

An Epidemiological Study on Endoparasites of Domestic Rabbits (*Oryctolagus cuniculus*) in Egypt with Special Reference to Their Health Impact

(Kajian Epidemiologi pada Endoparasit Arnab Domestik (*Oryctolagus cuniculus*) di Mesir dengan Rujukan Istimewa kepada Kesan ke atas Kesihatan Mereka)

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ABSTRACT

The present study was designed to study the enteric parasites infection in domestic rabbits in Upper Egypt and its impact on their health. A total of 298 faecal samples were collected from different farms in Upper Egypt. Each faecal sample was screened for parasitic isolation during May 2011- October 2012. Furthermore, The Eimeria species from samples containing isolated and sporulated oocysts were morphologically identified under microscope. Additionally, the Sheather's sugar flotation and Modified Zeihl Neelsen techniques were used to detect the *Cryptosporidium* species oocyst. According to results of the present study, the overall enteric parasite infections in rabbits was 68.12%, with 5.7% samples were found to be positive for helminths (*P. ambiguus*) and 49% were found to harbour various species of protozoa (*Eimeria* spp. and *Cryptosporidium* oocysts). Influences of age, sex and breed on the prevalence were recorded. Also, there was strong significant seasonal trends in the prevalence of the recovered parasites. Eight species of *Eimeria* were detected. Concurrent infection with two to eight *Eimeria* species occurred most frequently. *Cryptosporidium* oocyst was detected in 45 rabbits (15.1%). To the best of author's knowledge, this the first report of *E. piriformis* and *Cryptosporidium* species in rabbits in Egypt. Histopathological changes were indicative of inflammatory reactions brought about by parasitic infection with *Eimeria* species and its consequent irritating effects on intestine. The current study showed the characteristics of the prevalence of rabbit enteric parasite infection in Egypt and provided relevant 'baseline' data for assessing the effectiveness of future control strategies against these parasites in Egypt.

Keywords: Egypt; helminth; pathology; prevalence; protozoa; rabbits

ABSTRAK

Penyelidikan ini telah dijalankan untuk mengkaji jangkitan parasit enteron pada arnab domestik di Mesir Atas dan kesannya ke atas kesihatan mereka. Sebanyak 298 sampel tinja telah diambil dari ladang yang berlainan di Mesir Atas. Setiap sampel tinja ini telah disaring untuk pengasingan parasit sepanjang Mei 2011-Oktober 2012. Selain itu, spesies *Eimeria* daripada sampel yang mengandungi oosista yang dipencil dan disporulat secara morfologi telah dikenal pasti di bawah mikroskop. Di samping itu, pengapungan gula Sheather dan teknik ubah suai Zeihl Neelsen telah digunakan untuk mengesan oosista spesies *Cryptosporidium*. Menurut keputusan kajian ini, jangkitan keseluruhan parasit enteron dalam arnab adalah 68.12% dengan 5.7% daripada sampel didapati positif untuk helmint (*P. ambiguus*) dan 49% didapati mempunyai pelbagai spesies protozoa (*Eimeria* spp. dan *Cryptosporidium* oosista). Pengaruh umur, jantina dan baka secara lazim telah direkodkan. Selain itu, terdapat pengaruh aliran musim secara lazim bagi parasit yang diambil. Lapan spesies *Eimeria* telah dikesan. Jangkitan serentak dengan dua hingga lapan spesies *Eimeria* berlaku paling kerap. Oosista *Cryptosporidium* dikesan pada 45 arnab (15.1%). Sepanjang pengetahuan penulis, ini merupakan laporan pertama *E. piriformis* dan spesies *Cryptosporidium* pada arnab di Mesir. Perubahan patologi menunjukkan tindak balas keradangan yang disebabkan oleh jangkitan parasit dengan spesies *Eimeria* dan kesannya merengsa pada usus. Kajian ini menunjukkan ciri kelaziman jangkitan parasit enteron pada arnab di Mesir dan menyediakan data 'garis dasar' untuk menilai keberkesanan strategi masa hadapan terhadap kawalan parasit di Mesir.

Kata kunci: Arnab; helmint; kelaziman; Mesir; patologi; protozoa

INTRODUCTION

In recent years, rabbit production became well established in Egypt. Most of the rabbit population in Egypt is in the hands of small holders, while the rest belongs to the commercial sector as a good source of protein (Lebdah & Shahn 2011). In addition to this commercial value, they are also used for the diagnosis of infectious diseases, in the

production of vaccines and other biological substances of public health and veterinary importance (Harcourt 2002). Intestinal parasites, both protozoa and helminths, are one of the main enteropathogen of rabbits, especially in neonates. Some of these parasites are responsible for direct and indirect losses that are attributed to acute illness and death, premature slaughter and diminution of productive

potential such as decreased growth rate, weight loss and late maturity of slaughter stock (Hansen & Perry 1994), as well as to important zoonotic emerging and re-emerging infections, such as cryptosporidiosis.

