Original Article

Investigation of Enteric Parasites with a Focus on Zoonotic Parasites in the Feces of *Galliformes*

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ABSTRACT

Galliformes have a global distribution and are bred by humans as domestic animals or game birds. The world's poultry population is increasing due to high consumer demand for poultry products. Due to the increasing growth of *Galliformes* breeding, this study aimed to investigate gastrointestinal parasites in the feces of some species of *Galliformes*. In the present study, 100 distinct fecal samples were collected from seven different *Galliformes* species, including rural chickens, peacocks, partridges, pheasants, turkeys, quails, and guinea fowls. Sampling of each bird was performed separately, and these birds were kept in cages. In order to identify gastrointestinal parasites, the samples were evaluated using the Clayton-Lane method, the Modified Ziehl-Neelsen, and the Trichrome staining methods. Among 100 birds sampled, 53% were positive for gastrointestinal parasites. Moreover, 30%, 18%, and 5% of birds were infected with *Eimeria spp.*, nematodes, and *Giardia spp.*, respectively. The highest rate of parasitic infestation in *Galliformes* was related to protozoan infections, and the highest rate of protozoan infection was related to *Eimeria* (30%). In addition, complex infestation was not detected. Considering that most parasitic infections have been detected in apparently healthy birds, it is recommended to observe hygiene to regularly disinfect cages and reduce the density of birds in the nests. Due to the zoonotic potential of *Giardia* and the contamination of some birds with this parasite, this issue should be considered by public health officials, bird sellers' markets, owners, and breeders of these birds.

Keywords: *Galliformes*, Gastrointestinal parasites, Zoonotic diseases, Feces

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1. Introduction

The *Galliformes* are among the most important orders of birds in the world (1) and have a beneficial role for humans because they are widely used as food, plumage, and trading (2). The *Galliformes* include chicken, turkey, pheasant, partridge, quail, and other landfowl. The *Galliformes* are often reared by humans for their meat and eggs, or they are hunted as game birds. This order involves approximately 290 species that live in all continents except Antarctica and includes three important families: *Phasianidae* (e.g., turkeys, chicken, pheasants, quail, partridges, grouse, and peafowl [peacocks]), *Odontophoridae* (New World quail), *Numididae* (guinea fowl) (3). The local or exotic breed of domestic fowl, *Gallus gallus domesticus*, is raised by people to produce eggs and meat as an animal protein source (4). Lack of protein, especially animal proteins, is one of the most important aspects of malnutrition that affects many people in different parts of the world. Helminthic diseases such as cestodes and ascarids are important parasitic disorders that affect poultry production. Ascariodiosis is the most common helminthic disease in poultry (5). Approximately a hundred helminth species have been identified in wild and domestic birds. Chickens with ideal feeding and keeping conditions and those that are genetically suitable will not have ideal growth or lay eggs if they are infected with parasites (6). In Africa and Asia, some helminth species are widely distributed in poultry (7). Intestinal parasites are a common problem in Nigeria (5). The most commonly recorded species include *Ascaidia galli*, *Heterakis gallinarum*, and *Capillaria annulata* (8); however, just a few reports address the importance of helminth species in commercial production systems. Morgenstern and Lobsiger (1993) (9) reported that the prevalence of *Ascaridia galli*, *Capillaria* species, and cestodes in Switzerland was about 20-30% in free-range systems. Among different protozoan parasites, *Trichomonas gallinae* (particularly in pheasant chicks), *Histomonas meleagridis* (in all *Galliformes* but particularly in turkeys, pheasants, and partridge if in close contact with chickens), *Hexamita spp*. (in turkeys), *Eimeria spp.*, *Giardia spp.*, and *Cryptosporidia spp.* have significant pathogenic importance (3). *Cryptosporidium* is a protozoan that is considered a zoonotic disease (10). Zoonotic transmission plays a key role in cryptosporidiosis epidemiology (11). There are many reports of infection by people in direct and close contact with livestock and poultry (10). Cryptosporidiosis is an important parasitic disease that causes diarrhea and gastroenteritis in humans and animals worldwide (12). *Giardia* is another protozoan with a wide geographical and host distribution and has been reported in most avian species (13). The evidence obtained in the last two decades has introduced giardiasis as a zoonotic disease (14), which indicates the importance of zoonotic transmission of *Giardia spp.* between humans and birds (14). Symptoms of giardiasis vary from asymptomatic to watery diarrhea, nausea, abdominal pain, and weight loss (14). The main symptoms of acute giardiasis in humans include diarrhea, flatulence, epigastric cramps, nausea, vomiting, and weight loss (15). There is particular concern regarding *Giardia spp.* since the parasite is easily spread between humans and can cause long-term side effects (16). The transmission of *Giardia spp.* and *Cryptosporidium spp.* is oral-fecal (11). Infections with *Cryptosporidium spp.* and *Giardia spp.* mainly occur in developing countries due to poverty and limited access to adequate nutritional resources (17). 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 sub-clinically, weaken the immune system, and increase the bird's susceptibility to other infectious agents, endoparasites of birds should be detected and treated (13). Considering the increase in the rearing of *Galliformes* and the importance of zoonotic parasites, the main objectives of the current study were to investigate gastrointestinal parasites, including worms and protozoa in the feces of some *Galliformes* species with a focus on zoonotic parasites.

