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Intestinal protozoan infections in stunting and diarrheic native turkey poults (*Meleagris galopavo*) in Iran

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ABSTRACT
Sixty cases of one- to seven-week old stunted diarrheic native turkey poults from the native turkey-raising provinces of Iran were examined for the presence of intestinal protozoan infections. Forty-seven cases out of 60 had one or more intestinal parasites. Based on fecal, histological, and transmission electron microscopic examinations, *Cryptosporidium spp*., *Hexamita sp.p*, *Cochlosoma sp.p*, *Coccidia spp*., *Chilomastix spp*., and *Trichomonas spp*. were identified. The protozoan infections were associated with diarrhea and unthriftiness.

Keywords: Native Turkey Poults; Protozoan Infections; Iran

INTRODUCTION
A number of protozoan parasites cause gastrointestinal tract infections in turkey poults. Such infections occur when the gut immune system is not developed enough and thus is incompetent to combat the pathogenic microorganisms. A contaminated environment, improper functioning of the immune system, malnutrition, genetic susceptibility, and a combination of these factors could be responsible for turkey poults that are prone to various gut infections. The most important protozoan parasite infections in turkey poults, according to the literature, are due to the *Cryptosporidium*, *Hexamita*, *Cochlosoma*, *Eimeria*, *Trichomonas*, and *Histomonas* parasites (Hofstad et al. 1978 and Swayne et al. 2013). The protozoan *Chilomastix*, which occurs in turkey gut lumen, is considered nonpathogenic (Hofstad et al. 1978; Swayne et al. 2013). However, in an experimental infection in specific pathogen-free chickens with *Chilomastix gallinarum*, *Tetratrichomonas gallinarum*, and *Tritrichomonas eberthi*, some of the birds developed the disease and showed clinical manifestations of emaciation, diarrhea, and dilatation of caeca (Friedhof et al., 1991). These pathogenic protozoans cause diarrhea, poor growth, growth retardation, weight loss, and mortality (Cooper et al. 1995; Gharagozlou et al.2006; Gharagozlou and Dezfoulian 2009; Dezfoulian et al., 2010a,b; Swayne et al. 2013). The tissue changes of villous, crypt, and inflammatory reactions of the gut have a detrimental impact on the functioning of the gastrointestinal tract, which in turn results in the malabsorption of essential nutrients, as well as stunting, unthriftiness, and mortality. As described by Barnes and Guy (1995) and Barnes et al. (1996), in addition to protozoan organisms, several other microorganisms could cause stunting and diarrheic syndrome in turkey poults. They include viruses (*Rotavirus*, *Coronavirus*, *Birnavirus*, *Enterovirus*, *Adenovirus*, *Astrovirus*, *Reovirus,* and *Parvovirus*) and bacteria (*Salmonella*, *Escherichia coli, Campylobacter*, *Bacteroides*, and

Clostridia). In this syndrome, co-infection with two or more microorganisms may alter the severity of the clinical manifestations—for example, a co-infection by a virus and protozoa (Guy et al. 1987; Guy et al. 1988). Based on previous studies conducted by the authors, in the present paper, the occurrence of protozoan infections in native turkey poults of Iran with clinical manifestations of diarrhea and poor growth or growth retardation have been described. The native turkeys raised in free-run conditions could be a natural source of pathogenic protozoa for commercial turkey industries. Nevertheless, the identification, treatment, and control of the protozoan infections in the native turkeys could be a fruitful approach to combat pathogenic protozoan parasites.

MATERIALS AND METHODS

A total of 60 stunting diarrheic native turkey poults from the turkey-raising areas of Iran—specifically the Mazandaran and Lorestan provinces—were examined clinically. The fecal samples were obtained. The birds were sacrificed, and the necropsy procedure was carried out for each bird. The outer and internal organs were inspected for any pathological lesions. The gastrointestinal tract, including the bursa of Fabricius, was removed as a whole and a part of the organ fixed in 10% formaldehyde solution was processed in an automatic tissue processor to prepare paraffin sections; meanwhile, another part was fixed in 10% formaldehyde solution, post-fixed in 2.5% glutaraldehyde solution, and processed through the standard method for transmission electron microscopic examinations (Bozzola and Russel, 1999). The Harris hematoxylin and eosin method, May-Grunwald Giemsa method, the Kinyoun-modified Ziehl–Neelsen stain paraffin sections (Lee and Lunaht, 1968), and copper grids containing thin sections stained with uranyl acetate and lead citrate were assessed by a light microscope and a Philips 208 S transmission electron microscope, respectively. The specimens from the gut contents and fecal samples were stained and processed for protozoological examinations (Table 1).

