



Coccidiosis in Sistan: the prevalence of *Eimeria* species in native chicken and its histopathological changes

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Received: 19 April 2018 / Accepted: 6 June 2018 / Published online: 22 June 2018
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Abstract

Coccidiosis is an important disease in the poultry industry caused by species of protozoan parasites belonging to the genus *Eimeria* (phylum Apicomplexa); it results in a great economic loss all over the world. The aim of this study was to determine the frequency and diversity of *Eimeria* species, the effect of season on the prevalence of coccidiosis in poultry, and the evaluation of its histopathological changes in native infected chickens in different parts of Sistan. A total of 2792 samples selected through random cluster sampling of chickens from areas of Sistan (Zabol, Hirmand, Nimrooz, and Zehak) and their infected organs especially intestines were examined for histopathological studies. Out of the 2792 samples processed, 585 samples were found to be positive for coccidiosis with a prevalence of 20.95%. Coccidiosis was initially diagnosed on the basis of fecal examination and confirmed by the presence of sporulated oocysts and pathomorphological alterations in the intestines. Five *Eimeria* species identified by morphometry were *Eimeria acervulina*, *E. maxima*, *E. brunetti*, *E. necatrix*, and *E. tenella*. Based on the results of the experiments, the percentage of coccidiosis infection in cold seasons (October–March) was higher than in the warm seasons (April–September). Histopathological lesions revealed loss of epithelial tissue, congestion of blood vessels which indicate disruption followed by hemorrhage, severe muscular edema, and necrosis of the submucosa of the intestine and cecum. There was loss of intestinal villi, disruption of cecal mucosa, and clusters of oocysts seen. Also, there was massive infiltration by a large number of inflammatory cells including eosinophil, lymphocyte, and plasma cells. Several merozoites, schizonts, microgametes, and macrogametes were found in the epithelial cells.

Keywords Coccidiosis · *Eimeria* · Native poultry · Sistan · Histopathological changes

Introduction

Coccidiosis is one of the most common and important diseases in the poultry industry caused by species of protozoan parasites belonging to the genus *Eimeria* (phylum Apicomplexa) (Gharekhani et al. 2014; Nematollahi et al. 2009). Coccidiosis after *salmonellosis* is the second important disease of poultry and can lead to enormous economic losses both in the traditional and industrial poultry production. Modern and industrial poultry-breeding programs have been

shown to lead to much more production efficiency and increased poultry density than native and traditional methods; however, application of various prevention and treatment programs in broiler and layer poultry farms cause diseases with subclinical symptoms and drug resistance, and they failed to completely eradicate coccidiosis (Chapman 2009 and Sureshkumar et al. 2004). Although there are many factors that include improper reproductive management, inadequate nutrition and storage place, no consumption of dietary supplements and no application of prevention programs and timely treatments against parasitic diseases in traditional poultry farming which may cause the extensive spread of disease at the farm and, consequently, significant reduction in the poultry production (Eslami et al. 2009).

Coccidian infection can affect all species and races of poultry at different ages, but like many other parasitic diseases, the clinical signs of the disease occur most in young birds (3 to 6 weeks) (Gharekhani et al. 2014). Severity of coccidiosis in young birds depends on the number of ingested sporulated

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oocytes, parasite species, the immunity level, nutritional conditions of the host, and presence of other infectious diseases. In addition, the place of parasite establishment and the severity of injuries, shape and size, evolution of parasite inside the body, symptoms of the disease, and mortality rate are not the same in different species which are unique in their types (Yakhchali and Fakhri 2018). Among the nine *species* of coccidian affecting the domesticated poultry including *Eimeria maxima*, *E. brunetti*, *E. necatrix* and *E. tenella*, *E. acervulina*, *E. praecox*, *E. hagani*, and *E. mitis*, there are seven species that are clinically significant, so that *E. praecox*, *E. mitis*, and *E. mivati* may cause mild or moderate morbidity, whereas *E. tenella*, *E. necatrix*, and *E. maxima* may cause severe morbidity (Jadhav et al. 2011). Coccidiosis transfer between the hosts is done through the oocyst shedding.

