

Research Article

Occurrence of *Eimeria* species in Naturally Infected Domestic Rabbits (*Oryctolagus cuniculus*) in North of Karbala Province, Iraq

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ABSTRACT

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Coccidiosis has an economic impact for poultry and livestock industries. The current study examined the prevalence of *Eimeria* infections in domestic rabbits in musaib city, North of Karbala province. A total of forty-eight faecal samples were taken from several market places located across the city. Individual fresh fecal samples were taken 24 hours prior in containers placed beneath the cages. After being collected, the excrement was moistened, placed in plastic bags, kept, and chilled at 4°C until it was examined. Freshly collected non-sporulated oocysts in each sample were put in Petri dish have 2.5% (W/V) aqueous solution of potassium dichromate. Every faecal sample was collected and then sent to a lab for oocyst detection. The identification of an infection was based on the physical traits. Sporulated oocysts were used to validate the identity of the samples containing *Eimeria* species, and they also provided morphological identification under a microscope. The prevalence of the population with coccidial illnesses was 34 out of 48 which equal for 70.8% . No significant differences were found between male and female at $P \geq 0.05$. Thirty-four rabbit infected with *Eimeria* species were present and identified from oocyst features. *Eimeria stiedae* and *E. magna* were the most prevalent species (25% and 21%), respectively. The results showed that the prevalence and distribution of coccidiosis was high among the rabbit population in musaib city, North of Karbala province. In conclusion, the epidemiological result must be taken into consideration in order to minimize the economic losses caused by coccidiosis.

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INTRODUCTION

Rabbits are a potential livestock resource that can provide high-quality meat that have more protein and less fat and cholesterol than other meats. Numerous *Eimeria* species are the source of the serious diseases known as rabbit coccidiosis, which costs the rabbit industry a great deal of money. Coccidiosis causes economic losses due to its negative impact on weight gain, growth, feeding efficiency, and death in weaning rabbits (Scialfa *et al.*, 2021). Only a small number of the eleven coccidian species that infected domestic and wild rabbits were linked to actual illnesses (Athanasίου *et al.*, 2023). Eimeriosis is a significant issue in rabbit farming, regardless of the maintenance approach. The disease influence the animals across all age groups, which lead to reduce body weight, impaired feed conversion, increased morbidity, and ultimately mortality (Shkromada and Nedzheria, 2020). Rabbit coccidia infects certain regions of the gut and at varying levels within the mucosa (Pakandl, 2013). Rabbits serve as a substitute for meat, wool, fur, economic, medical and are also often maintained as pets and used in research experiments (Ütük *et al.*, 2015; Shkromada and Nedzheria, 2020; Rabie *et al.*, 2022). Rabbits often suffer from coccidiosis, which is a significant health issue (Bachene *et al.*, 2018). Generally, rabbits are infected by a range of parasites, including ectoparasites and endoparasites. two forms of *Eimeria* spp. could be distinguished the first one is *E. stiedae* which can be deemed the most causative agent of hepatic coccidiosis and the second form is *Eimeria* spp. Which causes the intestinal coccidiosis (Rabie *et al.*, 2022). Endoparasitosis in rabbits is a frequent occurrence, particularly in domestic breeding environments. The findings indicated that rabbits bred for reproduction can carry coccidian infections without reveal any symptoms, and they have the ability to release oocysts at any point during their reproductive cycle. The morphological properties of coccidia oocyst were important for identifying the *Eimeria* species (Scialfa *et al.*, 2021). *Eimeria stiedae* infects the bile duct, resulting in hepatic coccidiosis in domestic and wild rabbits. Hepatic coccidiosis, which is caused by the parasite *E. stiedae*, leads to a significant infection, widespread outbreaks, and fatalities in juvenile rabbits (Ütük *et al.*, 2015). The identification of *Eimeria* species has traditionally relied on the examination of the morphological characteristics of sporulated oocysts (Li *et al.*, 2016). In the current study, to assess the infection of *Eimeria* oocysts in fecal samples of domestic rabbits were analyzed based on unsporulated oocyst morphology traits and sporulated method and considered the first study in north of Karbala province, Iraq.