Table 1. Clinical manifestations and pathological findings in the stunted diarrheic native turkey poults.

Protozoan diseases	Clinical signs	Gross lesion	Microscopic lesion
Cryptosporidiosis	diarrhea, growth retardation, severe emaciation, ruffled feathers	small intestinal distention, fluid, mucus and gas accumulation in cecum	villous atrophy, crypt hypertrophy, infiltration of mononuclear cells in the lamina propria
Coccidiosis	diarrhea with mucus secretion, intestinal wall edema	thickening of the wall of the small intestine with mild petechiae	glandular epithelial destruction, mild inflammatory cells infiltration
Hexamitiasis (Spironucleosis)	watery diarrhea, emaciation, growth retardation	small intestinal distention with frothy yellow water fluid	crypt dilation of jejunum and ileum, glandular epithelial hyperplasia and destruction, inflammatory cells infiltration in the lamina propria
Cochlosomiasis	growth retardation, diarrhea	small intestinal distention, intestinal wall edema	fusion of villi, villous edema, inflammatory cells infiltration in the lamina propria

RESULTS

In the present study, 47 cases out of 60 were infected with one or more protozoan organisms as follows. The total number of birds infected with *Cryptosporidium, Hexamita*, *Eimeria*, *Cochlosoma*, and *Chilomastix* in the 47 infected cases was 17 (36.2%), eight (17.0%), six (12.8%), one (2.1%), and 30 (63.8%), respectively (Table 2). In 34 cases out of 47, only a single protozoan infection was diagnosed. *Cryptosporidium spp*. was found in seven cases (14.9%, Fig. A, B), *Hexamita spp*. in five cases (10.6%, Fig. C, D), *Eimeria spp*. in a single case (2.1%, Fig. E, F), and *Chilomastix spp*. in 21 cases (44.7%, Fig. G). Simultaneous infections with two parasites included *Cryptosporidium spp*. and *Hexamita spp*. in two cases (4.3%), *Cryptosporidium spp*. and *Cochlosoma spp*. in a single case (2.1%, Fig. H, I), *Cryptosporidium spp*. and *Eimeria spp*. in two cases (4.3%), *Cryptosporidium spp*. and *Chilomastix spp*. in five cases (10.6%), *Hexamita spp*. and *Chilomastix spp*. in a single case (2.1%), and *Eimeria spp*. and *Chilomastix spp*. in three cases (6.4%) (Table 2). Also, in three cases out of 47, concurrent infection with more than two protozoan organisms were recorded: *Cryptosporidium spp*., *Eimeria spp*. and *Chilomastix spp*. in two cases (4.3%), and *Cryptosporidium spp*., *Hexamita spp*. and *Chilomastix spp*. in a single case (2.1%).

Table 2. The number and percentages of involvement of a single or multiple protozoan species in the GI tract infection in stunted diarrheic native turkey poults.