Parasite multiplies in the intestine and causes tissue irritations and bleeding in the gastrointestinal tract. On the other hand, due to the proliferation of the protozoon in the tissues of the digestive system, intake of nutrients and their absorption will face difficulty. With the expansion of injuries, other infections such as intestinal inflammation caused by bacteria may occur. Ultimately, weight loss occurs due to dehydration, anemia, and a decrease in immunity against other infections causing irreparable damage to the poultry.

There have been numerous studies on the prevalence of coccidiosis among the native and industrial poultry in different parts of Iran and worldwide, but there have not been any recent studies regarding the prevalence of coccidiosis among poultry and diversity of *Eimeria* species in four areas of Sistan, region in the northeast of Iran. This study was aimed to determine the prevalence of infection and *Eimeria* species in native poultry, the effect of season factor in the prevalence of coccidiosis, and the histopathological changes in the tissues in order to differentiate *Eimeria* species and coccidiosis from other intestinal diarrhea diseases in the poultry.

Materials and methods

Sampling and microscopic examination

In order to determine the prevalence and intensity of infection of different *Eimeria* species, sampling was conducted from regions in Sistan including Zabol, Hirmand, Nimrooz, and Zahak from October 2015 to October 2016. A total of 2792 fecal samples were collected from indigenous chickens at the age of 4 weeks and 2.5 years using randomized cluster sampling. The collected data included age, sex, hygiene conditions, type of bedding, and history of diarrhea and appearance of the studied poultry. The studied poultry received no vaccination against coccidiosis, and the nesting or site conditions were inappropriate (these poultry was mainly kept in wooden boxes covered with sack or in the nests built from low-value

materials or in some rural homes in stables with other animals). Some cardboard cartons were placed in different parts of the bed to collect the sample. To prepare *Eimeria* oocysts, fresh fecal samples were collected from the bed of the birds and then they were transferred to the laboratory of parasitology in the faculty of veterinary at the University of Zabol for examination.

The presence of fecal oocysts was determined by using Clayton-Lane flotation method (Eslami and Ranjbar-Bahadori 2005 and Hendrix 1998). For oocyst sporulation, 10 to 20 g of fecal samples infected with *Eimeria* oocysts were mixed with 60 ml of potassium dichromate 2.5% and placed within a petri dish. To prevent the growth of fungus, the glass was washed with 1% sodium bicarbonate already. The container was kept in autoclave at 25–27 °C for 5–10 days until oocyst sporulation was completed. The lid of the container should be removed daily for aeration.

Then, the contents were centrifuged by sedimentation, and the sediment containing oocysts was tested, using flotation to view sporulated oocysts (Nematollahi et al. 2009 and Meireles et al. 2004). *Eimeria* species was determined based on morphological indices of oocyst and sporocysts (shape, color, wall structure, micropyle, presence or absence of remaining oocyst and sporocysts, and static body), and micrometer characteristics were evaluated using an eyepiece scale calibrated by means of a stage micrometer (oocyst shape, length, and width) (Soulsby 1986). The results were analyzed using SPSS software with a significance level of $p < 0.05$.