MATERIAL AND METHODS

Location

The study was conducted among the rabbit populations in north of Karbala province, Iraq, which is located between the northern latitudes of 32.616667° and eastern longitudes of 44.033332°E.

Fecal samples

Fecal samples were collected randomly from different locations in the studied area and examined for the presence of oocysts. In these local markets, faecal samples were collected from 48 healthy rabbits. On the other hand While no consider the age factor was ignored due to the different sources of rabbits. Individual fresh fecal samples were taken 24 hours prior in containers placed beneath the cages. After being collected, the excrement was moistened, placed in plastic bags, kept, and chilled at 4°C until it was examined. By collecting, plastic tubes were used to collect samples. Then transferred directly to the laboratory (Department of Parasitology, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq) to analysis. A bout 100 g of fresh faecal pellets were collected from each animal alone, which considered as one sample. Each faecal sample was checked for infection then the type of infection was estimated and calculated as single, dual and triple infection.

Parasitological analysis

The prevalence and intensity of coccidial infection were detected by the coprological methods. The disease was diagnosed by fecal examination, oocyst sporulation. The species were determined based on oocyst morphology to identify the species composition (Karaer, 2001). Freshly collected non-sporulated oocysts in each sample were put in Petri dish have 2.5% (W/V) aqueous solution of potassium dichromate (K₂Cr₂O₇ at 24-26°C) with a good aeration (Ütük *et al.*, 2015). When the sporozoites inside the sporocysts were completely developed, the sporulation time was calculated (Murshed *et al.*, 2023).

Statistical analysis

The statistical package SPSS was used for data analyses, and a value of $P < 0.05$ was considered a significant difference in comparison. No significant by many factors genders, health status, parasitic species distribution and type of infections.

RESULTS

The result showed that out of the 48 examined rabbits, 34 were positively infected with *Eimeria* spp. In the examined rabbits, the total prevalence of *Eimeria* spp. was 70.8% (34/48), and co-infection with more than species of the genus *Eimeria* could be observed in all positive rabbits. Concerning the genders, the prevalence of *Eimeria* spp. was 76.7% (23/30) in male rabbits while it was 61.1% (11/18) in female rabbits. Significant differences were not observed ($P \geq 0.05$)(Table 1).

Table 1. Prevalence infection of health status and genders.

			Gender type		Total
			Male	Female	
Health status	Non-infected	N	7	7	14
		%	23.3%	38.9%	29.2%
	Infected	N	23	11	34
		%	76.7%	61.1%	70.8%
Total			30	18	48
X ²		1.318	P value	0.251	No sig.

The morphological characteristic of the recovered from the seven different *Eimeria* species were distributed at different percentages. High of *E. stiedae* were (25%), *E. magna* were (21%), *E. perforans* were (15%), *E. coecicola* and *E. exigua* were (11%), *E. piriformis* were (9%), while less prevalence in *E. media* was (8%) (Table 2). No significant difference of *Eimeria* prevalence was confirmed between males and females. Seven species of *Eimeria* spp. Among infected rabbits in Karbala were identified by coprological parasitological analysis as *E. stiedae*, *E. perforans*, *E. media*, *E. coecicola*, *E. exigua*, *E. magna* and *E. piriformis*.

Table 2. Prevalence of the percentage of *Eimeria* species in male and female.

Genders	Type of <i>Eimeria</i> species						
	<i>E.stiedae</i>	<i>E.perforans</i>	<i>E.media</i>	<i>E.coecicola</i>	<i>E.exigua</i>	<i>E.magna</i>	<i>E.piriformis</i>
Male	10	6	2	5	5	6	2
%	28	17	6	14	14	17	6
Female	3	2	2	1	1	5	3
%	18	12	12	6	6	29	18
Total	13	8	4	6	6	11	5
%	25	15	8	11	11	21	9

The total relation type of infection was 55.9%(19/34) in single infection, 32.3%(11/34) in dual infection and 11.7% (4/34) in triple infection. The total infection was (67.7% and 32.3%) in male and female, respectively. However in single, dual and triple infection were 60.9% (14/23), 21.7% (5/23) and 17.4%(4/23) in male, respectively. In female's have single, dual and triple infection were 45.4% (5/11), 54.5% (6/11) and 0% (0/11) respectively. (Table 3).