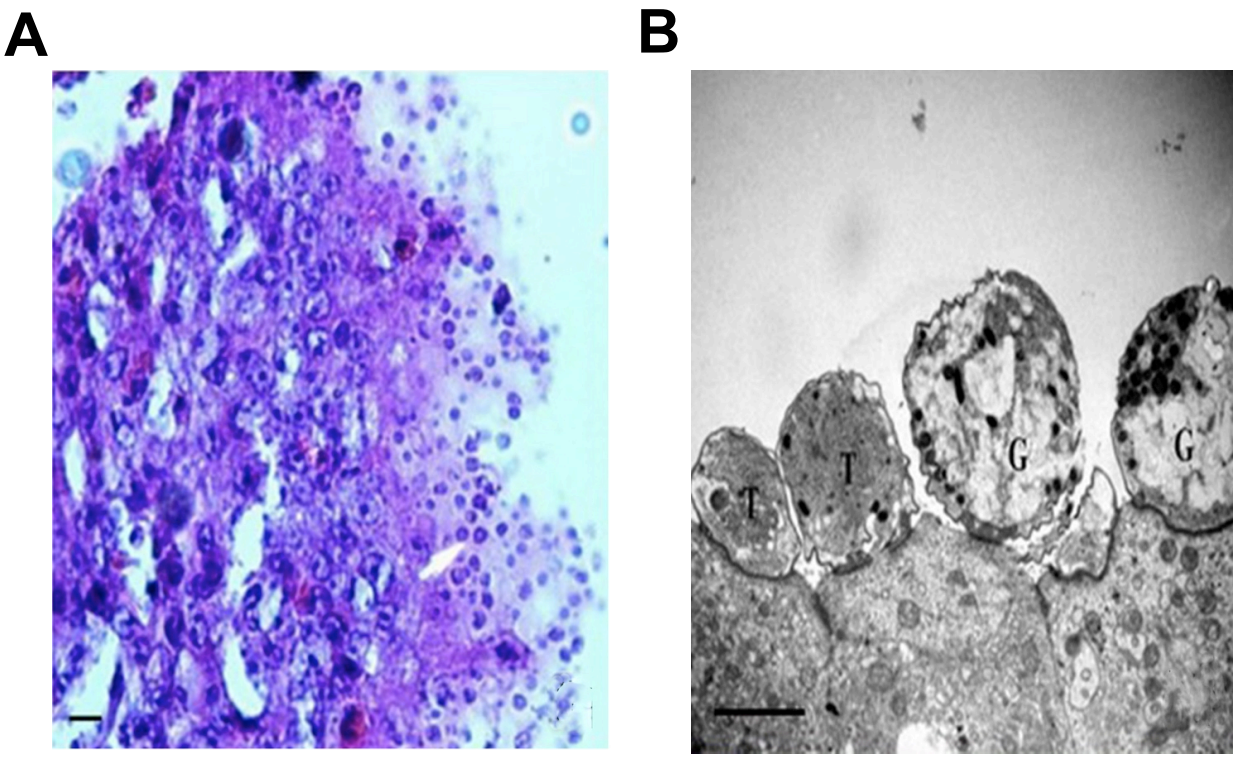
Protozoan	*Cryptisporidium spp*.	*Hexamita spp*.	*Eimeria spp*.	*Cochlosoma spp*.	*Chilomastix spp*.
Cryptosporidium spp.	7 (14.9%)	2 (4.3%)	2 (4.3%)	1 (2.1%)	5 (10.6%)
Hexamita spp.	2 (4-3%)	5 (10.6%)	-	-	1 (2.1%)
Eimeria spp.	2 (4.3%)	-	1 (2.1%)	-	3 (6.4%)
Cochlosoma spp.	1 (2.1%)	-	-	-	-
Chilomastix spp.	5 (10.6%)	1 (2.1%)	3 (6.4%)	-	21 (44.7%)
Sum	17 (36.2%)	8 (17.0%)	6 (12.8%)	1 (2.1%)	30 (63.8%)

