Prevalence of coccidiosis in local breed rabbits (*Oryctolagus cuniculus*) in Baghdad province

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Abstract

The study aimed to investigate the prevalence of *Eimeria* species in local breed rabbits. Eighty local breed rabbits of both sexes were examined. The results revealed, that (58) rabbits were infected with a total prevalence rate of (72.5%). The intestinal *Eimeria* were recorded the higher prevalence rate (55%) compared with the liver *Eimeria* (3.75%), and the mixed infection of intestinal and liver *Eimeria* (13.75%). The infection with oocyst of *Eimeria perforans* species display the highest prevalence rate (31.25%) followed by the *E. piriformis*, *E. irresidua*, *E. media*, *E. flavescens*, *E. coecicola*, *E. exigua*, *E. intestinalis*, *E. magna* and *Eimeria stiedae* with an prevalence rate of (27.5, 11.25, 17.5, 28.75, 13.75, 18.75, 23.75, 28.75 % and 17.5%) respectively. The mean and the SE of the length and the width of *Eimeria* species oocyst were found as: *Eimeria stiedae* (32.5±0.64) x (20.5±0.30) (µm), *E. perforans* (20.07±0.64) x (14.21±0.40), *E. piriformis* (24.38±0.41) x (16.54±0.29), *E. irresidua* (24.38±0.41) x (16.54±0.29), *E. media* (27.14±0.96) x (17.10±0.76), *E. flavescens* (30.30±0.59) x (19.93±0.46), *E. coecicola* (32.22±1.07) x (19.90±0.62), *E. exigua* (32.22±1.07) x (19.90±0.62), *E. magna* (33.78±0.62) x (20.52±0.58) and *E. intestinalis* (26.5±0.39) x (17.6±0.24) (µm). The mixed infection was most common with no significant differences appeared between males and females.

Key words: Prevalence, coccidiosis, intestinal *Eimeria*, liver *Eimeria*, rabbits.

الانتشار الوبائي لداء الاكريات في الأرانب المحلية (*Oryctolagus cuniculus*) في محافظة بغداد

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الخلاصة

هدفت الدراسة الى معرفة الانتشار الوبائي لأنواع الايميريا التي تصيب الأرانب المحلية من خلال فحص 80 أرنباً محلياً من كلا الجنسين. بلغت عدد الحالات الخمجة 58 وبنسبة خمج كلية 72.5% . وكانت الإصابة بالايميريا المعوية اعلى (55%) مقارنة مع الإصابة بالايميريا الكبدية (3.75%) والإصابة المشتركة للايميريا المعوية والكبدية (13.75%). شكّل الخمج بنوع *Eimeria perforans* أعلى نسبة خمج بلغت (31.25) يليه الخمج بكل من الأنواع: *E. piriformis* ، *E. irresidua* ، *E. media* ، *E. flavescens* ، *E. coecicola* ، *E. exigua* ، *E. intestinalis* ، *E. magna* و *Eimeria stiedae* وبنسبة 27.5، 11.25، 17.5، 28.75، 13.75، 18.75، 23.75، 28.75 و 17.5 %على التوالي. بلغت ابعاد المعدل الحسابي والخطأ القياسي للطول والعرض لأكياس بيض الايميريا في الارانب المحلية للأنواع التالية: *Eimeria stiedae* (32.5±0.64) x (20.5±0.30)، *E. perforans* (20.07±0.64) x (14.21±0.40)، *E. piriformis* (24.38±0.41) x (16.54±0.29)، *E. irresidua* (24.38±0.41) x (16.54±0.29)، *E. media* (27.14±0.96) x (17.10±0.76)، *E. flavescens* (30.30±0.59) x (19.93±0.46)، *E. coecicola* (32.22±1.07) x (19.90±0.62)، *E. exigua* (32.22±1.07) x (19.90±0.62)، *E. magna* (33.78±0.62) x (20.52±0.58) و *E. intestinalis* (26.5±0.39) x (17.6±0.24) مايكرون. الخمج المختلط كان الأكثر شيوعاً مع عدم وجود فرق معنوي في الإصابة بين الذكور والإناث.