2. Materials and Methods

In the present study, 100 distinct fecal samples were taken from seven different *Galliformes* species, including chickens, peacocks, partridges, pheasants, turkeys, quails, and guinea fowls in Ahvaz, Iran, from November 2021 to July 2022, and were kept in cages. In order to identify gastrointestinal parasites, distinct fecal samples were collected from apparently healthy birds from bird shops and bird sellers' markets and diseased birds from Ahvaz rural areas, referred to the Department of Avian Medicine, Ahvaz, Iran. Most of the diseased birds had nonspecific signs such as lethargy, losing weight, anorexia, vomiting, and diarrhea. Clinical symptoms were recorded based on the owner's history and external physical examination. The number and species of sampled birds are listed in Table 1; sampling of each bird was performed separately. In order to collect fresh feces, first, each bird was moved from the cage where the birds were kept collectively to the sampling cage. Approximately 30 min after placing the sterile paper sheets on the bottom of the sampling cage, fresh feces were collected from the bottom of the cage using sterile wooden spatulas and then kept in separate sterile vials and immediately sent to the laboratory for further processing. Afterward, two smears were prepared from each sample, one for the Modified Ziehl-Neelsen

(ZN) and the other for Trichrome staining, and after drying, the smears were fixed with pure methanol (18).

2.1. Modified Ziehl–Neelsen staining:

Modified ZN staining (Kinyoun's modification of acid-fast staining) was carried out on smears made from fresh samples. The slides were screened under 100x magnification of a light microscope for the diagnosis of the *Cryptosporidium spp.* (18).

2.2. Trichrome staining method:

All samples were analyzed by the Modified Trichrome to detect *Giardia spp*. The slides were screened under 100x magnification of a light microscope (18). Furthermore, the feces were examined using the centrifugal stool flotation technique (Clayton-Lane) to identify helminthic and protozoan parasites (19). In the Clayton-Lane method, a saturated solution of sugar (Sheather's solution) and a saturated zinc sulfate solution were employed. In positive samples, 2.5% potassium dichromate was used for the detection of coccidia oocysts**.**

2.3. 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 ($K_2Cr_2O_7$) (20).

Table 1: The number of collected samples, and the percent of positive samples in *Galliformes*.

Common name	Number of birds sampled	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. (%)	No. of positive for nematode spp.	Positive samples for nematode spp. (%)
Quail	20	10	50 ± 3.3*	0	0	0	10	50 ± 6.2
Peacock	7	3	42.85 ± 7.1	0	0	0	4	57.14 ± 1.1
Chicken	20	7	35 ± 6.3	0	0	0	0	0
Partridge	13	4	30.76 ± 5.5	0	0	0	0	0
Turkey	15	4	26.6 ± 3.3	3	20 ± 1.2	0	0	0
Pheasant	10	0	0	2	20 ± 3.7	0	0	0
Guinea fowl	15	2	13.33 ± 2.1	0	0	0	4	26.66 ± 2.3
Total	100	30	30 ± 6.1	5	5 ± 4.3	0	18	18 ± 6.8

3. Results

The results of the present study indicated that 53% of birds were positive for gastrointestinal parasites. In addition, 30%, 18%, and 5% of birds were infected with *Eimeria spp.*, nematodes, and *Giardia spp.*, respectively. (Figure 1 and Table 1). This study demonstrated that the highest rate of contamination was related to bird shops and bird sellers' markets, which increases the risk of infection in these centers.

All sampled birds were negative for the *Cryptosporidium spp.* Infection with *Giardia* trophozoites was detected only in pheasants and turkeys, and the infection rate was 20% for them. No *Giardia* trophozoites were detected in other species. Oocysts of *Eimeria spp.* were detected to be 50%, 42.85%, 35%, 30.76%, 26.6%, 13.33%, and 0% in quail, peacock, chicken, partridge, turkey, Guinea fowl, and pheasant, respectively.