Table 3. Relation of type infection with genders.

Gender	Type of infection							
	Single	%	Dual	%	Triple	%	Total	%
Male	14	60.9	5	21.7	4	17.4	23	67.7
Female	5	45.4	6	54.5	0	0	11	32.3
Total of infected animals	19	55.9	11	32.3	4	11.7	34	

The oocysts of 7 *Eimeria* species collected from the domestic rabbits in the present study are illustrated in Figs. 1–2.

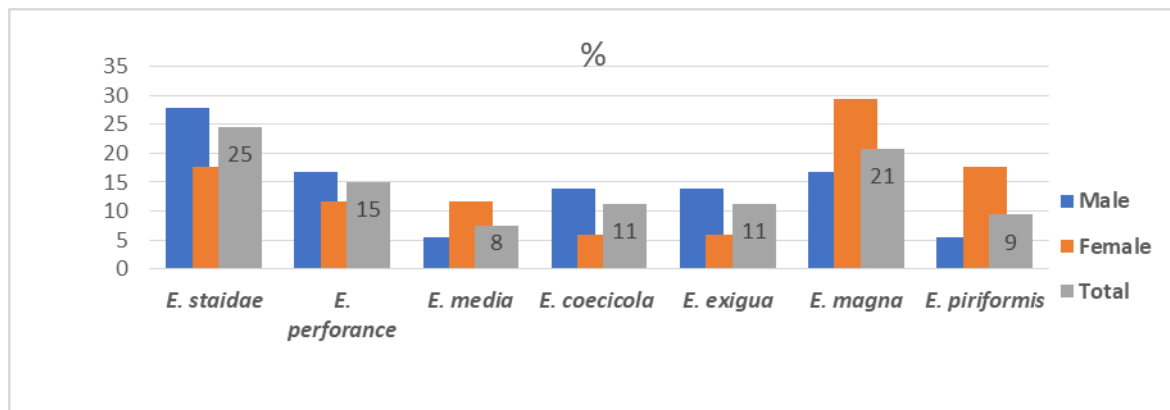


Figure 1. Distribution of the percentage of *Eimeria* species.

