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Original Research Article

The occurrence and distribution pattern of *Eimeria* species among domestic pigeons in Minia, Egypt

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ABSTRACT

Avian coccidiosis is an important parasitic disease affecting poultry and causes high economic losses in poultry industry, which acts as an important sector in the Egyptian national income. It is caused by genus *Eimeria* that belongs to subphylum apicomplexa. It affects domestic pigeons causing great losses, particularly in squabs. So, this study was conducted to investigate the prevalence of *Eimeria* species infecting domestic pigeons (*Columbia livia*) in Minia province. Intestines of 400 domestic pigeons collected from various poultry butcher shops as well as 103 pooled fecal samples of household pigeons were microscopically examined by the flotation technique. The recovered oocysts were morphologically identified. The overall prevalence of *Eimeria* species infection was 27.0% (108/400) and 72.82% (75/103) in the intestinal and pooled fecal samples respectively. Seasonally, the highest infection rate in the pooled fecal samples was in both spring and autumn (80.0%), while the lowest was in summer (56.0%). Meanwhile, the infection rate of the intestinal samples was the highest in winter (33.33%) and the lowest in autumn (20.0%). Moreover, The monthly infection rate of intestinal samples revealed that September and April had the highest rates;(55.0% and 41.62%). However, no infection rates were found in October and November, while monthly infection rate of fecal samples recorded that January and February showed 100% infection rate. Meanwhile, March and August, showed the lowest rate of infection 28.57% and 42.86% respectively. The morphological identification revealed the presence of four *Eimeria* species, *Eimeria labbeana, E. columbarum E.columbae* and *E.labbeana*-like, in pigeons in Minia province. Further studies are recommended to investigate the life cycle and molecular differentiation of *Eimeria* species infecting domestic birds.

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1. Introduction

The domestic pigeons, *Columba livia domestica*, have a worldwide distribution. It is derived from the rock pigeons (*Columba livia*), which is the oldest world's domesticated bird (Croome and Shields, 1992;Yang et al., 2016). Pigeons are usually reared for meat production, gaming, showy purposes, and hobby and recently as laboratory animals (Cooper, 1984; Harlin, 1994; Radfar et al., 2011; Sood et al., 2018).

Coccidiosis in pigeons is caused by protozoan parasites of the genus *Eimeria* (Coccidia Eimeriidae), (Tenter et al., 2002; Yang et al., 2016). Coccidiosis of pigeons may occasionally be seen in young squabs aged 4 weeks to 4 months particularly when they reared intensively with poor hygienic measures, while elder ones serve as carrier and remain apparently healthy (Soulsby, 1982; Ali et al., 2015). It affects the intestinal tissue causing rough feathers, anorexia and greenish watery diarrhea with mucus, dehydration, loss of body weight and results in mortality (McDougald, 2003; Abdul Latif et al., 2016; Mohammed et al., 2017; Donget al., 2018).

There are four species of *Eimeria* species in pigeons: *E. columbae*, *E.columbarum*,*E. labbeana*and *'E.labbeana*-like (Yang et al., 2016). Theyare characterized by varying degrees of virulence. *E. labbeana* is the most prevalent species (Yang et al., 2016). Itwas first described by Pinto (1928), while *E. columbae* was described by Mitra and Das Gupta (1937) and *Eimeria columbarum* by Nieschulz (1935), Aleksandra and Pilarczyk (2014) and Yang et al. (2016). The *E. labbeana*-like was molecularly identified by Yang et al. (2016). Pigeon's coccidiosisis is subclinical, however, outbreaks of coccidiosis may occur causing a high mortality among nestlings and young birds (VanReeth and Vercruysse, 1993; Sari et al., 2008; Yabsley, 2008; Ali et al., 2015). The *Eimeria* infection in pigeons lofts occurs via the spread of oocysts by aerosol of dust from dried feces or debris fromnests or footwear. The contaminated food/water is a potential source of infection (Saikia et al., 2017).

Therefore, this study aimed to investigate the prevalence of natural infection and morphological identification of *Eimeria* species in domesticated pigeons in Minia province.

2. Materials and methods

2.1. Study area and sampling

A total of 400 gut samples of pigeons squabs suspected for coccidiosis were collected from different poultry butcher's shops during the period from October 2017 to September 2018 in Minia city, which located at Upper Egypt, 241 km south from Cairo (coordinates: 28.1003°N 30.7582°E). The intestines were collected after evisceration. Each one was collected separately in plastic bag in an ice tank then it was transported to the laboratory of Faculty of Veterinary Medicine, Beni- Suef University. One hundred and three freshly deposited fecal samples from households located at different regions of Minia city were collected. The pigeons houses contained 3000 bird; each house had 20-50 birds of various ages.