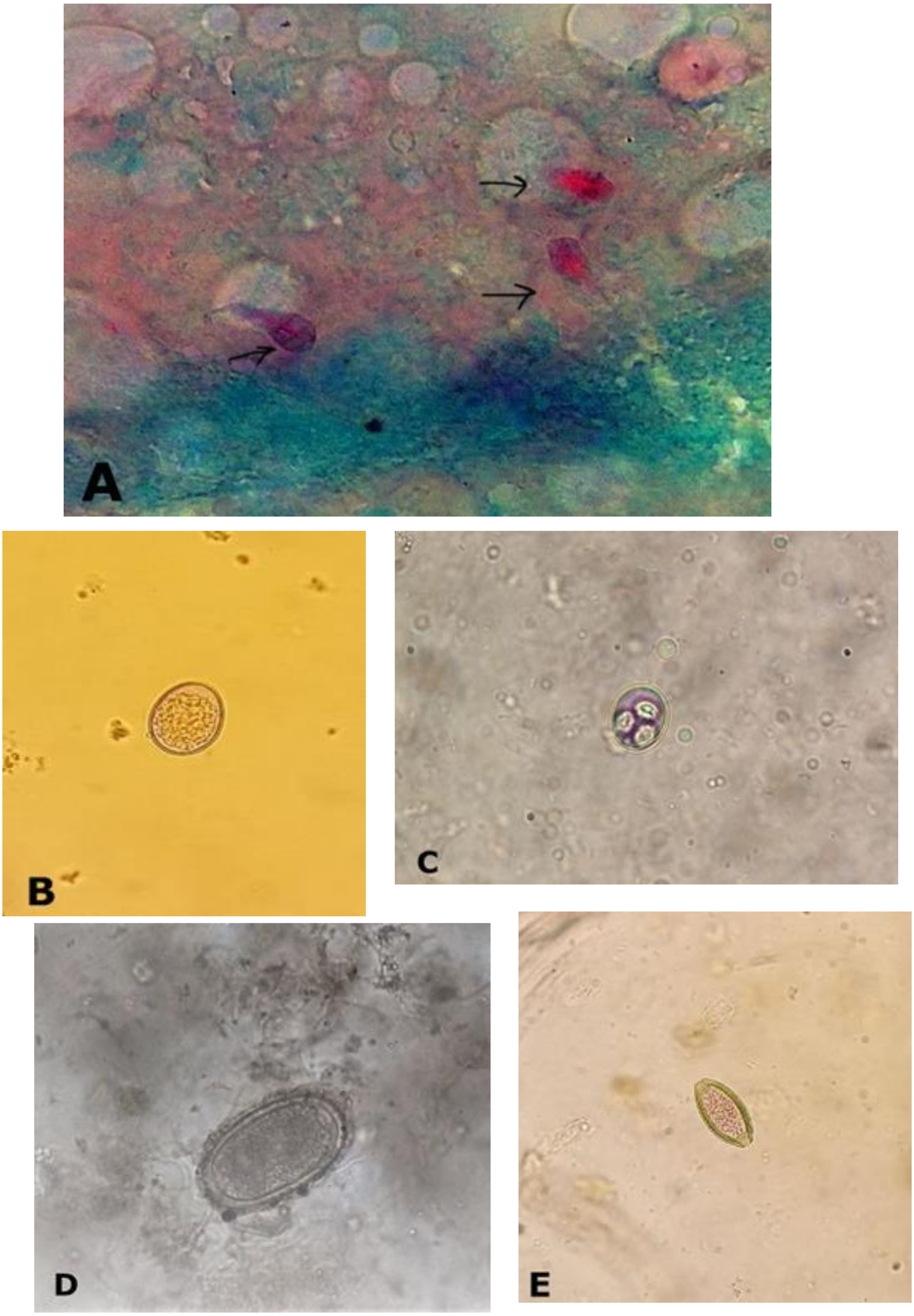

Figure 1: The gastrointestinal parasites in feces of *Galliformes* in Ahvaz.

(A): *Giardia spp*. trophozoite (1000×),(B):Non-sporulated oocyst of *Eimeria spp*. (400×), (C): Sporulated oocyst of *Eimeria spp*. (400×), (D): Suspected to *Ascaridia galli* egg (400×), (E): Suspected to *Capillaria spp*. egg.