The localizations of the parasites are presented in Table 3. Based on a microscopic examination of tissue sections stained with Harris hematoxylin and eosin method, May-Grunwald Giemsa method, or the Kinyoun-modified Ziehl–Neelsen method, *Cryptosporidium spp*. was found to be localized all over the gut epithelium, including on the duodenum, jejunum, ileum, cecum, colon, cloaca, and bursa of Fabricius. However, the organism mostly tends to be localized on the jejunal and ileal epithelium (Table 3). In all the cases infected with *Hexamita spp*., and in most of the *Eimeria*-infected cases, the organisms were localized in the jejunum and ileum (Table 3). However, in some cases with *Eimeria* infection, the organisms were found in the duodenum, cecum, and colon. Based on the morphological characteristics of oocysts, five species of *Eimeria* were identified in these stunted diarrheic turkey poults, including *E. adenoeides* (More and Brown 1951), *E. meleagridis* (Tyzzer 1929), *E. dispersa* (Tyzzer 1929), *E. innocua* (Moore and Brown 1952), and *E. meleagrimitis* (Tyzzer 1929) (Hofstad et al. 1978; Dezfoulian et al. 2010a). *Hexamita* was found within the crypts of the jejunum and ileum. *Cochlosoma* was observed in the jejunum, ilium, and cecum. *Chilomastix* was identified mostly in the cecum and in a few cases in the colon (Table 3). The clinical manifestations and macroscopic and microscopic findings in the birds infected with a single protozoan infection are presented in Table 1. Growth retardation and chronic diarrhea are associated with chronic pathological changes of the infected intestinal mucosa including crypt and villous changes, and infiltration of a sub-acute to chronic inflammatory cell in the lamina propria (Fig.E, F). In the cases of natural co-infection with two or more protozoan parasites, the assessment of clinical

manifestation and pathological changes of intestinal mucosa as compared to the single protozoan infection seem to be subjective due to the discrepancy of the data. The degree of severity of the disease related to the interaction of two or more parasites does not appear in the text or in the table. Nevertheless, it could be suggested that simultaneous infection with two or more intestinal protozoan parasites could aggravate the severity of the intestinal lesions and clinical manifestations. In addition, the May-Grunwald–Giemsa staining method of the intestinal contents in the several cases of the affected birds revealed the presence of the *Trichomonas spp*. organism (Fig. J). However, this finding has not been followed by additional studies since the parasite was not seen below the ventriculus.

Table 3. Tissue localization of protozoan parasites in 47 stunted diarrheic native turkey poults.

Protozoan	Sample number (%)	Tissue localization						
		Duodenum	Jejunum	Ileum	Cecum	Colon	Cloaca	Bursa of Fabricius
Cryptosporidium spp.	17 (36.2%)	2	14	14	3	1	1	1
Hexamita spp.	8 (17.0%)	-	3	5	-	-	-	-
Eimeria spp.	6 (12.8%)	2	5	5	1	1	-	-
Cochlosoma spp.	1 (2.1%)	-	1	1	1	-	-	-
Chilomastix spp.	30 (63.8%)	-	-	-	30	4	-	-



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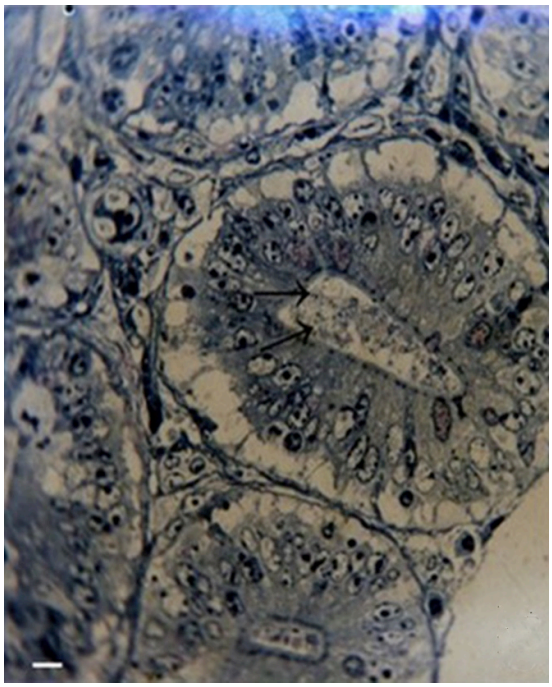

