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ORIGINAL ARTICLE

First report of birds infection by intestinal parasites in Khorramabad, west Iran

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Abstract Parasitic infections in birds are omnipresent, even when they occur in low amounts, may result in subclinical diseases. There aren't any studies, based on Iranian data, investigating the prevalence of intestinal parasitic infections in some birds' species. We conducted a cross-sectional study between December 2011 and December 2012. The fecal samples were taken from 451 birds including hen, turkey, sparrow, pigeon and decorative birds. The samples screened for intestinal parasitic infections using direct smear, formalin–ether concentration technique, modified Ziehl–Neelsen staining, Culture in RPMI 1640 medium, sporulation with potassium dichromate and Trichrome and Giemsa staining. Out of 451 birds' species, 157 (34.8 %), were infected with one or more type of intestinal parasites. We identified two nematode, two cestoda species and five protozoan parasites species. No trematodes were found in the samples studied. The parasites identified among birds involved *Raillietina* spp. (4.2 %) and *Eimeria* spp. (7.1 %) were the most common helminthes and protozoa respectively. From total of birds study, 12 (2.7 %) and 6 (1.3 %) have two and three mixed infections respectively. Intestinal parasitic infections are common in birds in west Iran. The future studies are needed in order to determine to which extent the infections influence mortality and performance of the birds.

Keywords Prevalence · Intestinal parasites · Birds · Helminthes · Protozoa

Introduction

Traditional free-range poultry are performed by the some families in small holdings. This trade is great importance in urban production systems in small communities throughout the developing world (Pande et al. 1993). The food and agriculture organization (FAO) has expected that around 14 milliard poultry be present worldwide and of these, 75 % are in developing countries (FAO ACA 2001; Poulsen et al. 2000).

Internal and external parasites of poultry are common in the tropics where the standard of husbandry is poor and climatic conditions are favorable for their increase (Abebe et al. 1997; Imura et al. 2012). The effects of parasitism on birds are often severe including malnutrition, retarded growth, low egg production, susceptibility to other infections and death in young birds (Radfar et al. 2012). The common internal parasitic infections occur in birds include cestodes, nematodes and coccidians. Free-range scavenging birds are in direct contact with parasite vectors, soil and feces. On the other hand, lack of hygiene, direct contact with humans, captivity conditions and the physical environment (e.g. rainfall, humidity, and ambient temperature) provide optimum conditions to maintain parasites populations. Some bird parasites may have a zoonotic potential,

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and direct or indirect contact with infected specimens can cause human diseases (Corrêa and Corrêa 1983). The probability of zoonotic disease transmission is influenced by many factors, such as time of infection, latent period, stability of the agent when exposed to the environment, population density, animal handling, virulence, and route of infection (Corrêa and Corrêa 1983; Marietto-Gonçalves et al. 2008).

Knowledge about farm conditions is essential for developing the best prevention program, enabling the recognition of factors that influence the possibility of incidence of the disease (Graat et al. 1998). Wet litter, bad ventilation, high density of birds, improper coccidiostat consumption and contaminated feeders are samples of bad managements that exaggerate intestinal parasites problems (Ruff 1993). Potentially zoonotic agents, such as *Cryptosporidium* spp., *Giardia* spp., *Encephalitozoon* spp. and *Enterocytozoon* spp. have also been observed in birds (Graczyk et al. 2008).

At the moment, information on the geographical distribution of parasitic diseases among birds species in Iran is still very limited. Due to the risk of human exposure to zoonotic agents found in birds and need to understand the epidemiology of the various birds parasites in order to plan strategies to bolster increased productivity of free-range birds, we investigated the prevalence of intestinal parasites in birds at Khorramabad, west of Iran.

Materials and methods

Study area

The study was carried out in Khorramabad, the capital and largest urban center in Lorestan state, west of Iran between December 2011 and December 2012. Khorramabad is at a height of about 1,125 m above sea level with a latitudinal position of 33° 29′ 16″ North, 48° 21′ 21″ East. The city has a population of 354,855 based on 2011 census ("Statistics in Iran, I.B.O. population census" 2011). The temperature mean value is 17.60 °C, however, during the cooler periods (November and March) the average temperature drops to about 5 °C, relative humidity mean value is 46.08 % and precipitation mean monthly value is 42.74 mm ("Available from this site: http://www.climate-charts.com/Locations/i/IR40782.php").