Passalurus ambiguus infections of rabbit are not generally thought to be very pathogenic, although fatal massive infections in young rabbits have been recorded (Eira et al. 2007).

Coccidiosis of rabbits are ubiquitous infections caused by obligatory intracellular protozoan parasites belonging to the genus *Eimeria* and considered as a major cause of significant morbidity and mortality (Bhat et al. 1996). Although mortality can result from heavy infection by these parasites, the majority of infections result in morbidity due to lower weight gain and diarrhea (Renaux et al. 2003).

Cryptosporidium is a ubiquitous enteropathogen and has been reported from many geographical regions of the world. Although the first report of rabbit *Cryptosporidium* was noticed in 1912 (Tyzzer 1912), the concerns regarding *Cryptosporidium* infection in rabbits have only occurred in recent years due to a few sporadic human cases and a serious waterborne outbreak of cryptosporidiosis caused by *Cryptosporidium cuniculus* (previously named as *Cryptosporidium* rabbit genotype) (Chalmers et al. 2009; Molloy et al. 2010; Robinson et al. 2008).

Despite the importance of rabbits as good source of animal protein in Egypt, very few parasitological studies of enteric parasite infection in rabbits have been done (Abdel Megeed et al. 2005; Abu-Akkada et al. 2010; Arafa & Wanas 1996; Atta et al. 1999). Since the knowledge on rabbit enteric parasite is rather scarce, the present study was undertaken. The present study deals with the basic epidemiological survey of natural prevalence of enteric parasite infections among rabbits, which will facilitate the establishment of an efficient control system for rabbit parasites in Egypt.

MATERIALS AND METHODS

ANIMALS AND PARASITOLOGICAL TECHNIQUES

In the present study, 298 rabbits *Oryctolagus cuniculus* of both sexes, different age groups and breeds randomly collected from different localities in Upper Egypt (small farmers, private and Governmental rabbitries), Egypt were investigated. Fresh faecal samples were screened for enteric parasite infections.

The studied rabbits were housed in galvanized wire cages (40 cm high × 50 cm width × 60 cm length) and fresh water was automatically available at all time. These rabbits were fed with commercial standard rabbit diet without any anti-parasitic or anti-coccidian drugs prior to sampling.

For detection of helminth and coccidian infections, concentration floatation technique was performed as each faecal sample was homogenized in 100 mL tap water, and then 2 g of the mixture was put into 60 mL of saturated salt solution (Carvalho et al. 2011). Furthermore, the

suspension was then emptied into a modified McMaster chamber (Coudert et al. 1995). Five min later, the oocysts within the chamber were counted under the microscope and the oocyst per gram (OPG) was calculated to estimate the degree of infection. Additionally, the Sheather's sugar flotation and Modified Zeihl Neelsen techniques were used to detect the *Cryptosporidium* species oocysts (Henriksen & Pholenz 1981).

SPECIES IDENTIFICATION

Oocysts of *Eimeria* species were purified as previously described by Kvičerová et al. (2008) and were sporulated by shaking in a 2.5% potassium dichromate solution at $25 \pm 3^\circ\text{C}$ for 7 days to ensure good aeration and stored at 4°C until morphological identification at laboratory of Parasitology- Faculty of Veterinary Medicine, Quena, South Valley University. Concentrated oocysts in each sample were identified based on their sizes and morphological characteristics (shape, colour, form index, presence or absence of the micropyle and its cap, presence or absence of residual, polar and Stieda bodies) of the oocysts and sporocysts (Kvičerová et al. 2008). To ensure that species identification is valid, at least 50 sporulated oocysts from each species were observed and measured. Additionally, the *Cryptosporidium* oocyst appeared red on green background in the examined fecal smears.

POST MORTEM AND HISTOPATHOLOGICAL EXAMINATIONS

Furthermore, post mortem examination was carried out on 40 dead rabbits. The obtained material was evaluated through macroscopic analysis of each liver and gall bladder and inspected for nodules typical characteristic of infection with *Eimeria stiedae* followed by liver smear treatment (Sivajothi et al. 2014). Histopathologically, intestines of the dead rabbits were thoroughly examined for the gross lesions and specimens were collected in 10% formalin. Tissues were processed as per standard technique and 5-6 μm thick paraffin embedded sections were cut for histopathological examinations after staining with H. & E. (Carlton & McGavin 1995).

STATISTICAL ANALYSIS

The significance of differences was analyzed using chi-square (χ^2) using the Statistical Package for Social Science version 15.0 (SPSS Inc., Chicago, IL) and $p < 0.05$ was considered significant.