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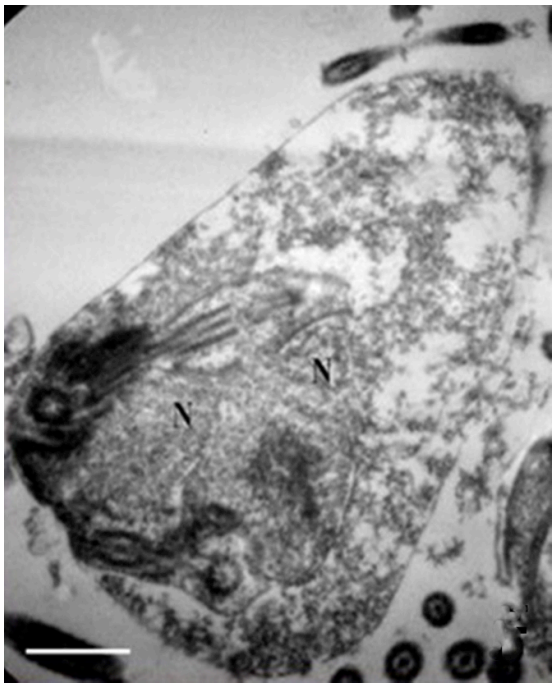

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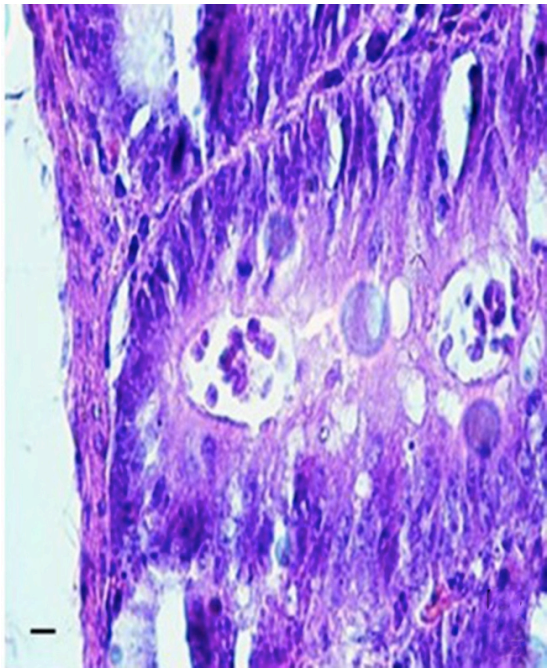

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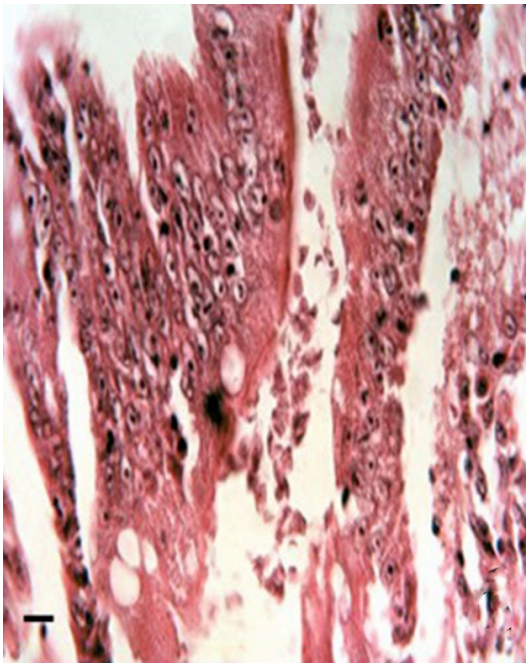

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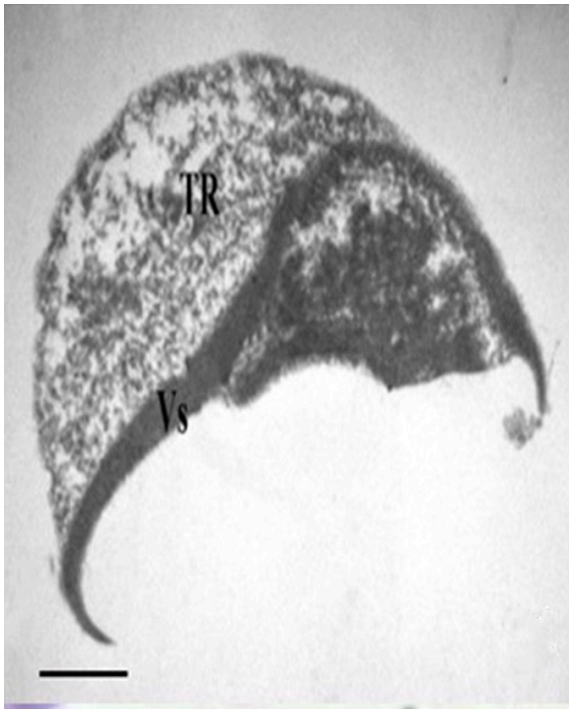