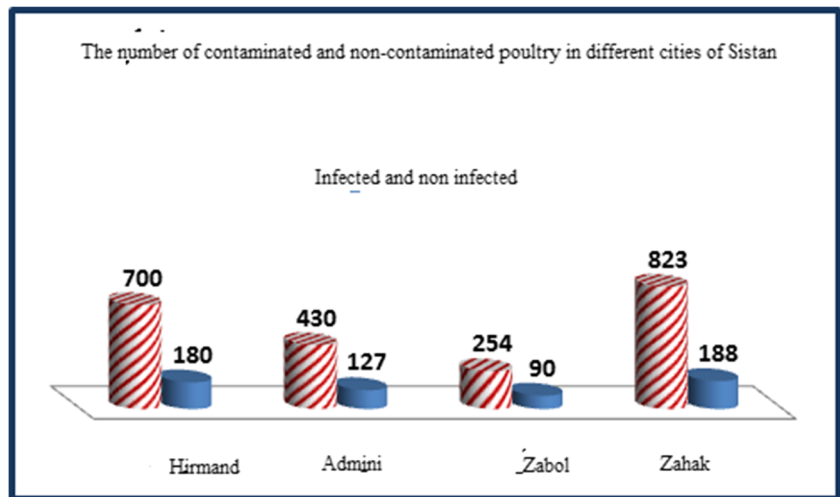
Autopsy and histopathological tests

For histopathological tests, chickens with symptoms of the disease were autopsied. During autopsy, suspected infection organs were prepared from different regions of the intestine of tissue sample after a visual examination. To detect pathological lesions, the samples were placed in 10% buffered formalin and then microscopic sections were prepared with a diameter of 5 to 6 μm using conventional immersion paraffin method, and the prepared slides were stained using Hematoxylin and Eosin technique. The tissue structure of the stained sections from different areas of the intestine was examined by light microscopy, and micrographs were taken from different stages of evolution of the parasite (Luna 1968 and Sharma et al. 2015).

Results

According to the results, out of the total 2792 examined fecal sample collected from native chickens from different parts of Sistan (Zabol, Hirmand, Nimruz, and Zahak), 585 (20.95%) were positive for *Eimeria* oocysts of domestic poultry (Fig. 1).

Fig. 1 Mean and percentage of infection in different regions of Sistan



In addition, the average rate of chickens infected with *Eimeria* oocysts per gram (OPG) of fecal sample was obtained in all regions of Sistan (19.99%), and the maximum and minimum rates of OPG were belonged to *E. acervulina* and *E. tenella* species, respectively (Table 1).

As the result shown in Table 1, *E. acervulina* with 37 oocysts per gram of fecal sample (35.23%) had highest levels of infection and *E. tenella* with five oocysts per gram of fecal sample (4.76%) had the lowest level of infection. The infection rate of *E. maxima* (25.71%) and *E. brunetti* (19.04%) were ranked second and third after *E. acervulina*.

Prevalence of *Eimeria* infection in different parts of Sistan In Adimi area, 557 fecal samples were collected from domestic poultry in different seasons. According to the results, the highest and the lowest rate of infection were related to *E. acervulina* with 10 oocysts per gram of sample (37.04%) and *E. tenella* with two oocysts per gram of sample (7.41%), respectively.

E. maxima and *E. brunette* were 22.22% with six oocysts and *E. necatrix* was 11.11% with three oocysts per gram of sample with the highest infection rate, respectively.

In Zahak, a total of 1011 stool samples were collected from the domestic poultry in different seasons. According to the results, the highest and the lowest rates of infection were related to *E. acervulina* (37.41%) and *E. tenella* (3.44%), respectively.

In Zabol, a total of 344 fecal samples were collected from the domestic poultry in different seasons.

According to the results, the highest and the lowest rate of infection were related to *E. acervulina* (33.33%) with seven oocysts per gram of sample and *E. brunetti* and *E. necatrix* (19.04%) with four oocysts per gram of sample, respectively.

In addition, in Hirmand, 880 fecal samples were collected from the domestic poultry in different seasons. According to the results, the highest and the lowest rates of infection were belonged to *E. acervulina* (28.57%) and *E. tenella* (7.14%), respectively (Table 2).

The mean and percentage of *Eimeria* infection in different seasons of the year in different areas of Sistan using SPSS software

Sistan and Baluchestan Province is located in the southeast part of Iran, with an area of 131,935 km² and is the second largest province in Iran. Sistan region with an area of 15,197 km² is situated in the north-east of the province and is bordered with Afghanistan country. The climatic condition in Sistan is hot and dry, and there are only really two seasons in the city: dry and wet. The average annual temperature in Zabol city is 22.2 and 22.9 in Zahak city. Also, the average annual rainfall in Zabol, Zahak, and Hirmand cities is 59,46.1,44 mm, and humidity has been reported 39 and 33% in Zabol and Zahak cities, respectively. The present study was carried out in two cold and warm periods (October–March) and (April–September) in different parts of Sistan. According to the results of the experiments, from 2792 native chickens

Table 1 The total number of *Eimeria* oocysts per gram (OPG) of poultry feces

<i>Eimeria</i> species	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. brunetti</i>	<i>E. necatrix</i>	<i>E. tenella</i>
Mean OPG	37	27	20	16	5
Percent	35.32	25.71	19.04	15.23	4.76