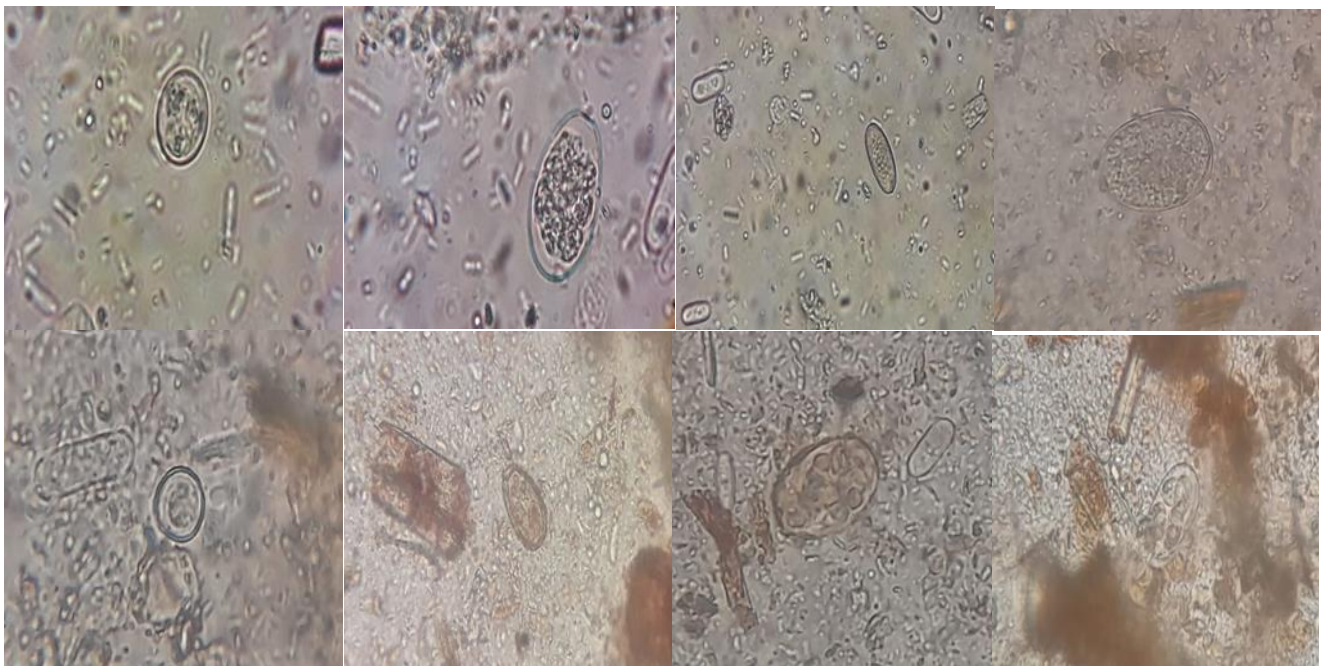


Figure 2: From left the above photo unsporulated and the under photo sporulated. The name from left, *E. exigua*, *E. magna*, *E. coecicola*, *E. perforans*

DISCUSSION

Eimeria spp. is one of the most protozoal parasites that has an important economic issue in the rabbit industries and production, which cause a disease called coccidiosis (Basiaga *et al.*, 2020). Rabbit coccidiosis is a common important disease caused by different intestinal and non-intestinal *Eimeria* species (Yuan *et al.*, 2022). In the current study, carpological examination was used for the detection of the presence of *Eimeria* spp. Preparation of slides and sporulation of oocyst are the main steps of the test and basically the diagnosis is depend on the morphological features (Yin *et al.*, 2016; Hamid *et al.*, 2019; Temim *et al.*, 2019; Al-Sadi and Al-Khafaji, 2000; Pilarczyk *et al.*, 2020; Gökpınar *et al.*, 2023) they were used sporulation and identification according to morphological characteristic in 2.5% potassium dichromate.

Importantly the results of this study showed that the prevalence of *Eimeria* spp., infection in domestic rabbits in musaib area, North of Karbala province was 70.8% (34/48) of the total tested samples. Thus, the high rates could be due to live with infected animal at the same cage, or less using of treatment drugs, stress factors and contaminated food. Our findings agree with the previous published evidence include Yin *et al.*, (2016) who recorded high prevalence of coccidial infection in rabbits (10-90%) in Sichuan Province, southwest China. Additional study from Indonesia has found that the prevalence rate was 70.3% (527/750) from fecal samples (Hamid *et al.*, 2019). The obtained result was in line with (Elhendy *et al.*, 2018), was 84% (42/50) in Assiut,

Egypt. While other studies were showed high prevalences as (Basiaga *et al.*,2020), was 100% in Poland and Ukraine, (Nicollas *et al.*,2022), in the Philippines,100%. In contrast, lower prevalence rates of infections were reported with different seasons of the year ranged from 42–15% in autumn-winter period, and 19–6% in spring-summer (Shkromada and Nedzheria,2020). (Temim *et al.*, 2019 was 47.6% in North of Algeria), (Rabie *et al.*,2022 was 50% in Egypt) and (Gökpinar *et al.*,2023, was 37.3% in Ankara and Kırıkkale provinces, Turkey). moreover, most of the previous reports in Iraq were showed various prevalence rates such as (Al-Sadi and Al-Khafaji, 2000, was 17% in Nenevah,). (Sulaiman,2005, was 57.14%, in Mosul). (Al-Moula,2005 was 76. 6% in Mosul), (Khider *et al.*, 2015, was 72.5%, in Baghdad) ,(Faraj, 2017, was 19% in Baghdad), and (Marhoon *et al.*,2018, was 61.82% in Al-Diwaniyah).