الكلمات المفتاحية: مدى الانتشار، الكوكسيديا ، الكوكسيديا المعوية ، الكوكسيديا الكبدية ، الارانب

Introduction

Rabbit meat is used as a good source of animal protein, and some breeds are reared for fur production as well as for medical and biological purposes (1). Coccidiosis is a parasitic disease caused by intracellular protozoon of the genus *Eimeria* (2). Rabbit coccidiosis is a common and widespread protozoal infection (3, 4). It is an important economic disease, especially, in breeding and rearing establishments where sanitation measures are poor. It has two forms: hepatic caused by *E. stiedae* and intestinal caused by other fourteen *Eimeria* species (5). These species *are E. media, E. magna, E. irrestidua, E. piriformis, E. exigua, E. intestinalis, E. coecicoIa, E. neoleporis, E. nagpurensis, E. leporis, E. flavescens, E. matsubayashii* and *E. perforans* (5, 6, 7), and these cause significant mortality in domestic rabbits where kids are the most susceptible; however, infected adults become carriers of the disease and are the source of infection (2). These coccidians can lead to retarded growth, diarrhea and even mortality thus causing considerable economic losses (8, 9, 10,and 11). Due to the importance of rabbits coccidiosis this study was conducted to investigate the prevalence of different *Eimeria* species in Baghdad province.

Materials and methods

A total of 80 local breed rabbits (*Oryctolagus cuniculus*) were collected from local markets at different areas in Baghdad during the period of December /2012 to September/ 2013. The sex and weight were recorded. Blood was taken directly from the heart. Rabbits were Slaughtered and dissected. The gastrointestinal tract was splitted longitudinally, scraped, and the contents washed in a 100 mesh screen and liver was dissected. Parasite identifications were made utilizing conventional parasitological techniques (12), and histopathological examination was conducted (13). Fecal samples were collected from the intestine of each rabbit, and examined for the presence of *Eimeria* oocysts by centrifugal flotation technique using saturated salt solution (specific gravity: 1.20). The coccidial oocysts were sporulated in 2.5% (w/v) potassium dichromate solution (K2Cr2O7) in Petri dishes at 27ºC for 7 days. They were identified on the basis of the morphological characteristics using a light microscope (14, 15). The morphometric data and the specific characteristics of unsporulated and sporulated oocysts were recorded (1). The features of sporulated oocysts including shape, shape index, size inner and outer wall, cap and time of sporulation, micropyle and residium were measured and the identity of species of coccidia was determined by the keys previously described by researchers (6, 7, 11, and 16). The differential count of leucocytes was made from blood stained with Giemsa (17). Statistical analysis of means were performed by using statistical package for social science (SPSS 2008), Version 16, and for determination of a significant differences by using one way analysis ANOVA (18).

Results

The total infection rate of *Eimeria* was found (72.5%); where the intestinal *Eimeria* was recorded a higher infection rate (55%) compared with Liver *Eimeria* (3.75%) and mixed infection of intestinal and liver *Eimeria* (13.75%). Also, females had a higher *Eimeria* infection rate (76%) compared with males (66.66%); but the liver *Eimeria* infection rate in males was a higher (6.66%) than females (2%); While intestinal *Eimeria* in females was recorded a higher infection rate (60%) compared to males (46.66%). Also in the mixed infection (intestinal and liver *Eimeria*) the females had a higher infection rate (14%) than males (13.33%) (Table 1).

Infection rate of *Eimeria stiedae* according to sex:

The total infection rate of Eimeria stiedae in rabbits (17.5%), which were (20%) in males and (16%) in females (Table 2).

***Eimeria* species:**

The results showed that the species of intestinal *Eimeria*; they are (*E. perforans, E. piriformis, E. irresidua, E. media, E. flavescens, E. coecicola, E. exigua,*

Table (1) The total infection rate of *Eimeria spp*. in local breed rabbits.