Table 2 Frequency and percentage of infection of different species of *Eimeria* in different areas of Sistan

Variables	<i>E. acervulina</i>	<i>E. maxima</i>	<i>E. brunetti</i>	<i>E. necatrix</i>	<i>E. tenella</i>
Adimi	10 (37.04%)	6 (22.22%)	6 (22.22%)	3 (11.11%)	2 (7.41%)
Zahak	12 (41.38%)	7 (24.13%)	4 (13.8%)	5 (17.24%)	1 (3.45%)
Zabol	7 (33.33%)	6 (28.57%)	4 (19.05%)	4 (19.05%)	0 (0%)
Hirmand	8 (28.57%)	8 (28.57%)	5 (17.86%)	5 (17.86%)	2 (7.14%)

sampled in different cities of Sistan, the percentage of infection in the hot and cold seasons was reported to be 18.5 and 22.4%, respectively (Table 3).

As can be seen in Table 4, from a total of 557 native chickens sampled and tested in different seasons of the year in Adimi, the infection percentage in the warm season was found to be 15% and 29% in the cold season. In addition, from a total of 1011 indigenous chickens sampled and tested in different seasons of Zahak city, 16.9% were infected to *Eimeria* in warm season and 19.8% in cold season.

Moreover, from a total of 344 indigenous chickens sampled and tested in different seasons of Zabol, 33.3% were infected with *Eimeria* in warm season and 21.3% in cold season.

In Hirmand, from a total of 880 studied indigenous chickens, the infection percentage in the warm season was 18.5% and it was 21.3% in the cold season. According to the results, there is a significant relationship between seasonal variables and infection with a 95% confidence level.

Histopathological results

For the histopathological results, different sections were taken from submucosa of intestine and caecum of infected poultry and were stained with H&E. In tissues infected with coccidiosis, there were focal lesions with irregular margins in white color in the duodenum which indicated disruption and loss followed by necrosis of submucosa of intestine and caecum under the mucosal epithelium and gastric gland as well as massive infiltration by a large number of inflammatory cells including eosinophil, lymphocyte, and plasma cells. There was also infiltration of inflammatory cells between the muscle layer of the intestinal necrosis and disruption of the intestinal tracts, intestinal wall dilatation, and severe hemorrhage in the affected tracts. In addition, the intestinal thickness was

Table 3 Prevalence of *Eimeria* infection in different seasons of the year in different areas of Sistan

Variables	Infection					
	Infected		Non-infected		Total	
	Number	Percent	Number	Percent	Number	Percent
Warm season	192	18.5	845	81.5	1037	1100
Cold season	393	22.4	13.62	0.776	1755	100

significantly reduced in the affected tracts. The tissue sections of the intestine showed the parasite's evolutionary in the mucosa and submucosa, between disrupted and necrotic tissues, and no hemorrhagic and proliferative lesions were observed (Fig. 1).

In the histopathological study, oocysts and *macrogametes* were found in the epithelial cells of the intestinal mucous gland in Fig. 2a.

There was also schizont (development stages of coccidiosis) in the intestinal *villi necrosis* and disruption of enterocytes and infiltration of various cells in the intestine (Fig. 2b).

In comparison with healthy necrosis of the intestine and disruption of the mucosa and submucosa of the intestine, isolation of enterocytes with intestinal villi and infiltration of inflammatory cells between the muscle layer and some fibrous proliferation were seen in the intestinal coccidiosis (Fig. 2c, d).

In epithelial cells of the glands under the mucosal infected cecum, isolated oocyst inside the duct followed by inflammatory and fibrotic cells were also found.