RESULTS

SURVEY RESULTS

As shown by the preliminary coproscopic survey, the overall enteric parasite infections in the present investigation was 68.12% with 5.7% samples were found to be positive for helminths and 49% were found to harbour various species of protozoa as depicted in Table 1.

TABLE 1. Prevalence of parasitic infections in rabbits, found by faecal examination

Total no. of examined rabbits	Enteric parasites								Total no. of infected cases	Total %
	Helminth eggs		Protozoa				Mixed infection			
			<i>Eimeria</i> spp.		<i>Cryptosporidium</i> spp.					
No. of infected cases	%	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%			
298	17	5.7	101	33.9	45	15.1	40	13.4	203	68.12

Coproscopic analysis showed that the studied rabbits were infected with different parasite species including one nematode and two protozoan parasites.

Among the protozoan; the coccidia identified were *Eimeria* spp. and *Cryptosporidium* spp., while the identified helminths were *Passalurus ambiguus*.

With regard to protozoan parasites, the same table displayed that *Eimeria* species were the most prevalent species with an infection rate of 33.9%, while *Cryptosporidium* spp. was represented in 15.1% of the observed samples. Additionally, the mean *Eimeria* oocyst count was 20380 oocysts/g, while the oocyst load of *Cryptosporidium* spp. was very minimal since the smears showed 2 oocysts /slide.

Through this study, eight species of *Eimeria*, namely *Eimeria media*, *E. intestinalis*, *Eimeria coecicola*, *E. magna*, *Eimeria exigua*, *Eimeria perforans*, *Eimeria flavescens* and *E. piriformis*, were identified from naturally infected rabbits in Egypt with the later species was recognized and described for the first time in Upper Egypt.

E. perforans and *E. magna* were generally the most predominant species, while, *E. media*, *E. coecicola*, *E. flavescens*, and *E. exigua* were less common and *E. intestinalis* and *E. piriformis* were relatively rare as illustrated in Table 5).

Concurrent infections with more than one *Eimeria* species were commonly recorded in rabbits. Most of the rabbits in the present study carried two to eight species. The percentages of single and mixed infections of different *Eimeria* species in rabbits were shown in Figure 1.

Influence of age, sex and breed on the prevalence of *Eimeria* infection in rabbits was shown in Tables 2 and 3. From these tables it was evident that the prevalence of coccidian oocysts in young rabbits (47.1%) was higher than in adult rabbits (3.3%) and the differences were statistically significant ($p < 0.05$). Additionally, the prevalence was significantly higher in female rabbits (45%) compared to the male (2.7%), which is consistent with previous observations. On the other hand, coccidian oocysts were found in 48.5% (98/202) of faecal samples from Newzeland rabbits, 9.1% (1/11) from English rabbits and 7.7% (2/26) from Rex rabbits; with no infections among the other breeds; these values were significantly different ($p < 0.05$).

Seasonally, the prevalence was highest in winter (63.3%), particularly during rainfall months, followed by spring and autumn seasons where the infection rates were 40% and 33% respectively, while the least was recorded during summer season (6%) (Table 4). This marked difference was found to be statistically significant ($\chi^2 = 53.57$).

The results of the present study showed that *E. stiedae*, the causative agent of hepatic coccidiosis and one of the most important and well-known species of *Eimeria* in rabbits was not found in this area.

In respect to *Cryptosporidium* species, forty five samples were found to be positive for *C. cuniculus* (15.1%), adult were found more often infected (26 out of 90, 28.9%) than kitten rabbit (19 out of 208, 9.1%) and the difference was statistically significant. Additionally, the prevalence

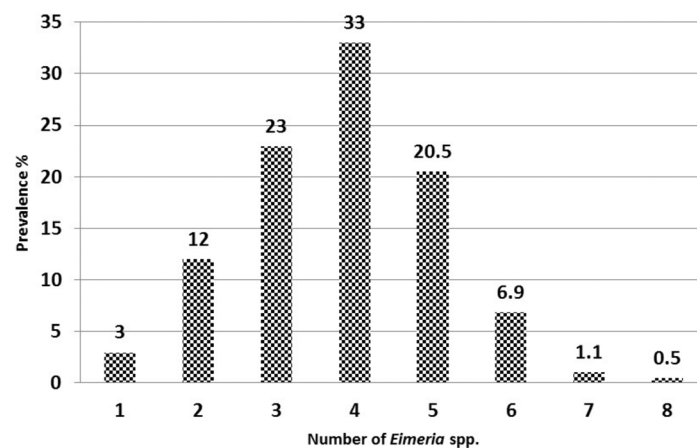
FIGURE 1. Frequencies of *Eimeria* species isolated from infected rabbits

TABLE 2. Prevalence of parasitic infections in rabbits, distributed by age and sex