Sex	*Eimeria*			Total Infection (%)
	Liver (%)	Intestinal (%)	Mixed (%)	
Males (30)	(6.66) 2	(46.66) 14	(13.33) 4	(66.66) 20
Females (50)	(2) 1	(60) 30	(14) 7	(76) 38
Total (80)	(3.75) 3	(55) 44	(13.75) 11	(72.5) 58

Table (2) Infection rate of *Eimeria stiedae* according to the sex in local breed rabbits.

Sex	No. of Exam. Animals	Infected	Percentage (%)
Males	30	6	(20)
Females	50	8	(16)
Total	80	14	(17.5)

Table (3) Infection rate of each species of *Eimeria* in local breed rabbits.

Types of *Eimeria*	No. of Exam. Animals	No. of Infected animals	No. of samples	Percentage (Total)%	Percentage (Species)%
E. perforans	80	58	25	31.25	43.10
E. piriformis			22	27.5	37.93
E. irresidua			9	11.25	15.51
E. media			14	17.5	24.13
E. flavescens			23	28.75	39.65
E. coecicola			11	13.75	18.96
E. exigua			15	18.75	25.86
E. intestinalis			19	23.75	32.75
E. magna			23	28.75	39.65

Table (4) Microscopic examination for oocyst of different species of *Eimeria* in the local breed rabbits. (M±SE= Mean ± Standard error)

Species	Length x Width M±SE	Sample	Shape	Wall	Micropyle	Residual body	Site of infection
E.stiedae	(32.5±0.64) x (20.5±0.30)	14	Ovoid	Yellow to light pink	Present and small	Present	Liver
E.perforans	(20.07±0.64) x (14.21±0.40)	25	Ellipsoid	Colorless to light pink bi-layered	Indistinct	Present small	Duodenum
E.piriformis	(24.38±0.41) x (16.54±0.29)	22	Piriform	Yellow-brown bi-layered	Present	Non	Cecum and colon
E.irresidua	(24.38±0.41) x (16.54±0.29)	9	ellipsoid	Light yellow bi-layered	Prominent	Non	Cecum and Ilium
E.media	(27.14±0.96) x (17.10±0.76)	14	Ellipsoid	Brown thick	Present	Present large	Jejunum, Ilium and colon
E.flavescens	(30.30±0.59) x (19.93±0.46)	23	Broad ellipsoid or ovoid	Yellow to brown bi-layered	Prominent	Non	Ilium, cecum and colon
E.coecicola	(32.22±1.07) x (19.90±0.62)	11	Ovoid – cylindrical	Yellow to brown bi-layered	Present small	Present	Ilium, cecum and colon
E.exigua	(32.22±1.07) x (19.90±0.62)	15	Spherical	colorless	Non	Non	Ilium
E.magna	(33.78±0.62) x (20.52±0.58)	23	Ovoid to ellipsoid	Orange- red-brown bi-layered	Prominent lipped	Present large	Jejunum, ilium
E. intestinalis	(26.5±0.39) x (17.6±0.24)	19	Pyriform or ovoid	Light brown	present	Large, granular	Jejunum, ilium

E. intestinalis, *E. magna*); the highest infection rate (31.25%) was recorded in *E. perforans* followed by *E. magna* and *E. flavescens* 28.75 % while *E. irresidua* showed the lowest infection rate (11.25%) (Table 3). The mean and the SE of the length, the width and the characters of the *Eimeria* species oocyst were found as: *Eimeria stiedae* (32.5±0.64) x (20.5±0.30) (µm), *E. perforans* (20.07±0.64) x (14.21±0.40), *E. piriformis* (24.38±0.41) x (16.54±0.29), *E. irresidua* (24.38±0.41) x (16.54±0.29), *E. media* (27.14±0.96) x (17.10±0.76), *E. flavescens* (30.30±0.59) x (19.93±0.46), *E. coecicola* (32.22±1.07) x (19.90±0.62), *E. exigua* (32.22±1.07) x (19.90±0.62), *E. magna* (33.78±0.62) x (20.52±0.58) and *E. intestinalis* (26.5±0.39) x (17.6±0.24) (µm) (Table 4).