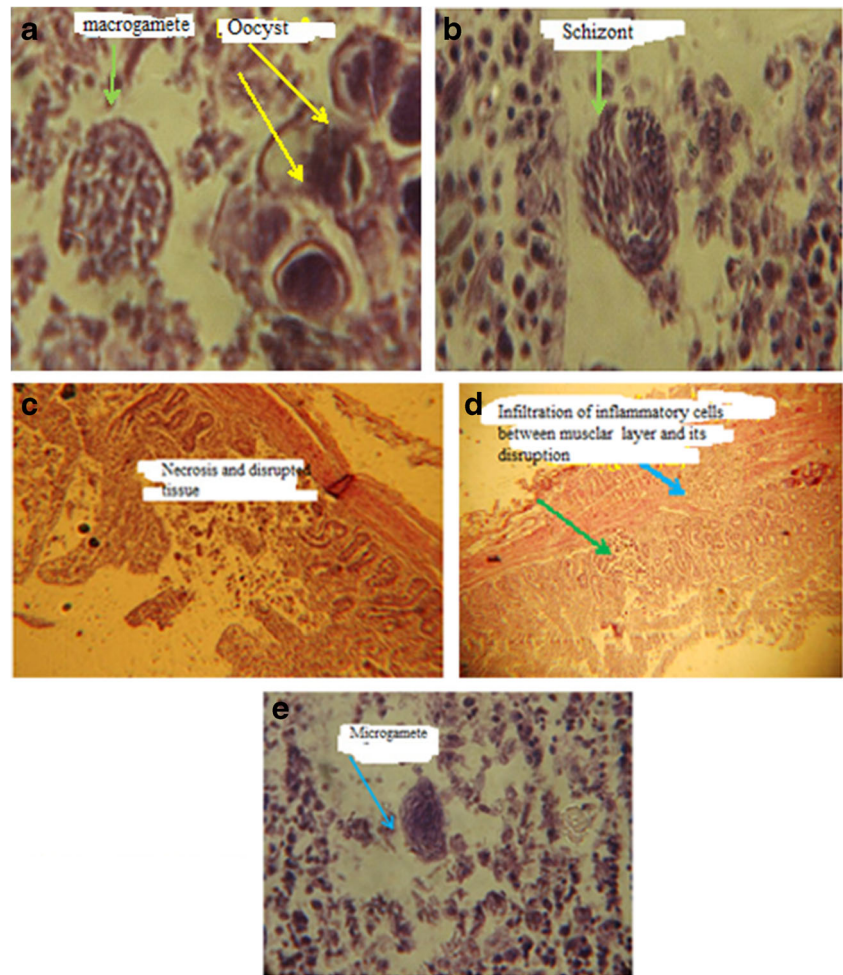
Discussion

Due to low income and economic poverty to provide the family's dietary protein in the Sistan region and to help the family economy through the sale of domestic poultry meat, native chicken farming is performed in every rural home; therefore, it is necessary and vital to investigate the prevalence of coccidiosis for controlling parasitic diseases in this area. Indigenous poultry breeding is usually defined as a traditional breeding system and practice with low production and returns where poultry is usually kept in closed stables and densely

Table 4 Prevalence and percentage of *Eimeria* infection in warm and cold seasons in different areas of Sistan

Variables	Warm season		Cold season		Significance level <i>p</i>
	Infected	Total	Infected	Total	
Adimi	37 (10%)	247	90 (29%)	310	0.0001 (0.05)
Zahak	70 (16.9%)	415	118 (19.80)	596	0.045
Zabol	55 (23%)	239	35 (33.3%)	105	0.05
Hirmand	50 (18.5%)	270	130 (21.30)	610	0.05

Fig. 2 **a** Oocyte and macrogamete, **b** evolutionary development of coccidiosis in necrosis tissues and disrupted regions, **c** necrosis and tissue disruption, **d** necrosis and degradation of the mucosa and intestinal mucus, **e** microgamete



packed with other domestic animals such as cattle, sheep, and goats for many years, and the food they need can also be supplied from the surroundings. There are numerous factors including keeping large number of livestock and poultry in a small and compact place in non-sanitary nourishment which contributes to the proliferation and transmission of live sporulated oocysts through water, feed, bedding, and even insects and beetles in the bed. Constant oocyst repulsion through infected poultry feces can lead to the persistence of contamination, mortality of the infected, and decline in growth and productivity. In addition, coccidiosis is of great importance in large and dense poultry practices and has been suggested as an important indicator of growth in the poultry industry (Rahbarie and Adib Hesami 1995). Since the infection of local poultry of Sistan with *Eimeria* species had never been studied in different regions of Sistan (Adimi, Zabol, Zahak, and Hirmand), the present study aimed to clarify the status of coccidiosis and determine the diversity of *Eimeria* species in local poultry of Sistan region. During this study, 2792 fecal samples were collected from domestic poultry in four cities of Sistan and Baluchestan Province in different seasons.