This difference in prevalence was showed to be associated with the variation in environmental factors geographical area such as the temperature, sporulation of oocysts, increase humidity, the use of chemoprophylaxis, ground breeding systems, kinds of feeding and number of samples were examined (Rabie *et al.*,2022). Thus, the practicality of detecting *Eimeria* infection in small rabbit farms is in the assessment of unsporulated oocyst shape, which aids in the control and prevention of rabbit *Eimeria* infections (Li *et al.*,2016).

From our finding , the prevalence of *Eimeria* spp. was 76.7% (23/30) in male rabbits while it was 61.1% (11/18) in female rabbits. *P* value non-significant (Table 1). these results were consistant with Khider *et al.* (2015) and Heker *et al.* (2017) who found no significant effects on the prevalence of *Eimeria* between male and female and *Eimeria* species. Also in accordance with the present results Pilarczyk *et al.*, (2020) showed no significant variation between male and female in the extensity of *Eimeria* infection. Similarly, the earlier reports of Al-Moula,(2005) who showed that the prevalence rate of infection in Mosul was 23/30 (76.6%) in domestic rabbits and mixed infection was more frequent with no significant variation between genders . Other reports were confirmed hepatic coccidiosis infection in ten hindered from rabbits (*Oryctolagus cuniculus*) from Baghdad city. The total infection rate was 19%, with no significant difference at the level of ($P>0.01$) (Faraj, 2017). The difference species distribution with clinical or without clinical signs may be increase probability of infection in animals. The findings indicate that local rabbits are asymptomatic and carriers of coccidian parasites and can shed oocysts all the time throughout the reproductive cycle (Scialfa *et al.*,2021). The oocyst stage of *Eimeria* spp. found ubiquitously in the environment and increases the risk of disease dissemination (Hamid *et al.*,2019).

Many of studies have been recorded about distribution of *Eimeria* species with different prevalences. In the current study, seven *Eimeria* species were identified include *E. stiedae* were (25%) the highest prevalence among the spp., followed by *E. magna* were (21%), *E. perforans* were (15%), *E. coecicola* and *E. exigua* were (11%), *E. piriformis* were (9%), and the lower prevalence was seen with *E. media* was (8%). Which is consistent with (Yin *et al.*,2016) who reported co- infection in Sichuan Province, southwest China 9 species of *Eimeria* were confirmed from positive samples. *Eimeria perforans* was the most prevalent species (42.73 %), *Eimeria media*(35.45%), *E. irrisidua* (34.55%), *E. magna*(31.82%), *E. intestinalis*(23.64%), *E. coecicola* (8%), *E. piriformis* and *E. flavescens* (6.36%). Other study was agreed with this study in Banyumas Regency, Indonesia. The prevalence 90.11% from 475 rabbits and were *E. exigua*, *E. perforans*, *E. flavescens*, *E. intestinalis*, *E. stiedae*, *E. vejdoskyi* and *E. caecicola*. They were showed that gender did not have a significant relationship ($P> 0.05$) (Indrasanti *et al.*,2020). While in Poland seven species were reported from 91 rabbits: *E. magna*, *E. media*, *E. perforans*, *E. stiedae*, *E. coecicola*, *E. exigua*, and *E. irrisidua*. From this infection demonstrated a significantly higher infection was *E. magna* (Pilarczyk *et al.*,2020).