2.1.2. Laboratory investigations

The intestinal samples of squabs were opened by a scissor and the contents removed in clean plastic cups. The collected intestinal contents and fecal samples were examined by direct microscopy and floatation technique according to Solusby (1982).

2.1.3. Morphological identification of *Eimeria* species

The recovered oocysts were collected by the concentration floatation technique using saturated sodium chloride solution. They were washed by PBS and were conducted in aqueous solution of potassium dichromate 2.5% (W⁄V) and incubated at 28^{o}C temperature and 60-80% humidity for 48- 72 hour (Reid and Long, 1979, Gad elhaq et al., 2015). Species identification based on morphological criteria including oocyst shape and size. The oocysts size was determined by measuring length and width of 25 oocystsin given at least 5specimens having similar

morphological featuresby using ocular micrometer (Sloss et al., 1994; Yang et al., 2016; Saikia et al., 2017).

3. Results

3.1. Prevalence of *Eimeria* species among pigeons at Minia province

3.1.1. Intestinal samples

The overall prevalence of *Eimeria* species infection in the examined intestinal samples of pigeons was 27.0% (108/400). The highest infection rate (33.33%) was recorded in winter. While autumn recorded the lowest lowest rate of infection 20% (Table 1).The monthly infection rate revealed that September and April had the highest rates (55.0% and 41.62%). However, no infection rates were found in October and November (Table 2).

3.1.2. Fecal samples

The coprologic examination resulted in a high infection rate (72.82%; 75/103). Seasonally, the highest rate of infection was in both spring and autumn (80.0%), while the lowest was in summer (56.0%), (Table 1). Moreover, the seasonal infection showed that all fecal samples examined in both January and February recorded 100% infection rate. Meanwhile, March and August, showed the lowest rate of infection 28.57% and 42.86% respectively (Table 2).

3.2. Morphological identification of *Eimeria* species

According to the morphological features of the *Eimeria* species, it has been found that 4 species were detected; *E. labbeana*, *E. labbeana*-like, *E. columbarum and E.columbae* (Fig 1). *E.labbeana* was the most prevalent species (Table 2). Sporulation time showed that *E.labbeana* and *E.columbae* was completed through 24 hour, while *E. columbarum* oocysts sporulated through 48 to 72 hour.

Table 1. Seasonal prevalence of *Eimeria* species infection in fecal and intestinal samples of pigeons at Minia province

Season	Intestinal samples			Fecal samples		
	Examined	Infected		Examined	Infected	
		Number	%		Number	%
Winter	60	20	33.33	18	13	72.22
Spring	170	48	28.24	20	16	80.00
Summer	120	30	25.0	25	14	56.00
Autumn	50	10	20.0	40	32	80.00
Total	400	108	27.0	103	75	72.82

Table 2. Monthly prevalence of *Eimeria* species infection in fecal and intestinal samples of pigeons at Minia province

Month	Intestinal samples			Fecal samples		
	Examined	Infected	%	Examined	Infected	%
January	20	5	25.00	5	5	100
February	20	7	35.00	6	6	100
March	21	7	33.33	7	2	28.57
April	55	23	41.82	7	6	85.7
May	52	15	28.85	7	4	57.14
June	60	14	23.33	7	4	57.14
July	34	4	11.76	6	3	50.00
August	35	3	8.57	7	3	42.86
September	40	22	55.00	10	8	80.00
October	24	0	0	9	8	88.88
November	18	0	0	11	10	90.91
December	21	8	38.09	21	16	76.19
Total	400	108	27%	103	75	72.81

Table 3. Identification of the recovered *Eimeria* **species infecting pigeons at Minia province**

Species	Shape	Shape Index Average*	Average size (µm)		Sporulation time	References
			Average length	Average width		
E. columbarum	Sherical or subspherical Without oocystic residual body, with sporocystic residual body, no micropyle	1.00-1.25	22.5 - 17.5	20. - 17.5	48-72 hr	Nieschulz, 1935 and Yang et al., 2016
E. labbeana	Oval or subspherical Without oocystic residual body, with sporocystic residual body, no micropyle	1.04-1.33	20- 17.5	16.25-12.5	24 hr	Pinto, 1928, Levine, 1942 and Yang et al., 2016
***E. labbeana*-like**	Subspherical, oocyst residuum and a polar granule were present. The micropyle was absent, sporocyst residuum was present. A spherical-ellipsoid posterior refractile body was found in the sporozoites	1.03-2.00	27- 22.5	26- 12.5	24-48 hr	Yang et al, 2016
E. columbae	Spherical with oocystic residual body, sporocystic residual body and no micropyle	1.08-1.25	16.25-12.5	10-15	24 hr	Mitra and Das 1937 and Yang et al, 2016