Animals		<i>P. ambiguus</i>			<i>Eimeria</i> spp.		<i>Cryptosporidium</i> sp.	
		No. of examined rabbits	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%
Kitten	Female	158	10	6.3	98	62	19	12
	Male	50	2	4	0	0	0	0
Total kitten (up to one year)		208	12	5.8	98	47.1	19	9.1
Adult	Female	62	4	6.5	1	1.6	22	35.5
	Male	28	1	3.8	2	7.1	4	14.3
Total adult (Over one year)		90	5	5.6	3	3.3	26	28.9
P value			0.9		<0.0001		<0.0001	
Total	Female	220	14	6.4	99	45	41	10.6
	Male	78	3	3.8	2	2.7	4	5.1
P value			0.4		0.0001		<0.01	
Total		298	17	5.7	101	33.9	45	15.1

TABLE 3. Prevalence of enteric parasites infection in different rabbit breeds

Rabbits breeds	<i>P. ambiguus</i>			<i>Eimeria</i> spp.		<i>Cryptosporidium</i> sp.		
	No. of examined rabbits	No. of infected rabbits	%	No. of infected rabbits	%	No. of infected rabbits	%	
English sp.	11	1	9.1	1	9.1	1	9.1	
Babion sp.	13	0	0	0	0	3	23.1	
Newzeland sp.	202	3	14.9	98	48.5	21	10.4	
Flander sp.	4	0	0	0	0	1	25	
Rex sp.	26	4	15.4	2	7.7	4	15.4	
Belgic sp.	11	3	27.3	0	0	3	27.3	
Shenshella sp.	11	1	9.1	0	0	3	27.3	
California sp.	20	5	25	0	0	9	45	
P value		<0.0005		<0.0001		0.0005		
Total		298	17	5.7	101	33.9	45	15.1

was significantly higher in female rabbits (10.6%) compared to the male (5.1%). On the other hand, *Cryptosporidium* oocysts were found in 45% (9/20) of faecal samples from California rabbits, 27.3% (3/11), from English rabbits and 25% (1/4) from Flander rabbits; with variable rates ranging from 9.1% - 23.1% among the other examined breeds; these values were found to be significantly different ($p < 0.05$) as recorded in Tables 2 and 3.

Seasonally, from the samples collected, *Cryptosporidium* oocyst was more prevalent in the summer season (24.1%), followed by autumn and spring where the infection rates were 15% and 11.6%, respectively, while the lowest rate was in winter (8.3%). This marked difference was found to be statistically significant ($\chi^2 = 8.3$) (Table 4).

A nematode, *Passalurus ambiguus*, was detected in 17 out of 298 of the examined samples with an overall infection

rate of 5.7%. Younger animals tend to be susceptible to helminthiasis as compared to adults. However, these differences were not significant ($p > 0.05$). In addition; relatively higher prevalence of helminthiasis was observed in female animals (6.4%) compared to male (3.8%) but the difference was not significant ($p > 0.05$) as indicated in Table (2).

In regard to rabbit breed, the most susceptible to helminth infections seem to be Belgic rabbits (27.3%) and the least susceptible English and Babion rabbits (9.1%). This marked difference was found to be statistically significant ($\chi^2 = 22.4$) Table (3).

Seasonally, the main feature was a peak incidence of *P. ambiguus* during summer (16.9%) followed by a gradual fall in spring (3.2%); with no observed infection in winter and autumn seasons. These differences were found to be significant ($p < 0.05$) as depicted in Table (4).

TABLE 4. Seasonal prevalence of parasitic infections in rabbits, found by faecal examination

Season	Internal parasites					
	<i>P. ambigua</i> eggs		Protozoa			
			<i>Eimeria</i> spp.		<i>Cryptosporidium</i> spp.	
	No. of infected cases	%	No. of infected cases	%	No. of infected cases	%
winter (n=60)	0	0	38	63.3	5	8
spring (n=95)	3	3.2	38	40	11	11.6
summer (n=83)	14	16.9	5	6	20	24.1
autumn (n=60)	0	0	20	33	9	15
P value	<0.0001		<0.0001		<0.05	

TABLE 5. Percentage of faecal samples infected with each coccidia species in rabbits

Eimeria species	Rabbit kitten up to one year No = 70		Adult rabbits More than one year No = 30		Doe No = 72		Buck No = 28		Total No = 100	
	No	%	No	%	No	%	No	%	No	%
	<i>E. perforans</i>	67	95.7	5	16.7	67	93.1	5	17.9	72
<i>E. magna</i>	62	88.6	6	20	6	88.6	62	21.4	68	68
<i>E. flavescens</i>	38	54.3	6	20	38	52.8	6	21.4	44	44
<i>E. coecicola</i>	30	42.9	6	20	30	41.7	6	21.4	36	36
<i>E. exigua</i>	25	35.7	0	0	25	34.7	0	0	25	25
<i>E. media</i>	20	28.6	0	0	20	27.8	0	0	20	20
<i>E. intestinalis</i>	10	14.3	2	6.7	10	13.9	2	7.1	12	12
<i>E. piriformis</i>	6	8.6	2	6.7	6	8	2	7.1	8	8

MORPHOLOGICAL FEATURES OF THE RECOVERED ENTERIC PARASITES

The most common features of the recovered enteric parasites were summarized as illustrated in Table 6 and Figure 2.

