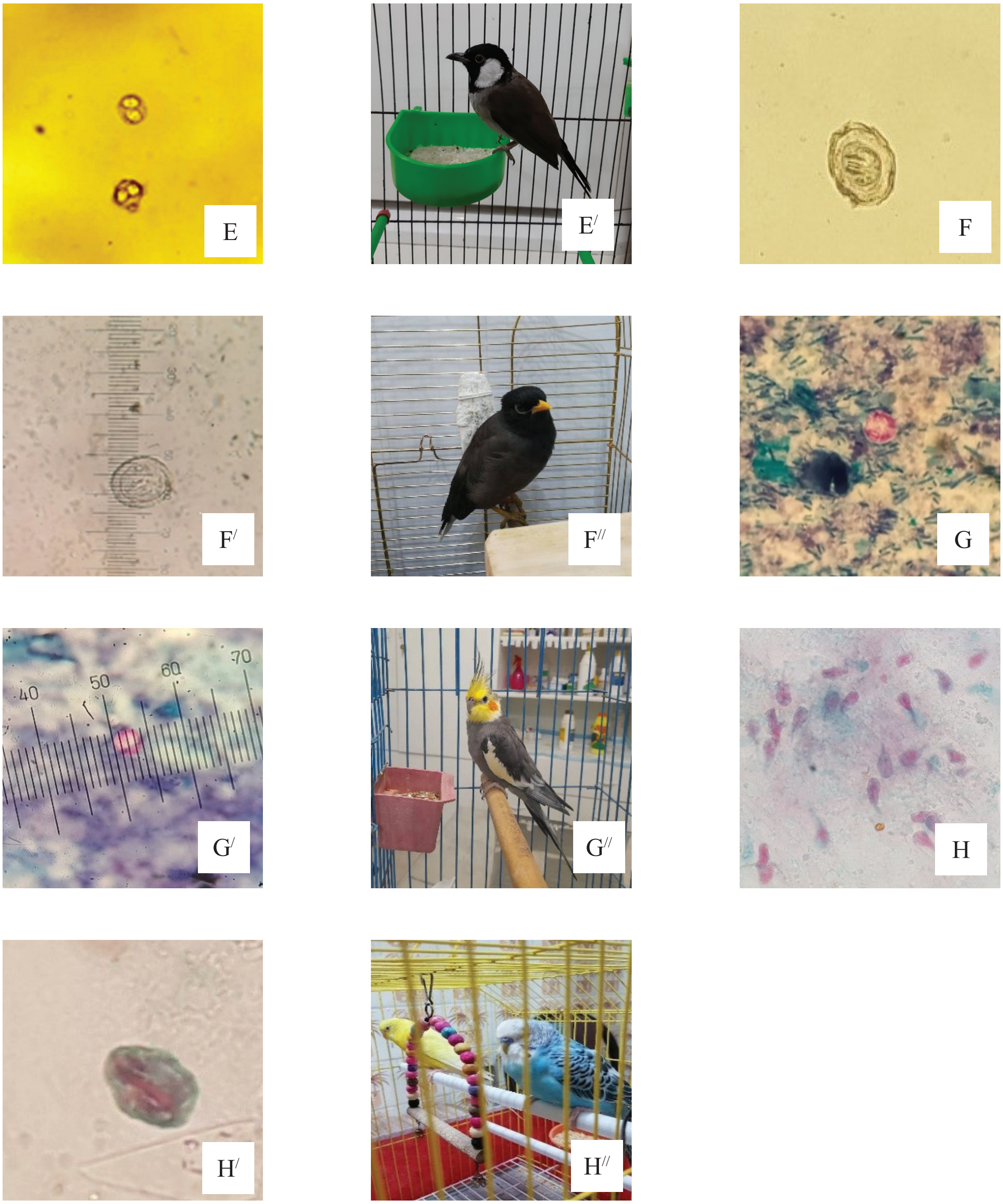


Figure 1: The gastrointestinal parasites in fecal samples of *Passeriformes* and *Psittaciformes* in Ahvaz.