Sample collection

A total 451 fecal samples were collected from the hen ($n = 201$), turkey ($n = 59$), sparrow ($n = 105$), pigeon ($n = 37$) and decorative birds ($n = 49$). Fecal samples from each decorative bird collected from the bottom of each birdcage. For other birds, we used plastic covers that lay on the floor in birds' nests and obtained fecal samples from it. Samples collected in labeled, leak-proof, and clean plastic stool cups and brought to the laboratory immediately.

Parasite processing and identification

At least 1 g of fresh stool sample was suspended in a tube containing 10 ml of 0.85 % saline. The suspensions were sieved through an 80 mesh. The strained suspension was centrifuged at 700 g for 5 min, and the supernatant was then decanted. The supernatant was discarded and diagnostic methods were performed on precipitates from each tube:

(1) Direct smear: The precipitates were examined microscopically following direct method. In brief, Direct microscopy of the smears in a saline (0.85 % NaCl solution) and Lugol's iodine was performed for the detection of trophozoites, cysts and ova of intestinal parasites.
(2) Formalin–ether concentration technique: The precipitates were adjusted to 7 ml with 10 % formalin, mixed well, and allowed to stand for 10 min. Later, 3 ml of diethyl ether was added. The tube was closed, vigorously shaken by hand for 1 min, and centrifuged at 700×*g* for 5 min. The debris plug was loosened, and the top three layers were discarded. Iodine stain preparation was made. The entire area under the cover slip was systematically examined using 10× and 40× objective lenses.
(3) 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 light microscope for identification of the coccidian parasites (Kuzehkanan 2011).
(4) Culture in RPMI 1640 medium: For motility, confirmation and detection of protozoan parasites the following method is used: A part of the precipitates was adjusted is added to RPMI 1640 with 5 % inactive fetal bovine serum at the 38 °C for 3–4 days.
(5) Sporulation with potassium dichromate: The precipitates were used for coccidian sporulation. Sporulation was performed in wet chamber at 24–26 °C in 2.5 % potassium dichromate solution ($K_2Cr_2O_7$).
(6) Trichrome and Giemsa staining: All samples were analyzed by Modified Trichrom and Giemsa stain especially for detecting Microsporidial spores and Histomonas meleagridis (Garcia 2009).

Results

In total of 451 fecal samples examined 157 (34.8 %) were intestinal parasitic infections positive. The prevalence of parasites species identified in urban birds is shown in Table 1. Four helminthes species were recovered and these involved two nematodes and two cestodes. *Raillietina* spp. was the most common helminthic parasite detected (4.2 %), followed by *Capillaria* spp. (2.4 %), *Ascarida* spp. (0.4 %) and *Hymenolepis nana* (0.2 %). *Cryptosporidium* spp. was the most common protozoan parasite detected (7.3 %), followed by *Eimeria* spp. (7.1 %), *Histomonas spp.* (6.5 %), *Trichomonas gallinae* (5.8 %) and *Amoeba* spp. (0.9 %). The most infected birds were sparrow (52.4 %), hen (34.8 %), turkey (28.8 %), decorative Birds (18.3 %) and pigeon (16.2 %), respectively. Mixed infections were found in 5.7 % of the birds, while 94.3 % had single infections. Among the mixed infections, 66.7 % had two species and 33.3 % had three species of parasites. Table 2 is showed mixed parasitic infections in birds study.

Discussion

Despite the several studies that have reported the prevalence of parasitic infection in this region (Badparva et al. 2012; Ezatpour et al. 2013; Kheirandish et al. 2014), this is the first investigation to determine the prevalence of intestinal parasites among bird's species in the Khorramabad, west of Iran. It is important to verify the presence of the above mentioned agents, due to the high risk of infection in professional workers, e.g., veterinarians, biologists, workers, tradesmen, and farm owners (Kašičková et al. 2009; Pérez Cordón et al. 2009). A limited number of studies have been performed about the prevalence of parasitic infections in birds in Iran. On the other hand, unlike some surveys that have studied only one species of bird, various species was investigated in present study. This rate (34.8 %) indicates a high incidence of parasitic infection in birds in the region.