Calculation of shape index =average length/ average width (L/W)

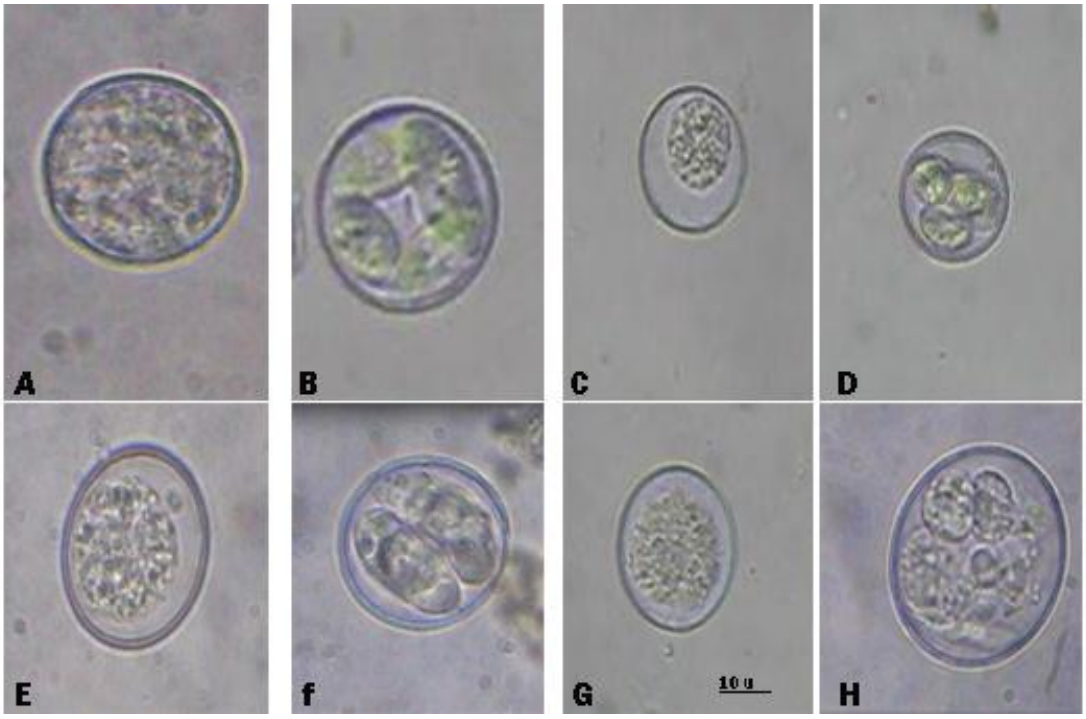

Fig. 1 *Eimeria* species of pigeons. *A. E. columbae* unsporulated oocysts, *B. E. columbae* sporulated oocysts, *C. E. columbarum* unsporulated oocysts *D. E. columbarum* sporulated oocysts, E. *E. labbeana*un sporulated oocysts F. *E. labbeana* sporulated oocysts, G. *E. labbeana*-like unsporulated oocysts *h.* H. *E.labbeana*-like sporulated oocysts

4. Discussion

Coccidiosis is the most prevalent intestinal parasitic disease of poultry caused by *Eimeria* causing high economic losses in the pigeons industry (Aleksandraand Pilarczyk, 2014; Mohammed et al., 2017) due to high mortality rates and the high cost of treatment. Domestic pigeons (*Columba livia domestica*) tend to be in close contact with humans and act as a source of protein, hobby, and recently as laboratory animals (Cooper, 1984; Harlin, 1994; Radfar et al., 2011).

The overall prevalence of *Eimeria* species infection was 27.00% (108⁄400) and 72.82% (75⁄103) for intestinal and fecal samples, respectively. This variation may be referred to being that intestinal samples were brought from young squabs aged 30 days from butchers shops, while fecal samples were brought from household rearing system. The results of intestinal samples were similar to those obtained by El-Madawy (2001) and Abdallah and Fetaih (1995) who recorded 28.7% and 23.4 %, respectively. While it was lower than that of Ahmed et al. (2013) who recorded an infection rate of 34.2 %. The results of fecal samples were similar to that of Ibrahim (1997) who reported it at 77.5% in Gharpia province. It was higher than of El-Sayed (2009) who reported it at 61.36% at Sharkia Governorate.On the other hand the results of this study were higher than those of Thabet (2015) who recorded 12.6% prevalence in upper Egypt, Assuit, Sohag and El-Wady El-Gadid and Mahmoud (2015) 10.86% in Assuit Governorate.