HISTOPATHOLOGICAL RESULTS

Macroscopically Intestines of naturally infected rabbits appeared pale in color, atrophied with extensive degree of the congestion, hemorrhagic with ulcerative wall and there was a mass of clotted blood in the intestinal lumen (Figure

TABLE 6. Morphological characteristics of sporulated *Eimeria* species from rabbits

<i>Eimeria</i> Species	Shape	Mean oocyst size		Mean sporooocyst size (µm)	cap	Micropyle	Oocyst residuum	Oocyst wall	Sporulation time
		Length (µm)	Width (µm)						
		<i>E. piriformis</i>	Ellipsoid						
<i>E. coecicola</i>	Elongated ellipsoid	30.52	17.56	17.47×9.88	-	+	+	Yellowish green	62
<i>E. flavescens</i>	Ovoid	29.22	16.70	12.98×6.78	-	+	-	Yellowish brown	56
<i>E. exigua</i>	Spherical	15.83	15.83	7.97×6.17	-	-	-	Purple	28
<i>E. intestinalis</i>	Pyriiform	28.29	17.08	12.03×6.04	-	+	+	Greenish brown	55
<i>E. perforans</i>	Ovoid	22.16	14.45	10.57×7.76	-	-	+	Greenish	36
<i>E. media</i>	Ovoid to ellipsoid	28.64	16.70	12.17×7.34	-	+	+	Light pink	36
<i>E. magna</i>	Ovoid	36.80	23.53	13.41×7.21	-	+	+	Yellowish brown	52

3(A)). Moreover, diarrhea was reported in 50 (16.8%) of rabbits and it was indicated by matted perineum, the presence of watery, mucoid and abnormally soft feces as shown in Figure (2(S) & 2(T)).

Microscopically Intestines of naturally infected rabbits had characteristic lesions manifested by severe degree of necrosis of the intestinal cells characterized by pyknotic and tiny nuclei with acidophilic cytoplasm were detected along intestinal tissues. Sloughing with desquamation of the epithelial lining at the lumen was observed. Moreover, atrophy of intestinal villi was noticed as well as different developmental stages of *Eimeria* spp. were noticed in crypts (Figure 3(B), 3(C), 3(D), 3(E) & 3(F)).

DISCUSSION

Parasitic infections pose a serious health threat and limit the productivity of livestock due to the associated morbidity and mortality. More specifically, plethora of parasitic diseases plays a detrimental role in rabbit's production leading to serious economic loss. Therefore, Knowledge of prevalence of enteric parasites and current species will help to minimize the economic losses in the rabbit industry, evaluate infection potential and control programs, especially for kits (Nwosu et al. 2007).

This study has provided for the first time detailed quantitative data on infections with enteric parasites in rabbits, which is relatively poorly studied and about which little is known.

The percentage of rabbit that bear one or more parasitic species in the present study was high (68.12%). The most

prevalent parasites found were *Eimeria* spp. (33.9%). Similar patterns were reported in other previous studies on rabbits (Biu & Nwosu 1998; Gurpartap & Khahra 1997). On the contrary, the prevalence rates were relatively higher than that previously recorded in Thrissur as the prevalence of coccidial infection in rabbits was 18.54% (Shameem & Devada 2005). These variations were likely due to wide usage of grass, silage and grain as rabbit diet, making the administration of anticoccidials in feed impracticable in small farms, although practice other than anticoccidials was also employed in rabbit farming, poor hygienic conditions and suboptimal temperatures were observed on some small individual rabbit farms, which can favour the occurrence of *Eimeria* spp. Infections.

Additionally, the present finding was lower than that reported among the European, Indian, Turkish and Taiwanese rabbits in previous studies as the overall prevalence of the coccidial infections in rabbits were 60%, 64%, 80% and 41.7%, respectively (Chandra & Ghosh 1990; Gonzalez-Redondo et al. 2008; Karaer 2001; Li et al. 2010). Variations in the percentage frequency of occurrence of coccidial infections worldwide are expected and this may be attributed to various factors, including the difference in environmental conditions prevailing in each region, meteorology and agro-ecology.