A first point to note is that our study confirms the findings of other studies that show coccidiosis is one of the most frequently reported disease of birds world-wide (Jatau et al. 2012). As *Cryptosporidium* spp. was the most common parasite detected (7.3 %). *Cryptosporidium* spp. are the important intestinal pathogens that have been reported in more than 30 species of birds worldwide (Qi et al. 2011). Each of the three species, *C. meleagridis, Cryptosporidium baileyi* and *Cryptosporidium galli* can infect numerous species of birds and may induce respiratory and renal diseases (Chvala et al. 2006; Fayer 2010; Shemshadi et al. 2011) and high mortality have been reported in infected birds with these species (Wang et al. 2011).

Second most frequent parasite was the protozoan of the genus *Eimeria* (7.1 %) that multiply in the intestinal tract and cause fluffy feathers, anorexia, and watery diarrhea with mucus, interruption of digestive processes or nutrient absorption, reduced weight gain, and increased susceptibility to other epidemic diseases such as mycoplasmosis and colibacillosis (Yegani and Korver 2008; Zhang et al. 2012). There are optimal conditions of temperature and humidity for the sporulation of the oocysts in our region. Studies in Tanzania, Ethiopia, India and Argentina, reported a higher prevalence of coccidiosis as compared to

Table 1 Prevalence of parasites in urban birds in Khorramabad, Iran

	Hen $n = 201$		Turkey $n = 59$		Sparrow $n = 105$		Pigeon $n = 37$		Decorative birds $n = 49$		Total $n = 451$	
	N	Inf (%)	*N*	Inf (%)	*N*	Inf (%)	*N*	Inf (%)	*N*	Inf (%)	*N*	Inf (%)
Protozoa												
Eimeria spp.	2	1	0	0	29	27.6	0	0	1	2	32	7.1
Trichomonas gallinae	17	8.4	7	11.8	2	1.9	0	0	0	0	26	5.8
Histomonas spp.	24	11.9	0	0	1	0.95	2	5.4	2	4.1	29	6.5
Amoeba spp.	0	0	2	3.4	1	0.95	0	0	1	2	4	0.9
Cryptosporidium spp.	3	1.5	7	11.8	20	19	1	2.7	2	4.1	33	7.3
Total protozoa	46	22.9	16	27.1	53	50.5	3	8.1	6	12.2	124	27.5
Helminthes												
Ascarida spp.	0	0	1	1.7	0	0	1	2.7	0	0	2	0.4
Hymenolepis nana	0	0	0	0	1	0.95	0	0	0	0	1	0.2
Capillaria spp.	9	4.5	0	0	0	0	1	2.7	1	2	11	2.4
Railleitina spp.	15	7.5	0	0	1	0.95	1	2.7	2	4.1	19	4.2
Total helminthes	24	11.9	1	1.7	2	1.9	3	8.1	3	6.1	33	7.3
Total of Parasites	70	34.8	17	28.8	55	52.4	6	16.2	9	18.3	157	34.8

Table 2 Prevalence of mixed parasitic infections in urban birds in Khorramabad, Iran

Mixed parasitic infections	*N*	Inf (%)
Infection with 2 parasites		
Ascarida spp. + *Capillaria spp.*	2	0.45
Eimeria spp. + *Cryptosporidium* spp.	4	0.9
Trichomnas spp. + *Railleitina* spp.	2	0.45
Eimeria spp. + *Amoeba* spp.	2	0.45
Trichomonas gallinae + *Histomonas* spp.	2	0.45
Total Infection with 2 parasites	12	2.7
Infection with 3 parasites		
Capillaria spp. + *Histomonas* spp. + *Hymenolepis nana*	1	0.22
Capillaria spp. + *Amoeba* spp. + *Histomonas* spp.	2	0.44
Capillaria spp. + *Histomonas* spp. + *Trichomonas gallinae*	3	0.66
Total Infection with 3 parasites	6	1.3
Total of mixed parasitic infections	18	4