Effects of *Eimeria spp*. on the differential leucocyte count (DLC):

Infection with *Eimeria* spp. was seen affect on the differential leucocyte count. The liver *Eimeria* had an increase in the neutrophils (40.67± 3.93) compared with intestinal *Eimeria* (40.52±2.28), but less than Liver and Intestinal *Eimeria* (40.82±3.19) while all these infected rabbits had an increase in neutrophils than the non-infected rabbits (43.36±3.16). While the rabbits infected with liver *Eimeria* shown significant increase ($p<0.05$) in monocytes (8.33 ± 0.33) compared to non-infected rabbits (5.95 ± 0.49). It worthwhile, that there was an increase ($p<0.05$) in basophil values in the infected with intestinal and liver *Eimeria* (1.18 ± 0.32). Also the liver *Eimeria* had an increase in eosinophils (3.33 ± 0.88) compared with intestinal *Eimeria* (1.86 ± 0.26) (Table 5).

Effects of season on the *Eimeria* infection rates:

The results showed the effect of seasons in the *Eimeria* infection rates. Spring showed a highest infection rate (81.3%) and the lowest infection rate was recorded in Winter (75.8%) (Table 6).

Effects of Eimeria infection on weight gain:

Infected Males had an increase in the body weight (1257.5±102.37g) compared with infected females (1104.6±100.15g) (Table 7).

Table (5) Effects of *Eimeria spp.* on the DLC in local breed rabbits.

Type of *Eimeria*	Mean ± SE (%)				
	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Intestinal	40.52 ± 2.28	49.93 ± 2.20	7.43 ± 0.55 AB	1.86 ± 0.26	0.25 ± 0.09 B
Liver	40.67 ± 3.93	47.67 ± 3.28	8.33 ± 0.33 A	3.33 ± 0.88	0.00 ± 0.00 C
Liver& Intestinal	40.82 ± 3.19	48.73 ± 3.22	5.91 ± 0.85 B	3.36 ± 0.69	1.18 ± 0.32 A
Non infected	43.36 ± 3.16	48.41 ± 3.40	5.95 ± 0.49 B	2.14 ± 0.60	0.18 ± 0.10 B

$P<0.05$

Table (6) Effect of seasons on *Eimeria* infection rates.

Season	No. of Examined Animal	*Eimeria* (%)
Winter	29	22(75.8)
Spring	43	35(81.3)
Total	72	57(79.1)

Table (7) Effect of *Eimeria* infection on weight gain of local breed rabbits according to sex.

Sex	Mean ± SE (g)
Males	1257.5±102.37
Females	1104.6±100.15

Discussion

This study has been carried out in order to investigate coccidiosis in the local breed rabbits. Till now, 15 *Eimeria* species are known which can infect rabbits, and all of them are parasites of the intestinal tract, except the *Eimeria stiedae*, which invades