(A): *Eimeria spp*. oocysts (400×), (A′): An apparently healthy zebra finch in which *Eimeria spp*. oocysts were detected. (B): *Eimeria spp*. oocysts (400×), (B′): This canary had symptoms of anorexia and feather picking and *Eimeria spp*. oocysts were detected, (C): spherical *Eimeria spp*. oocysts (18 µm diameter) (400×), (C′): An apparently healthy budgerigar in which *Eimeria spp*. oocysts were detected, (D): *Eimeria spp*. oocyst with a length of 21 µm (400×), (D′): *Eimeria spp*. oocyst with a width of 15 µm (400×), (D″): An apparently healthy zebra finch in which *Eimeria spp*. oocysts were detected. (E): sporulated oocyst of *Isospora spp*. (22 µm ×20 µm), (E′): An apparently healthy white-eared bulbul in which *Eimeria spp*. oocysts were detected, (F): *Hymenolepis spp*. Egg (100×), (F′): *Hymenolepis spp*. Egg (51 µm×42 µm) (400×), (F″): An apparently healthy Common mynah in which *Hymenolepis spp*. eggs were detected. (G) *Cryptosporidium spp*. (1000×), (G′) *Cryptosporidium spp*. (6 µm diameter) (1000×), (G″) An apparently healthy cockatiel in which *Cryptosporidium spp*. oocysts were detected, (H) *Giardia spp*. trophozoites (1000×), (H′) A cyst of *Giardia spp*. (1000×), (H″) An apparently healthy budgerigar in which *Giardia spp*. was detected.

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

Common name	Scientific name	Order	Number of birds sampled	Positive birds with clinical symptoms (%)	Positive birds without clinical symptoms (%)
zebra finch	***Taeniopygia guttata***	***Passeriformes***	23	0	60.86 ±9.94*
Common mynah	***Acridotheres tristis***	***Passeriformes***	14	7.14 ±0.48	50 ±6.19
Canary	***Serinus canaria***	***Passeriformes***	9	11.11 ±0.52	44.44 ±5.2
White-eared bulbul	***Pycnonotus leucotis***	***Passeriformes***	4	0	25 ±4.2
Budgerigar	***Melopsitacus undulates***	***Psittaciformes***	23	0	21.73 ±3.6
African gray parrot	***Psittacus erithacus***	***Psittaciformes***	5	0	20±2.2
Cockatiel	***Nymphicus hollandicus***	***Psittaciformes***	30	3.33±0.22	6.66 ±0.78
Rose-ringed parakeet	***Psittacula krameri***	***Psittaciformes***	5	0	0
Lovebird	***Agapornis roseicollis***	***Psittaciformes***	4	0	0
Alexanderine parakeet	***Psittacula eupatria***	***Psittaciformes***	2	0	0
Monk parakeet	***Myiopsitta monachus***	***Psittaciformes***	1	0	0
Total			120	2.5±0.79	28.33±5.7

* confidence interval (95%)

Mixed parasitic infection was not found in the samples. Among the positive birds, three birds had clinical symptoms which included a common mynah with symptoms such as anorexia and weakness (positive for *Eimeria spp.*), a canary with symptoms such as anorexia and feather picking (positive for *Eimeria spp.*), and a cockatiel with symptoms such as vomiting, loss of appetite, and diarrhea (positive for *Hymenolepis spp.*), and the other 34 positive birds were all apparently healthy birds kept in Ahvaz pet shops (Table 2).

Among *Passeriformes* and *Psittaciformes*, zebra finch, common mynah, canary, white-eared bulbul, budgerigar, African gray parrot, and cockatiel had the most infection rate, respectively but in other species, gastrointestinal parasites were not found.

DISCUSSION

The results of the present study showed the prevalence of gastrointestinal parasites in pet birds in Ahvaz at 30.83%, and *Passeriformes* had the most infection rate but the highest rate of infection with zoonotic parasites was in *Psittaciformes*. The zoonotic parasites detected in this study included *Cryptosporidium spp.* , *Giardia spp.* and *Hymenolepis spp.* (Heidari and Gharakhani, 2012; Ryan and Cacciò, 2013; Singh et al., 2020). These parasites have a wide host and wide geographical distribution and they mostly cause diseases in immunosuppressed persons. The findings of this study showed that pet birds, including *Passeriformes* and *Psittaciformes*, can be a reservoir 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 pet shops, which increases the risk of infection in these centers. Among the *Passeriformes*, the highest infection rate was related to zebra finch, common mynah, canary, and white-eared bulbul, respectively. Among the *Psittaciformes*, the highest infection rate was related to budgerigar, African gray parrot, and cockatiel respectively.

In this study, three birds (2.5%) were infected with the helminthic parasite, *Hymenolepis spp.*. In the present study, among the positive birds, one cockatiel with vomiting, loss of appetite, and diarrhea was positive for *Hymenolepis spp.* but the other birds which were positive for helminthic parasites had no clinical symptoms and were from pet 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 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) and these results were in contrast to the results of the current study in terms of the type of parasite, the number, and the percentage of helminthic parasites in the digestive tract.