the current work (Grainer and Baumann 2004; Lobago et al. 2005; Permin et al. 2002). It turns out that the Khorramabad's environment is suitable for its growing. In Beside the climatic conditions, many of these investigators did their work on intensive poultry management systems where differences in breed and general husbandry practices would account for the difference in findings. The contamination rate of Sparrows is estimated to be about 52.4 %. This means that due to the fly Sparrows in storage sites, including poultry, parasites are transmitted quickly.

The causative agent of histomoniasis, *Histomonas meleagridis* (6.5 %), is transmitted within a flock by direct lateral transmission (Hu and McDougald 2003). Histomoniasis (blackhead disease) mainly affecting the liver and caeca of birds. The severity of the disease varies over the different species. In turkey flocks, for example, mortality can be very high, whereas in chickens the symptoms are generally less severe (McDougald 1997). In normal conditions, there are two ways by which birds become infected with *H. meleagridis*: the ingestion of embryonated eggs of a caecal worm (*Heterakis gallinarum* as the protozoan has a unique association with the worm) which acts as the vector and through 'cloacal drinking', where protozoa which have been shed in the faeces are taken up by the cloaca (Powell et al. 2009). Nevertheless in current study we didn't seen *H. gallinarum*. Well as we not found *H. meleagridis* and most prevalence of histomoniasis are related to hen population (11.9 %). Some species that appear to be common in other countries were not found in this area. Reasons might be the geographical variations in the distribution of the parasites or the intermediate hosts of the parasites and sampling.

Fourth in the number of parasites was *Trichomonas gallinae* (5.8 %) that causes avian trichomoniasis and is the potentially most serious protozoan disease of nestling birds (Janmaat and Morton 2010). In some study, wet and sterile swab were taken from surface of mouth, throat and larynx of birds (Radfar et al. 2011). Unlike that, we used fecal samples (Radfar et al. 2011). Moreover we also were applied the additional tests such as culture and Trichrome and Giemsa staining for detection of *H. meleagridis* and *T. gallinae*.

We also note that prevalence of protozoa were four times more likely than helminthes. Researchers (Clayton and Moore 1997) reported that helminthes species are pathogenic for birds and can cause enteritis, ulceration, anorexia, emaciation and death. In our findings, the cestode *Raillietina* spp. (4.2 %) and the nematode *Capillaria* spp. (2.4 %) were the most prevalent helminthes. Trematodes were not reported during our study. All trematodes have indirect life cycle. The eggs usually hatch in water and include freshwater snail or dragonflies in their life cycles, and species require two or three intermediate hosts (Esch et al. 2002). The absence of lakes and thus the insufficient number intermediate hosts in this area might be reasons for the absence of tematodes. The results of the investigation are similar to a study from a semi-arid region (Hassouni and Belghyti 2006). Mixed infections caused by potentially zoonotic protozoa and helminthes were found in the present study. In our investigation the range of mixed infection was 4 % and about one-third had infected with three of parasites. Its noticeable the nematodes of *Capillaria* spp. are present in this group continuously.

Our investigation has brought to light information on the likely impact of parasites infections. In addition; information from this study may be a guide to certain control measures. Anyway, intestinal parasites can be controlled through effective management practices, including educate the breeders of birds, daily cleaning of cages, washing dishes, Quarantine new birds before entrance the flock, regular treatments of anthelminthic, anticoccidials drugs and dusting of birds with pesticides. Because the majority of birds in this study in contact with humans and the human environment, so as a reservoir can be transmitted to humans. In conclusion, prevalence of intestinal parasites in birds from the west region of Iran is fairly high. However, the impact of the parasitic infections on the well-being of the birds was not investigated and further studies are needed in order to determine to which extent the infections influence mortality, performance of the birds and quantify the possible relationship between weight gain and laying of eggs.

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Conflict of interest We declare that we have no conflict of interest.

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