exclusively the liver and the biliary tract (5). There is no significant difference in the prevalence of *E. stiedae* between male and female rabbits (P>0.05) (15). Oocysts of *Eimeria spp.* showed the highest percentage 72.5% that is agreed with (19) who recorded a high infection rate 73.9% in rabbits. Also (11) is found the prevalence in five provinces in domestic rabbits ranged from 73.36-87.54%. in Gansu province that had the highest prevalence of 87.54% which is followed by Shanxi, Ningxia, Xinjiang and Qinghai with prevalence of 78.09, 77.04, 74.99 and 73.36%, respectively. The overall prevalence of coccidial infections in Northwest China is 78.11% (1627/1622). (1) In Egypt he recorded an overall prevalence of 70% (70/100). Toula and Ramadan (20) reported that 90% of the examined rabbits are positive. More than a dozen *Eimeria* spp. are reported from the intestine of rabbits. Distinguishing morphologic features of the oocysts of intestinal *Eimeria* spp. is stated (21). However, with the possible exception of *E. neoleporis,* it appears that intestinal Coccidia of lagomorphs are host specific and that transmission to hosts of different genera does not occur. (21) About 14 species of *Eimeria* are examined namely, *E. stiedae* (17.81%; 289/1622), *E. media* (16.58%; 269/1622), *E. magna* (13.75%; 223/1622), *E. irrestidua* (23.98%; 389/1622), *E. piriformis* (8.69%; 141/1622), *E. exigua* (7.77%; 126/1622), *E. intestinalis* (9.31%; 151/1622), *E. coecicoIa* (10.05%; 163/1622), *E. neoleporis* (9.00%; 146/1622), *E. nagpurensis* (10.30%; 167/1622), *E. leporis* (6.04%;98/1622), *E.fIavescens* (8.75%; 142/1622), *E. matsubayashii* (6.98%; 113/1622) and *E. perforans* (19.61%; 318/1622). Kasim and Al-Shawa (22) reported that 73% of rabbits are found to have mixed infection with three Eimeria species. Kasim and Al-Shawa (22) reported that 73% of rabbits are found to have mixed infection with three Eimeria species. Also, Toula and Ramadan (20) reported five mixed Eimeria species and found that 90% of the examined rabbits are positive and that mixed infections with two to three species were most frequent. Eight species of Eimeria, namely *E. stiedae, Eimeria media, E. intestinalis, Eimeria coecicola, E. magna, Eimeria exigua, Eimeria perforans* and *Eimeria flavescens,* are identified from naturally infected rabbits in Egypt. Mixed infection with three different species occurred most frequently. *E. intestinalis* and *E. coecicola* are generally the most predominant species, while *E. magna, E. media* and *E. stiedae* are less common and *E.flavescence, E. exigua* and *E. perforans* are relatively rare (1). Coccidiosis usually spread from one rabbit to another through a rabbit's excrement or through soiled food or bedding (11). The first comprehensive study on the species spectrum of coccidia in hares in Austria is published by Kutzer and Frey (23). Other authors identified a total of eight coccidial species of which *Eimeria robertsoni, E. semisculpta* and *E. leporis* are the most common. (24) Chroust (25,26) who also reported eight species of which *E. robertsoni* and *E. leporis* caused infections in more than 70% of cases in the Czech Republic. (24) Visceral samples of 60 wild rabbits at necropsy, are selected and examined from districts of East Azerbaijan Province, where recorded *Eimeria magna* (5%) and *Eimeria steidae* 3.33%). (27) The prevalence is obviously low in rabbits whose cages have defecation device (self-cleaning) while the prevalence is high when rabbits are fed under crowded or poor sanitary conditions due to the primitive feeding equipment and hygiene status, coccidiosis in rabbits is widespread in rural areas in China (11). Zarzara *et al.* (28) found that the coccidian infection of liver is nearly 0.1%. While in Syria the abundance of infection is 4% (29). These variations may depend on geographical location, the difference in environmental conditions prevailing in each region, the rearing conditions, the number of samples examined, and the season of the year of study (30). In earlier studies by Catchpole and Norton (6) and Peeters and Geeroms (31), *E. stiedae* is not reported as a common protozoal infection in rabbits. (15). Gres et al. (32) reported the same finding in wild rabbits. The infection rate observed more in weanling rabbits in which there is no sex and

breeds differences. This isolate of *E. stiedae* multiplies in the epithelial cells of bile ducts and is considered as a pathogenic organism (30). *E. stiedae* like other *Eimeria* species enters into the intestinal wall, but it then migrates into the bile ducts, where it reproduces (33). The hepatic coccidiosis caused severe damage to the liver, particularly, in young rabbits and led to death (10,15). Functional tissue associated with immunity against coccidiosis is the gut associated lymphoid tissue (GALT) located in the intestine, along the mucosal layer and lamina propria. This lymphocyte forms act as an obstacle against the infection and takes part in the formation of antibody during the development of immunity against coccidiosis (34). The large amount of lymphocytes migrate actively to the mucosa of intestine confirms the presence of physiological response due to stimulation of sporozoites of *E. magna* that damages the intestine. (35) Intestinal Coccidia of the genus *Eimeria* are the most common protozoan parasites observed during the study (12).

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