It was demonstrated that using the Clayton-Lane method and observation of the wet slide under the microscope, 30 birds sampled (30%) were positive for *Eimeria spp.* oocysts. After sporulation of *Eimeria spp.* oocysts from the poultry species that were positive, an attempt was made to identify their species using reference books. Table 2 presents the obtained results based on measuring the dimensions of the sporulated oocysts (21). In addition, using the Clayton-Lane method, it was indicated that 18 birds sampled (18%) were positive for nematode eggs. The nematode eggs were detected to be 57.14%, 50%, and 26.66% in peacocks, quails, and guinea fowls, respectively, and the other species were negative. It is necessary to explain that the number of eggs counted per gram of feces (EPG) cannot completely determine the level of contamination in experimental or natural contaminations because EPG is influenced by the number of adult parasites in the digestive tract, host immunity, age of the host, stage of infection, sample collection time, and other factors (Table 3). Among the positive birds, only 13 sampled birds had clinical symptoms such as lethargy, anorexia, and diarrhea, and the rest of the positive samples were all apparently healthy birds (Table 4).

Table 2: Identified sporulated *Eimeria spp*

Eimeria spp.	**Length: 16.5 μm Width: 21.6 μm**	**Length: 15 μm Width 12 μm**	**Length: 21 μm Width: 15 μm**	**Length: 15 μm Width: 9 μm**
E.mivati		*		*
E.tenella	*		*	

Table 3: Average EPG of nematode eggs observed in each species.

	Peacock	**Quail**	**Guinea fowl**
average EPG	7	9	12

Table 4: The birds were sampled by species, scientific name, and health status of the birds

Common name	**Scientific name**	**Number of birds sampled**	**No. of Positive birds with clinical symptoms**	**The clinical symptoms**
Quail	***Coturnix coturnix***	**20**	**2**	**anorexia and diarrhea**
Peacock	***Pavo cristatus***	**7**	**5**	**vomiting, loss of appetite, and diarrhea**
Chicken	***Gallus gallus domesticus***	**20**	**2**	**anorexia, vomiting, loss of appetite, diarrhea, and weakness**
Partridge	***Alectoris chukar***	**13**	**-**	**-**
Turkey	***Meleagris gallopavo***	**15**	**2**	**anorexia and diarrhea**
Pheasant	***Phasianus colchicus***	**10**	**2**	**vomiting, loss of appetite, and diarrhea**
Guinea fowl	***Numida Linnaeus***	**15**	**-**	**-**
Total	**-**	**100**	**13**	**-**

Among the positive samples, 13 birds had clinical symptoms referred to the Department of Avian Medicine, Ahvaz, Iran. These samples involved two quails with anorexia and diarrhea, five peacocks with vomiting, anorexia, and diarrhea, two chickens with anorexia, vomiting, anorexia, diarrhea, and weakness, two turkeys with anorexia and diarrhea, and two pheasants with vomiting, anorexia, and diarrhea. The other positive birds were all apparently healthy (Table 4).