According to the accessible literature, the study of rabbit *Eimeria* in Egypt is very limited. Most of these studies were concerned with histopathology, biochemistry, and treatment of the hepatic coccidiosis, and they were done mostly with *E. stiedai* (Abdel Megeed et al. 2005; Abu-Akkada et al. 2010). In the present study, eight

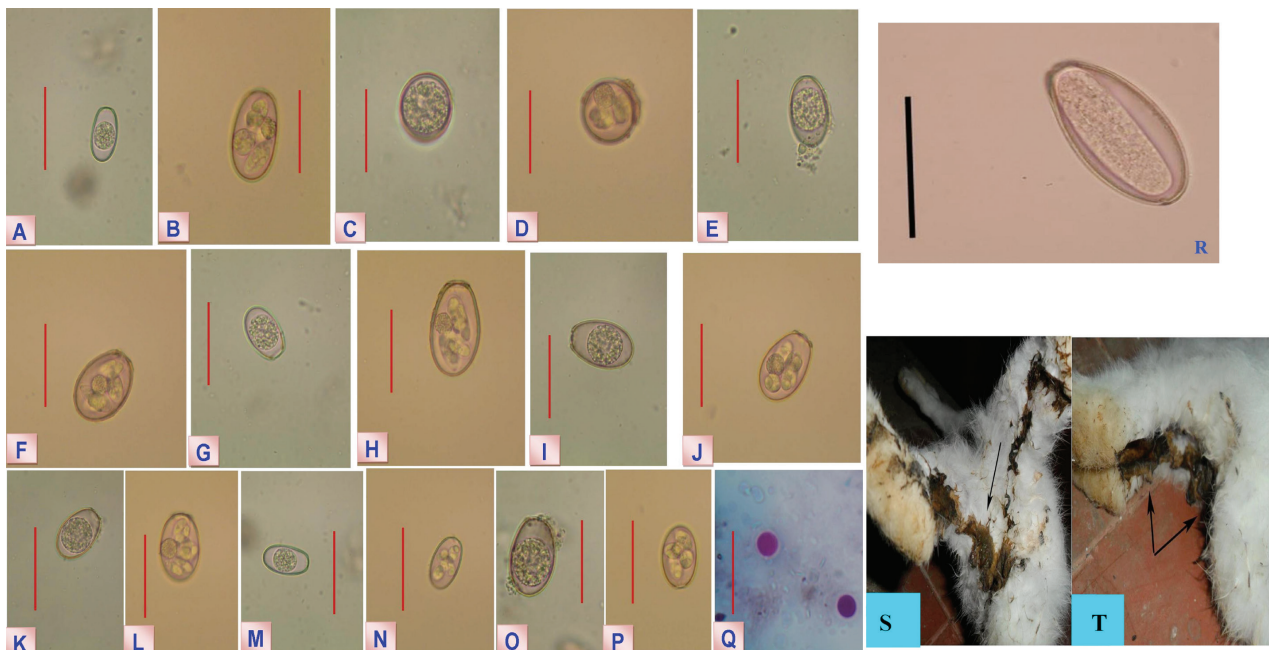


FIGURE 2. (A-Q) Coccidia species in rabbits (Bar = 20 µm); A&B: *E. coecicola*, C&D: *E. exigua*, E&F: *E. flavescence*, G&H: *E. intestinalis*, I& J: *E. magna*, K&L: *E. media*, M&N: *E. perforans*, O&P: *E. piriformis* and Q: *Cryptosporidium* oocyst (Bar = 50µm). (R) Helminth parasite stages identified, *Passalurus ambiguus* egg (Bar=20µm), Rabbits with severe diarrhea due to intestinal coccidiosis (S & T) (arrow)