In a survey by Badparva *et al.* on pet birds in Iran, the infection rate of helminthic parasites in the digestive system was 7.2% (*Ascaridia spp.* (0.4%), *Capillaria spp.* (2.4%), *Railleitina spp.* (4.2%) and *Hymenolepis spp.* (0.2%)((Badparva et al., 2015). In a survey by Prathipa *et al.* on caged parrots, in India, the infection rate of *Ascaridia spp.* in the digestive tract was 11.2%, the infection rate of *Capillaria spp.* was 20%, the infection rate of *Strongyloides spp.* was 2% and the infection rate of *strongyl spp.* was 1.6% (Prathipa et al., 2013). In contrast with this study, in the present study, all birds were negative for *Capillaria spp, Ascaridia spp.*, *Strongyloides spp.,* and *strongyl spp*.

In a study by Dewir and Elshanat in Egypt, the infection rate of gastrointestinal parasites in captive birds from pet shops was 31% which 26% of it related to helminthic parasites including *Ascaridia,spp.* at 13%, *Echinostoma spp*. at 9% and *Contracaecum spp.* at 4% (Dewir and Elshanat, 2017).

It seems that the reason for the difference between the results of the present study 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 which were positive for helminthic parasites had no clinical symptoms and were from pet shops. Only one cockatiel with digestive symptoms which was referred to a veterinary hospital was positive for *Hymenolepis spp.,* 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 this study, no infection with trematodes and nematodes 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 28.3%, of which, the rate of infection with *Eimeria spp.* was 20%, the rate of infection with *Isospora spp.* was 2.5%, the rate of infection with *Cryptosporidium spp.* was 1.66% and the rate of infection with *Giardia spp.* was 4.16%. In the current study, among the positive birds, a common mynah that suffered from anorexia and weakness which was positive for *Eimeria spp.* . Also, a canary that suffered from anorexia and feather picking, which was positive for *Eimeria spp.*, but most of the birds which were positive for protozoan parasites had no clinical symptoms and were from pet 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 study by *Papini et al.* in Italy, *Giardia duodenalis* (5.3%), coccidia (1.4%), cryptosporidium (4%) were detected, and *Cryptosporidium spp.* and *G. duodenalis* were exclusively found in *Psittaciformes* (Papini et al., 2012)

The results of this study are consistent with the present study. Similarly in the present study *Cryptosporidium spp.* and *Giardia spp.* were exclusively found in *Psittaciformes.*

In the study conducted by Badparva *et al.* in Iran, the infection rate of *Eimeria spp.* was 7.1% and the infection rate of *Cryptosporidium spp.* was 7.3% (Badparva et al., 2015).

In a study by Berkunsky *et al.* in Argentina, in wild blue-fronted Amazon, the rate of infection with *Eimeria spp*. was 25% and with *Isospora spp.* was 5% (Berkunsky et al., 2019).

In a study by Ahmadi Qarjeh *et al.* in Iran, in pet birds, the infection rate of *Cryptosporidium spp.* was 6.14% in *Passeriformes* and *Psittaciformes* (four canaries, two cockatiels, and one budgerigar)(Ahmadi Gharacheh et al., 2020).

The results of this study are in contrast with the present study and showed more infection rate of *Cryptosporidium spp.* in pet birds. In contrast, in the present study *Cryptosporidium spp.* was not found in *Passeriformes.*

In a study conducted by Sandoval-Rodríguez *et al.* in 2021 in Santiago, Chile, among 207 fecal samples from monk parakeets, 25 positive samples for *Giardia spp.*, and 10 positive samples for *Cryptosporidium spp.* were reported (Sandoval-Rodríguez et al., 2021). The results of this study are inconsistent with the present study, and in the present study, the *cryptosporidium* and *giardia* infection rates were lower than in the study by Sandoval-Rodríguez *et al.*, and in the present study, these parasites were detected in budgerigar, African gray parrot, and cockatiel but not detected in monk parakeets.

It seems that the reason for the difference between the results of the present study and some aforementioned studies about protozoan parasites includes the following: Geographical region, sample size, season, age, health status and species of birds, bird-keeping conditions such as long-term captivity, high density, and the nests' hygiene status may cause these differences.

CONCLUSION

In the present study, the examination of fecal samples of Ahvaz pet birds showed that most of the positive samples were from apparently healthy birds without clinical symptoms, and also most of the positive samples were reported from pet 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 pet shops. Considering that the pet 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 zoonotic parasites such as *Cryptosporidium spp.*, *Giardia spp.* and *Hymenolepis spp.* are present in pet birds in the Ahvaz area and should be considered by the owners of these pet birds, breeders, veterinarians, and public health organizations. According to the presence of these zoonotic parasites 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 pet birds in the Ahvaz area.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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