4. Discussion

The present study indicated the 53% prevalence of gastrointestinal parasites in *Galliformes* in Ahvaz, Iran. The detected zoonotic parasites in this study included *Giardia spp.,* and infection with *Giardia* trophozoites was detected only in pheasants and turkeys. This parasite has a wide host and geographical distribution and mainly leads to diseases in immunosuppressed people. The findings of the present investigation indicated that *Galliformes* can be reservoirs of zoonotic parasites, and humans can become infected in connection with these birds, and vice versa. In addition, it was reported that the highest rate of contamination was related to bird shops and bird sellers' markets, which increases the risk of infection in these centers. Among the positive samples, 13 birds had clinical symptoms referred to the Department of Avian Medicine, Ahvaz, Iran, and other positive birds were all apparently healthy with no clinical symptoms. This issue indicates the importance of detection, control, prevention, and treatment of subclinical infections, especially the identification of zoonotic parasites that have a direct impact on public health. In a study by *Papini et al.* in Italy (19), feces were taken separately from pet and zoo birds from 14 orders and 63 species. All samples were analyzed by the stool flotation method. A total of 35.6% of birds, including zoo 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%]) (19); these results were consistent with the findings of the present research in terms of detecting *Ascaridia spp.* in the digestive tract. In the present investigation, 18% of the sampled birds were positive for nematode eggs. The nematode eggs were detected to be 57.14%, 50%, and 26.66% in peacocks, quails, and guinea fowls, respectively, and the other species were negative. The feeding of domestic *Galliformes* includes a wide range of diets, and this habit makes them susceptible to parasitic infections, as many foods carry the infective stages of the parasites (4). This issue may account for the high prevalence rate of nematode detection in peacocks, quails, and guinea fowls, according to the present study. Moreover, the high rate of nematode infection may be due to environmental humidity because it facilitates larval growth and parasite transmission. The high rate of nematode infection in peacocks, quails, and guinea fowls could be due to the poor sanitary condition of the environment and lack of proper medicine for the birds, as observed by Permin *et al*. (1997) (8). In the present study, all birds were negative for cryptosporidiosis. The occurrence of mixed infection among different helminths and protozoa was also observed in peacocks, quails, and guinea fowls with *Eimeria spp.* and nematodes and in turkeys with *Eimeria spp.* and *Giardia spp.* Moreover, the present investigation is in line with the observation made by Islam (1985) (22), who noticed a mixed infection in domestic fowls in Zambia. In addition, Rao and Sharma (1992) (23) noticed mixed infections of *E.bateri* and *E.uzuro* in Japanese quail. It was also observed that nematodes and protozoan parasites were more common in young birds (42.2%) than those in adult quails (20%). Trematodes and cestodes were not recorded during the present study, which may be due to the lack of availability of snails, dragonflies, etc., since all trematodes have indirect lifecycles. These results are consistent with the observations of researchers who noted that nematodes and protozoa are common in quails, but tapeworms are sporadic, and trematodes and acanthocephalans are uncommon (24). The occurrence of gastrointestinal parasitism in *Galliformes* could be related to the fact that the *Galliformes* were free-ranging and had access to infective stages in the environment. This result was in agreement with a study conducted by Magwisha *et al.* (2002) in Tanzania (25), who observed a 100% prevalence of helminth infections in the free-range system regardless of the age and gender of the birds. This result was also in line with other investigations (26, 27). Some studies indicated that animals can be reservoirs of zoonotic parasites, and humans can become infected in connection with these animals (28-30). The present investigation demonstrated that *Galliformes* can be reservoirs of zoonotic parasites, and humans can become infected in connection with these birds and vice versa. However, the findings may be influenced by the season during which the survey was performed, the sample size, the geographical area, the presence of intermediate hosts in the investigated area, bird-keeping conditions, high density, and the nests' hygiene status. In the current research, most of the birds that were positive for parasites had no clinical symptoms and were from bird shops and bird sellers' markets. Among the positive birds, only 13 birds had clinical symptoms referred to the Department of Avian Medicine, Ahvaz, Iran, which included two quails, five peacocks, two chickens, two turkeys, and two pheasants and the other positive birds were all apparently healthy kept in birds shops and bird sellers' markets in Ahvaz, Iran. This finding demonstrates the importance of detection, control, prevention, and treatment of subclinical infections, especially the identification of zoonotic parasites that have a direct impact on public health. Out of 100 birds sampled, 53% were positive for gastrointestinal parasites. Moreover, it was revealed that 30%, 18%, and 5% of birds were infected with *Eimeria spp.*, nematodes, and *Giardia spp.*, respectively. The examination of feces of *Galliformes* in Ahvaz, Iran, indicated that most of the positive samples were from apparently healthy birds without clinical symptoms, and most of the positive samples were reported from bird shops and bird sellers' markets 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 therefore, cause a risk for immunosuppressed birds. This issue indicates the significance of periodic monitoring of all bird shops and bird sellers' markets. Considering that these centers in Ahvaz, Iran, were contaminated, observing hygiene and regularly disinfecting the cage beds is recommended. Moreover, it was shown that infection with the zoonotic parasite (*Giardia spp.*) is present in turkeys and pheasants and should be considered by the owners of these birds, breeders, veterinarians, and public health organizations. In addition, due to the sensitivity of immunosuppressed people to this zoonotic parasite, people with weak immune systems, such as those who suffer from rheumatism, AIDS, diabetes, hepatitis B, and other conditions, should not be in close contact with turkeys and pheasants in the Ahvaz area in Iran. Additionally, there is a need for continuous research on appropriate and preventive methods for controlling gastrointestinal parasites in *Galliformes*.

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Authors' Contribution

Study concept and design: Forough Talazadeh, Mohammad Hosein Razijalali

Acquisition of data: Majid Masaee Manesh, Fatemeh Khajeh

Analysis and interpretation of data: Forough Talazadeh, Mohammad Hosein Razijalali

Drafting of the manuscript: Forough Talazadeh

Critical revision of the manuscript: Forough Talazadeh

Ethics

The protocols used in the present research were approved by the Shahid Chamran University of Ahvaz Ethical Commission for Animal Experiments under verification number EE/1401.2.24.117815/scu.ac.ir.

Conflict of Interest

The authors declare there is no conflict of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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