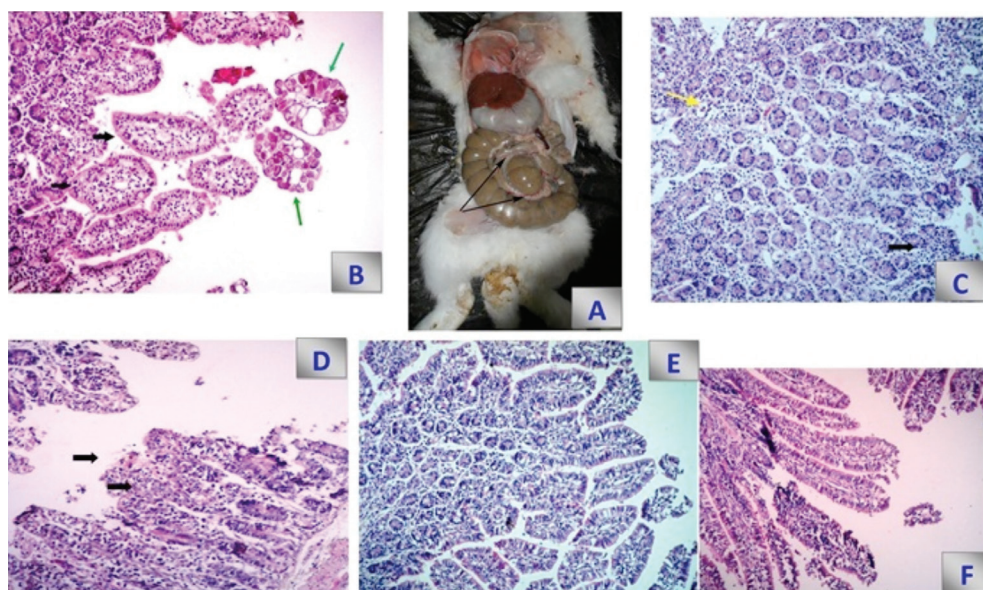


FIGURE 3. Intestines of naturally infected rabbit with *Eimeria* spp

A: showing congestion with thickened, hemorrhagic, ulcerated wall, B: Intestine showing necrosis of the cells with sloughing and desquamation of the intestinal lining (black arrow) and different developmental stages of *Eimeria* spp. (green arrow), C: Intestine showing severe inflammatory cells infiltration mainly lymphocytes (arrows), D, E & F: Intestine showing mild degree of sloughing of the epithelium lining, besides mild degree of lymphocytes infiltration (H&E $\times 40$).

species were recognized, of which 7 species was recorded previously (El-Shahawi et al. 2012) and one additional species *E. piriformis* was recognized and described for the first time in Egypt. Our descriptions of the sporulated oocysts of *Eimeria* from rabbits in Egypt vary slightly in size and other negligible characteristics from previous descriptions (El-Shahawi et al. 2012; Razavi et al. 2010). From different *Eimeria* species present in the rabbits of the present study, some species including *E. flavescens* and *E. intestinalis* are regarded as highly pathogenic organisms and may have led to hemorrhagic enteritis in rabbits (Li & Ooi 2009).

The influences of age, sex and breeds were also taken into consideration in the current study. Lower resistance or less immunity to coccidian infection in young rabbits than in older animals (Pakandl et al. 2008) is responsible for the high prevalence of coccidiosis in young rabbits (47.1%). Additionally, host sex exercise a great significant influence on parasitisation, as females harbored more infection compared to males, agreeing with various studies having reported this parasite in rabbits (Abdel-Baki & Al-Quraishy 2013).

Mixed infection with four *Eimeria* species was most frequent in the present study and in all previous studies as natural infection with a single *Eimeria* species are rare (Yakhchali & Tehrani 2007). These results could be attributed to that healthy rabbit could support the heavy infection with mixed species but stress factors encourage the onset of the disease, or likely due to the nature of the pasture which usually contaminated by various kind of parasites.

Referring to the influence of the season, with highest prevalence in winter season (63.3%) and the lowest was

in summer (6%). This indicated that favorable humidity and temperature hastened sporulation. Also the increase of infection was reported in areas of high humidity and rainfall (Rajkhowa 1996). Oocysts survive best in shaded moist condition with proper moisture and warmth condition (Soulsby 1986). Significant seasonal differences observed might be due management care, types of diet offered to the rabbits under the study.

In respect to the absence of *E. stiedae* in the present investigation, similar observation was reported previously in USA, France and Saudi Arabia, respectively (Al-Mathal 2008; Al-Rukibat et al. 2001; Gres et al. 2003).

In respect to *Cryptosporidium*, although *Cryptosporidium* was subsequently found in a broad range of animals, its impact was neglected until the early 1980s when it was found to be a common, serious primary cause of outbreaks of diarrhea in farm mammals (Angus et al. 1982). This is the first report of *Cryptosporidium* infection in pet rabbit in Upper Egypt area. Furthermore, the overall prevalence of *Cryptosporidium* in the present investigation was 15.1%. Similar results were reported in Japan, in a study of *Cryptosporidium* infection in juvenile pet rabbits, where the prevalence was 16.7% (Shiibashi et al. 2006). The result of this study thus implies that *Cryptosporidium* infection in rabbit, as well as the possible risk of transmission to humans by rabbit, is important in Egypt as it is elsewhere in the world. Hence, considerable attention should be paid to preventing the spread of the infection.

Our findings showed that there was significant difference ($p > 0.05$) between the rate of the infection in rabbit and the age, with highest peaks in adult (28.9%) compared to young rabbits (9.1%). Similar results have

been observed in pigs that the percentage of pigs shedding *Cryptosporidium* oocysts increased as the pigs got older (Morgan et al. 1999). This suggests that maternal antibodies protect animals from developing infections. On the contrary, the present data was not in line with that reported previously, as the age played an important role in the incidence of *Cryptosporidium* infection as young's more susceptible to infection with *Cryptosporidium* (Ohaeri & Iwu 2003).