J

Fig. (A) Numerous *Cryptosporidium* organisms within parasitoferous vacuoles of intestinal villi by hematoxylin and eosin staining method. Bar, 15 μm. (B) *Crytosporidium* trophozoites (T) and gametocytes (G) by transmission electron microscopic examinations. Bar, 15 μm. (C) The *Hexamita* (*Spironucleus*) organisms are located within the lumen of glands of *Lieberkuhn* which erroneously seemed to be binucleated under toluidine blue staining method, Bar= 10 μm. (D) Flagellated *Hexamita* (*Spironucleus*) with bilobed spiral nucleus (N) by transmission electron microscopic examinations. Bar=,1.5 μm. (E) Hematoxylin and eosin stained paraffin section from *Eimeria*-infected turkey poults. Deformity of the villi, large number of lymphoid cells within the lamina propria and *Eimeria* protozoan are seen in the intestinal mucosa. Bar, 0.3 mm. (F) A closer view of fig. (E). Bar, 30 μm. (G) *Chilomastix* in the lumen of *Lieberkuhn* glands of cecum by Giemsa staining method. Bar, 12 μm. (H) *Cochlosoma* in the intervilli spaces by hematoxylin and eosin staining method. Bar, 12 μm. (I) Trophozoite (TR) and Ventral Sucker (Vs) of *Cochlosoma* by transmission electron microscopic examinations. Bar, 2 μm. (J) Structure like axostyle (Ax) and undulating membrane (Um) of *Trichomonas* by Geimsa staining method. Bar, 8 μm.

DISCUSSION

Diarrhea, emaciation, and growth retardation are the most common clinical manifestations of the birds in our study (Table 1). As shown in Table 2, the frequency of the pathogenic protozoa in the poults from higher to lower is as follows: *Cryptosporidium* > *Hexamita* > *Eimeria* > *Cochlosoma*. It seems that the high frequency of *Cryptosporidia* infections in these poults could result from the organism's resistance to therapies and disinfectants.

Two species of pathogenic *Cryptosporidium* characterized in turkeys—namely *C. baileyi* and *C. meleagridis*—are important protozoa that infect turkeys (Dubey et al., 1990; Swayne et al. 2013). Although molecular genetics is the best choice for differentiating among the species, morphological characteristics like oocyst structure, lower severity of disease caused by C. *baileyi*, and location of the developmental stages are helpful in differentiation of the parasite from *C. meleragridis*. Cryptosporidiosis in birds usually manifests as intestinal, respiratory, and renal diseases. Many other organisms like *Eimeria* (which cause a sub-acute form of coccidiosis), *Hexamita*, and *Cochlosoma* as potential pathogens manifest similar clinical signs with intestinal cryptosporidiosis including non-bloody diarrhea and intestinal distention due to the accumulation of gas and mucoid materials. Therefore, the best method of examination for differential diagnosis between these intestinal protozoans is microscopic and molecular procedures. *Cryptosporidium* developmental stages could be detected in several anatomical sites of the gastrointestinal tract. *C. meleagridis* is usually confined in the small intestine, whereas *C. baileyi* is usually found in the cloaca and bursa of Fabricius (Swayne et al. 2013). Among intestinal protozoans of turkey poults, *C. meleagridis* appeared to be of a particular significance to public health (Fayer 1997; Akiyoshi et al. 2003; Joachim 2004). The oocysts contaminate the water and survive over a long period, which could be infectious for human beings. The most infective zoonotic form is *C. meleagridis* as a potentiated pathogen for humans and mice, particularly for immunodeficient or immunocompromised subjects. As mentioned in the results, based on the morphological characteristics of oocysts, five species of *Eimeria* have been identified in these stunted diarrheic turkey poults, including *E. adenoeides, E. meleagridis*, *E. dispersa*, *E. innocua*, and *E. meleagrimitis* (Dezfoulian et al. 2010a). *E. adenoeides* and *E. meleleagrimitis* are considered the most pathogenic, *E. dispersa* is mildly pathogenic, and *E. innocua* and *E. meleagridis* are seen to be non-pathogenic in turkeys (Swayne et al. 2013). Overall, the clinical disease related to *Eimeria spp*. in turkeys due to low-intensity infections might be complicated, especially when the bird is co-infected with other parasites. It is well established that *Cochlosoma* alone is responsible for clinical disease manifestations in turkey poults; however, when it is accompanied by other intestinal pathogens like *Hexamita*, they share their pathogenesis in different directions,