Globally, the prevalence of pigeon'scoccidiosis ranged from 50-100% (VanReeth and Vercruysse, 1993; Sari et al., 2008; Yabsley, 2008; Ali et al., 2015). Meanwhile, the current finding was higher than that of Mohammed et al. (2017) who recorded an infection rate of 19.44% in Nigeria.

The result of pooled fecal samples was 72.82% (75⁄103) which is higher than the findings of Joseph et al. (2017) who found the prevalence of 40.6% in Maiduguri Metropolis Borno State, Nigeria. Furthermore, Saikia et al. (2017) recorded 38.81% in Assam, India. Abdul Latif et al. (2016) recorded 23.0% in Pakistan and Sivajothi and Sudhakara Reddy (2015) recorded 31.0% in YSR district, Andhra Pradesh, India. In addition, Kim et al. (2015) recorded19.02% in Seoul, Korea and Dong et al. (2018) reported 52.8% in Shanghai, China. Meanwhile, these results were lower than those of Ali et al. (2015) who record prevalence of 84.0 % in Babylon province, Iraq. This discrepancy in rate of infection might be attributed to system of rearing, locality and veterinary medical care possibility.

The results of seasonal prevalence of *Eimeria* species in intestinal samples revealed that the highest infection rate was in the winter (30.0%) and the lowest prevalence was recorded in autumn 20.0%. While seasonal prevalence of *Eimeria* species infection in fecal samples showed that the highest infection rate appeared during the autumn and spring (80.0%) and the lowest prevalence was recorded in summer 56.0%.This result agrees with Ahmed et al. (2013), who recorded the highest seasonal prevalence was in winter (42.0%), although they found that the lowest incidence was in spring (20.4 %) in Gharbia governorate, Egypt.

The monthly prevalence of the intestinal samples was high in September while in the examined pooled fecal samples, it was revealed that January, February and November showed the highest prevalence. These differences in the results might be due to the different ages of birds during the study as well as different rearing systems, types and methods of sampling. The prevalence of pigeon coccidiosis worldwide is 50-100 % and the mortality might be 70% in juvenile birds (VanReeth and Vercruysse, 1993; Sari et al., 2008; Yabsley, 2008; Ali et al., 2015).

The morphological differentiation of pigeons *Eimeria* species is difficult due to incomplete descriptions and lack of measurements for many *Eimeria* spp.

(Duszynski et al., 2000). The morphological identification of *Eimeria* species infecting pigeons is mainly based on the shape and size. Oocysts of *E. columbarum* are spherical to ovoid and average 20.0 X 18.7 µm. Their double layered-wall is smooth and colorless. Mature oocysts contain an oocyst residuum (Nieschulz and Kokzidien, 1935). Sporulation lasts 24-38 hours, where the elliptical to spherical shaped-oocysts of *E. labbeana* are somewhat smaller than those of *E. columbarum*, which measures 15-18.9 urn X 14-17.5 µm (Hunt and Grady, 1976). No micropyle or oocyst residuum was present (Varghese, 1975). Anew species of *Eimeria* oocysts infecting domestic pigeons was recorded and molecularly analyzed by Yang et al. (2016) called *E. labbeana*-like. The sporulated oocysts were subspherical, with a smooth bi-layered oocyst wall. Oocysts measured 20.2- 16.1 (22.0 x 18.9-15.7 x 18.9) µm, oocyst length/width (L/W) ratio, 1.38. Oocyst residuum and a polar granule were present. The micropyle was absent. Sporocyst residuum was present (Yang et al., 2016).

According to the morphological identification of the revealed *Eimeria* species, it has been found that four species were reported; *E. labbeana*-like (27- 22.5 x 26 - 12.5 µm), *E. labbeana* (25- 20 x 22.5-12.5 µm), *E. columbarum* (22.5-20 x 20-17.5µm) and *E.columbae* (16.25x15- 12.5x 10µm). These measurements were more or less similar to those given by Pinto (1928); Nieschulz (1935); Mitra and Das (1937); Yang et al. (2016); Matsubara (2017). *E. labbeana* was the most prevalent species in both intestinal and fecal samples. Meanwhile, the previous studies in different localities in Egypt revealed the presence of two species of *Eimeria*; *E.labbeana* and *E.columbea* (Ahmed et al., 2013). Thus molecular techniques are urgently needed for accurate delimit species and infer phylogenetic relationships among *Eimeria* species (Yang et al., 2016).

In conclusion, the overall prevalence of *Eimeria* species infection in domesticated pigeons in Minia of both intestinal and fecal samples was 27.0% and 72.82% respectively. The morphological identification showed presence of four *Eimeria* species in domesticated pigeons in Minia; *E. labeanna*-like, *E. labbeanna*, *E. columbarum* and *E.columbae*. Further study is needed for differentiation of pigeons *Eimeria* species using molecular tools.

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