According to the results, out of 2792 samples processed, 585 samples were found to be positive for *Eimeria* oocyst with a prevalence of 20.95%. Regarding the *Eimeria* species of native poultry in Sistan region, there were four species of *Eimeria acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella* in the region. *E. acervulina* (35.23%) and *E. tenella* (4.76) species had the highest and lowest rates of infection in all regions of Sistan. The prevalence of *E. necatrix* and *E. brunetti* species in the cities of Zabol (Central Sistan) and Adimi (Northern Sistan) were ranked first and second after *E. acervulina* and *E. maxima*. In Hirmand, the prevalence of *E. brunetti* and *E. necatrix* were ranked the third and fourth, while, in Zahak (southern Sistan), the highest rate of infection was related to *E. necatrix* after *E. acervulina* and *E. maxima*. Many studies have been conducted on coccidiosis in Iran. In a study on poultry farms of Tabriz in 2009, five species of *Eimeria* including *E. tenella*, *E. maxima*, *E. acervulina*, *E. necatrix*, and *E. mitis* have been reported and the prevalence of poultry infection to *Eimeria* species were reported to be 55.96% (Nematollahi et al. 2009). In another study conducted in 2017 in Western Azerbaijan, the prevalence of infection to

Eimeria species was reported 21.53% and was found in *E. acervulina*, *E. maxima*, *E. tenella*, and *E. necatrix* respect of frequency (Yakhchali and Fakhri 2018).

In a study on broiler chicken in Hamedan in 2014, the prevalence of infection with oocyte coccidia was reported 75% and *E. tenella*, *Eimeria maxima*, *Eimeria acervulina*, and *Eimeria necatrix* species were also found in terms of frequency (Mehrabi and Yakhchali 2015). Charkhkar et al. carried out a study in order to identify *Eimeria* species in poultry based on morphological characteristics. In their research, 17 samples from five climatic zones were tested and a total of 25 *Eimeria* oocytes were identified. The highest frequencies were related to four species including *E. tenella*, *E. maxima*, *E. acervulina*, and *E. necatrix*.

Razmi and colleagues in Mashhad also demonstrated the highest rate of coccidiosis was observed in poultry over 6 weeks of age, so that 97% of the poultry were contaminated with *E. acervulina*, 41% to *E. maxima*, and 12% to *E. tenella* (Razmi and Kalideri 2000).

In poultry farms of Tabriz, five species of *Eimeria* including *E. tenella*, *E. maxima*, *E. acervulina*, *E. necatrix*, and *E. mitis* have been reported (Davari and Nematollahi 2004).

In Ethiopia, a study was conducted in the case of coccidiosis in broiler chickens from November 2009 to April 2010 and four species of *Eimeria* including *Eimeria tenella*, *Eimeria acervulina*, *Eimeria necatrix*, and *Eimeria brunetti* were identified. Among these species, *Eimeria brunetti* (34.3%) and *Eimeria tenella* (5%) showed the highest and the lowest prevalence, respectively (Dinka and Tolossa 2012).

In another study conducted by Muazu et al 2008. in Niger, among the 100 collected carcasses, 30 cases were infected with coccidiosis [10]. The highest prevalence was related to *Eimeria tenella* (10%) and then *Eimeria maxima*, *Eimeria acervulina*, and *Eimeria necatrix* ranked second to fourth with percentage of 9, 6, and 5, respectively.

Theorists believe that the development of any society is based on the native capital of the same region, as well as the climate and weather conditions of a region, that can be effective in parasitic outbreaks. Coccidian infection occurs in all ages but its clinical protests are restricted to young birds.