thus leading to more intensive intestinal injuries and therefore the clinical signs seem to become more complicated (Cooper et al. 1995; Lindsay 1999; Ghragozlou and Dezfoulian 2009; Bermudez 2013). As seen in Table 1, the clinical manifestations in the birds with a single protozoan infection have characteristics in common, such as diarrhea and growth retardation resulting from the pathological changes of the infected intestinal tissues. As species of the protozoa concerned, two species of *Cryptosporidium, C. meleagridis* and *C. baileyi* (bursa of Fabricius infection), a single species of *Hexamita* or *Spironucleus* (*H. meleagridis* or *S. meleagrids*), a single species of *Cochlosoma* (*C. anatis*), and several species of *Eimeria* as aforementioned, a single species of *Chilomastix* (*Chilomastix gallinarum*), and a single species of *Trichomonas* (*T. gallinae*) seem to be the protozoan parasite species associated with diarrhea and stunting or stunting in the native Turkey poults in Iran. It could be emphasized that co-infection with other microorganisms such as enteropathogenic viruses and bacteria may participate in these protozoan infections. However, it was not considered in the present study. For the best knowledge of the authors, this is the first paper related to protozoan infections of native turkey poults in Iran. Due to the importance of protozoan infections in the native turkeys, treatment, culling, and hygienic measures should be taken into consideration. There are no effective drug therapies or control measures described for the cryptosporidial infections since the organism resists antibiotics and antiprotozoal drugs or conventional disinfectants (MacDougald, 2013). A list of anticoccidial drugs is now available. It could be effective in the prevention and treatment of coccidiosis (McDougald and Fitz-Coy, 2013). To treat *Spironucleosis* infection, butynurate and chlortetracycline in the drinking water are recommended (Hess and McDougald 2013). Metronidazole for *Cochlosoma* (Bermudez, 2013) and dimetridazole for *Trichomonas* infections could be effective (McDougald and Fitz-Coy, 2013).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUBMISSION DECLARATION AND VERIFICATION

The authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

REFERENCES

Akiyoshi DE, Dilo J, Pearson C, Chapman S, Tumwine J, Tizipori S. 2003. Characterization of *Cryptosporidium meleagridis* of human origin passaged through different host species. Infect Immun. 71: 1828-1832.

Barnes HJ, Guy JS. 1995. Spiking mortality of turkeys (SMT) and related disorders-an update. pp.16-21. In: Proceeding 19th Annual North Carolina Turkey Industry Days Conference. Raleigh, NC.

Barnes HJ, Guy JS, Brown TP, Edens FW. 1996. Poult enteritis mortality syndrome ("spiking mortality of turkeys") and related disorders-an update. pp.1-8. In NCSU Quarterly Update to Poultry PEMS Task Force. April. North Carolina State University, Raleigh, NC.

Bermudez AJ. 2013. Miscellaneous and Sporadic Protozoal Infections. pp.1182. In Diseases of Poultry. Swanye DE, Glisson J, Mc Dougald LR, Nolan LK, Suarez DL, NairV. 13th Ed, Willy Blackwell. Hoboken, NJ, USA.

Bozzola JJ, Russel LD. 1999 .Electron Microscopy. 2 nd ed., Jones and Barllett Publishers, Boston, USA, pp: 17-147.