The present study also reports a significant difference ($p < 0.0001$) between the infection rates of *Cryptosporidium* sp. in female and male rabbit, with the female animals twice as likely to be at risk as the males. The reason for this disparity is not known, although this outcome could be attributed to the usual practice of having a higher female: Male ratio in a farm and also the retention of female animals for breeding. It could also be related to host intrinsic factors (genetics, physiology and immunology) and extrinsic factors (environment and management practices) (De Graaf et al. 1999).

The findings of the present study showed that seasons have a significant effect on the prevalence of infection in rabbits, with highest prevalence in summer (24.1%) followed by autumn and spring (15% and 11.6%, respectively) and lowest in winter (8%). Peaks in *Cryptosporidium* prevalence appear to correspond with warmer seasons in temperate and tropical climates (Bern et al. 2002).

The present study showed the overall prevalence of the recovered helminths, *P. ambiguus* to be 5.7%. This result was very close to the report on helminths prevalence in rabbit's in Jeonbuk area (Tae-Wook et al. 2003) and relatively higher than the previous findings in Turkey (Sürsal et al. 2014). However, the prevalence rates were relatively lower than that recorded previously in eastern Scotland (14.2%) (Boag 1985) and in Egypt (26.7%) (Ashmawy et al. 2010). These variations were likely to be due to difference in environmental condition, mangemental care, nature of pasture and the level of humidity from place to another place.

In the present study, the risk of infection to all age groups was the same. The young and adult rabbit had the same risk of infection. Similar risk of infection could be due to the fact that both young and adult rabbit were exposed to the same risk of infection by the infective stage of *P. ambiguus* due to the knowledge about the hygienic measures as kits remain in contact with their dams, as well as might be attributed to the nature of studying area. Additionally, the sex of rabbit was not significantly associated with prevalence of helminths in this study despite the higher frequency in females (6.4%) compared with males (3.8%). This finding supports with the general understanding of helminth infections that female animals are more susceptible to helminthiasis. It is assumed that sex is a determinant factor influencing prevalence of parasitism (Maqsood et al. 1996) and females are more prone to parasitism during pregnancy and peri-parturient period due to stress and decreased immune status (Urquhart

et al. 1996). Moreover, breed was found to be statistically significant as a risk factor, which has a great effect on the prevalence of helminths in examined breeds of rabbits. It seems to be associated with genetic and inheritable effect and to some extent management factors like rearing system. Regarding the distribution of infections according to seasons, a considerable difference was found in the prevalence of *P. ambiguus* among different season, with the highest value found in summer season 16.9% followed by spring 3.2%, without recorded infection in both winter and autumn seasons. Similarly, farmed rabbits were most often infested by *P. ambiguus* whose prevalence varied with season of the year, with the highest extensity 40% in the summer period and the parasite did not occur in autumn season (Nosal et al. 2006; Szkucik 2014). The reason for the higher prevalence during summer could be due to favorable ecological and environmental conditions of the study area. The high temperature and humidity during the summer provides a conducive atmosphere for luxurious growth of infective larvae on pasture.

Regarding to the histopathological results, the naturally infected rabbits with coccidia showed the presence of different developmental stages of *Eimeria* spp. invaded epithelial cells of the intestine. Furthermore, it had characteristic lesions manifested by severe degree of necrosis of the intestinal cells characterized by pyknotic and tiny nuclei with acidophilic cytoplasm were detected along intestinal tissues. Sloughing with desquamation of the epithelial lining at the lumen was also noted. Moreover, atrophy of intestinal villi was noticed. These findings were similar to the figures previously reported in the intestinal coccidiosis as the lesions were seen in the small and large intestine depending on the causative agent as the parasitized epithelial cells become necrotic, may result in ulceration, which appeared grossly as multiple white areas in the wall of intestine (Yakhchali & Tehrani 2007).

CONCLUSION

In conclusion, to the best of the author's knowledge this is the first report of *E. piriformis* and *Cryptosporidium* oocyst among studied rabbits in the study region. Additionally, the current study showed the characteristics of the prevalence of rabbit enteric parasite infection in Egypt and provided relevant 'baseline' data for assessing the effectiveness of future control strategies against these parasites in Egypt. Parasitic invasions, especially coccidiosis, have prominent effect on animal overall performance and economic benefits of rabbit production. Furthermore, The present figures indicates that persons handling or otherwise exposed to rabbits are at greater risk of zoonotic infection from *Cryptosporidium* and thus becoming a source of infection for others, because attention cannot be paid to personal hygiene, more extensive studies in rabbits in different areas are needed to better characterize the transmission of cryptosporidiosis and to assess the public health significance of these parasites.

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