Severity of coccidiosis in young birds depends on the number of ingested sporulated oocytes, parasite species, the immunity level, nutritional conditions of the host, and presence of other infectious diseases (Jahantigh and Salehi 2012). Although *Eimeria* infection in poultry has been reported from different parts of the world and Iran, its prevalence and species diversity are associated a host of reasons including type of bed composition, bed height, age of poultry, cleanliness and washing of drinking appliances, density of poultry per unit area, and the use of preventive and vaccination methods.

Pathologic changes in coccidiosis are variable, depending on the host, parasite species, and severity of infection. Infected intestine can be dilated such as balloon and discolored. Also,

changes on the mucosal surface of the intestine may be varying from dry crusts, formation of white caseous cores until filled with mucus, and fulminate hemorrhage into the intestine.

Our histopathological results in poultry infected with coccidiosis revealed necrosis and degradation of the tissue, mucosa, submucosa of intestine, their glands and caecum, and the muscle layer of the intestine caused thickening of the intestinal wall in the affected areas. In addition, the different stages of parasite evolution as well as infiltration of inflammation cells appeared in the damaged tracts along with reduction of intestinal wall thickness and, consequently, disruption and necrosis of intestinal tracts.

Rasheda and Bano (1985) also showed in their histopathological examination of poultry that the accumulation of oocysts in the intestinal tissue contributes to the disruption of the epithelial tissue, congestion of blood vessels with signs of hemorrhage, severe muscular edema and necrosis of submucosa of intestine and loss of intestinal villi followed by severe mononuclear cell penetration. The results of our findings regarding the presence of schizont, microgamete in epithelial cells, multiple changes in the colon and cecum, submucosal degeneration, and severe hemorrhage are consistent with the findings of studies by Soomro et al. 2001, Sood et al. 2009 and Sharma et al. 2015.

Our findings suggest that the prevalence of coccidiosis is more common in cold seasons (October–March) than warm ones (April–September) in Sistan. This difference in the prevalence of infection can be due to two factors of ambient temperature and humidity of the animal bed. Since *Eimeria* species undergo both sexual (gametogony) and asexual (schizogony) reproductions to produce oocysts within the intestine tract of animals and they are expelled through the feces, as a result, in cold seasons, which livestock and poultry can be kept longer in the closed environment and at their resting place and furthermore, the effect of the temperature and an increase in the humidity of the bed provide conditions for the growth of *oocyst sporulation*; it is likely that the poultry can be infected by coccidiosis and other parasitic diseases (McDougald and Reid 1997; Razmi and Kalideri 2000).

Additionally, Matter and Oester (1989) found in their study that high *oocyst sporulation* play an important role in epidemiology and the spread of disease among poultry. The results of our findings are also consistent with the results of the studies by Khan et al. (2006) and Graat et al. (1996), on the effect of seasons on the prevalence of coccidiosis. They found that the prevalence of coccidiosis occurs mostly in autumn compared with other seasons.

Ahad et al. (2015) also reported that the prevalence of coccidiosis is frequently observed in autumn rather than in summer, spring, and winter seasons, respectively. Since Sistan is a region where all four seasons do not occur and our research results were divided into cold and hot seasons,

the results of our study can partly contribute to and be effective in controlling the disease based on the abovementioned descriptions.

The results of this study on the prevalence of coccidiosis infection in native chickens of Sistan revealed the ratio of poultry affected with the disease in this area, which lacked clinical signs and histopathological changes became evident. Our findings indicated that seasonal contamination could greatly help determine the best time to prevent coccidiosis in the region during the year as well as the best way of keeping and rearing conditions of poultry, so that the severity of the infection in the poultry can be reduced. It should be noted that due to the presence of pathogenic species in native poultry in Sistan, receiving vaccination along with periodic or strategic treatments can greatly prevent the adverse effects of these parasites, including loss weight, lowered production, and decreased egg production during the development of poultry.

Acknowledgments This work has been supported by the Faculty of Veterinary Medicine of Zabol University, and the authors wish to express their deep sincere to Mrs. Rashki laboratory technician staff at the Faculty of Veterinary and habitants of Zabol, Hirmand, Adimi, and Zahak regions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval for the present study was duly obtained from and approved by the Institutional Animal ethics and Research committee of the Faculty of Veterinary Medicine, University of Zabol, Iran.

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