Cooper GL, Shivaprasad HL, Bickford AA, Nordhausen R, Munn RJ, Jeffrey JS. 1995. Enteritis in Turkeys Associated with an Unusual Flagellated Protozoan (*Cochlosoma anatis*). Avian Dis. 39: 183-190.

Dezfoulian O, Gharagozlou MJ, Rahbari S, Samani R. 2010. Coccidiosis due to various species of *Eimeria* in the stunted and diarrheic native turkey poults: Pathology and morphological characterization of oocysts. Archives of Razi Institute .65: 15-19.

Dezfoulian O, Gharagozlou MJ, Rahbari S. 2010. *Hexamita* infection associated with diarrhea and stunting in native turkey poults .Tropical Biomedicine. 27: 504-508.

Dubey JP, Speer CA, Fayer R. 1990. Cryptosporidiosis of Man and Animals. CRC Press, Boca Raton, FL, USA.

Fayer R. 1997. "Cryptosporidium and Cryptosporidiosis". CRC Press, Boca Raton, FL.

Friedhof KT, Kuhnigk C, Muller I. 1991. Experimental infections in chickens with *Chilomastix gallinarum*, *Teteratrichomonas gallinarum*, *and Tritrichomonas eberthi*. Parasitol Res. 77: 329-334.

Gharagozlou MJ, Dezfoulian O, Rahbari S, Bokaie S, Jahanzad I, Razavi ANE. 2006. Intestinal Cryptosoridiosis in Turkey in Iran. J Vet Med A. 53: 282-285.

Gharagozlou MJ, Dezfoulian O. 2009. *Cochlosoma* Infection in a Turkeys in Iran. Korean J Parasitol. 47: 393-395.

Guy JS, Levy MG, Ley DH, Barnes HJ, Gerig TM. 1987. Experimental reproduction of enteritis in bob white quail with *cryptosporidium* and *reovirus*. Avian Dis. 31: 713-722.

Guy JS, Levy MG, Ley DH, Barnes HJ, Gerig TM. 1988. Interaction of *reovirus* and *Cryptosporidium baileyi* in experimentally infected chickens. Avian Dis. 32: 381-390.

Hess M, Mc Dougald LR. 2013. Histomoniasis (Black head) and Other Protozoan Diseases of Intestinal Tract. pp.1167-1182. In: Diseases of Poultry. Swanye DE, Glisson JR, Mc Dougald LR, Nolan LK, Suarez DL, Nair V. 13th ed . Willy Blackwell. Hoboken, NJ, USA.

Hofstad MS, Calnek WB, Hemboldt CF, Yoder Jr HW. 1978. pp.805-812, 841-846. In: Diseases of Poultry. Iowa State University Press, Ames, IA, USA.

Joachim A. 2004. Human cryptosporidiosis: an update with special emphasis on the situation in Europe. Infect. Dis. Vet. Public Health. 51: 251-295.

Lindsay DS, Larsen CT, Zajac AM, Pierson FW. 1999. Experimental *Cochlosoma anatis* infections in Poultry. Vet Parasitol. 81: 21-27.

Lee G, Luna HT. 1968. Manual of HISTOLOGIC STAINING METHPODS of the Armed Forces Institute of Pathology, 3rd ed. The Blakiston Division McGRAW-HILL Book Company, NY, USA.

McDougald LR, Fitz-Coy SH. 2013. Coccidiosis. pp.1159, 1163-1165. In: Diseases of Poultry. Swanye DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair V. 13th ed., Willy Blackwell. Hoboken, NJ, USA.

McDougald LR. 2013. Cryptosporidiosis. pp.1170. In: Diseases of Poultry. Swanye DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair V. 13th ed., Willy Blackwell. Hoboken, NJ, USA.

Qureshi MA, Edens FW, Hawenstein G. 1997. Immune System Dysfunction During Exposure to Poult Enteritis and Mortality Syndrome Agents. Poul Sci. 76: 564-569.

Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair V. 2013. pp:1195-1201. In: Diseases of Poultry. 13th ed. Willy Blackwell. Hoboken, NJ, USA.