Ten species of *Eimeria* spp. were confirmed in Yogyakarta, Indonesia. *Eimeria flavescens* was 80%, *E. coeciola* was 78%, *E. perforans* was 61%, *E. exigua* was 37%, *E. media* was 33%, *E. stiedae* was 31%, *E. irrisidua* was 12%, *E. magna* was 11%, *E. intestinalis* was 10%, and *E. piriformis* was 10% (Hamid *et al.*,2019). Co-infection with more than species of the genus *Eimeria* could be observed in all positive rabbits. In rabbits concurrent infections are common and generally, and can infect more than one species of *Eimeria* targeting both the liver and intestine (Murshed *et al.*, 2023). Many factors could influence the prevalence of coccidial infections which include protozoan species, size of samples, strain of animal, test type of diagnosis, geographical regions and mixing of infected with non-infected at the same cage. (Gökpinar *et al.*,2022) was found the prevalence of infection from the cages with two or more rabbits (88.1%), higher than the prevalence from the cages with a single rabbit (12.1%).

The total infection was 55.9%(19/34) in single infection, 32.3%(11/34) in dual infection and 11.7% (4/34) in triple infection. The total infection was (67.7% and 32.3%) in male and female respectively. While single infection more prevalent from the dual and triple. (Table 4). Single infection means that animals are infected at least with one species. the current findings agree with the results of the previous published reports in Iraq and other countries, significant differences were reported between single and mixed infection, with infection in (two-seven) *Eimeria* species was detected infection in Sichuan Province, southwest China (Yin *et al.*,2016).

It has been reported that out of 750 samples with 2-6 species from examined samples. *E. flavescens* and *E. coeciola* were the major infection among *Eimeria* spp. ($p \leq 0.0001$). *Eimeria* spp. is identified in high infection with commonly mixed infections. Triple infection is more than one and dual infections (Hamid *et al.*,2019). Ten species are identified from 102 feces samples using Fuelleborn's flotation technique, and arrange prevalence of infection from the maximum to lower *E. perforans*, and *E. media*, *E. magna*, *E. exigua*, *E. coecicola*, *E. intestinalis*, *E. piriformis*, *E. flavescens*, *E. stiedae* ve *E. irresidua* (Gökpınar *et al.*,2023). Other study was confirmed ten species in Assiut, Egypt by parasitological examination. Overall prevalence were *E. perforans* (66.7%), *E. exigua* (26.2%), *E. media* (26.2%), *E. magna* (21.4%), *E. intestinalis* (19%), *E. coecicola* (19%), *E. irresidua* (19%), *E. piriformis* (14.3%), *E. flavescens* (7.1%) and *E. stiedae* (7.1%). (Elhendy *et al.*,2018). In the same examined samples more than one species of *Eimeria* oocyst was detected. *E. magna*; *E. flavescens*; *E. exigua*; *E. stiedae* and *E. coecicola*, Single-infection 13/90 (14.4%), dual-infection 39/90 (43.3%) and triple-infection 38/90 (42.2%) from Riyadh City, Al Kharj and ADilam in Saudi Arabia (Murshed *et al.*, 2023).

While Khider *et al.*, (2015) was recorded (72.5%, 58/80) with highly infection *E. perforans* was (31.25%), *E. piriformis* 27.5%, *E. irresidua* 11.25%, *E. media* 17.5%, *E. flavescens* 28.75%, *E. coecicola* 13.75%, *E. exigua* 18.75%, *E. intestinalis* 23.75%, *E. magna* 28.75% and *E. stiedae* 17.5%. The study conducted by Elhendy *et al.* (2018) revealed that 23.8% of the infected rabbits had a single infection of *Eimeria* spp., whereas 76.2% of the rabbits had mixed infections including two, three, or four *Eimeria* spp. The prevalence of mixed infection was equally distributed among males and females, with no statistically significant differences seen (Khider *et al.*, 2015). The prevalence of *Eimeria* infections in domestic rabbits, was 47.6% (197/414) from 414 faecal samples were collected from 50 farms in six regions in Medea province, North of Algeria. Eleven rabbit *Eimeria*'s species were present and identified from oocyst positive samples. *Eimeria magna* and *E. media* were the most prevalent species (47.6% and 47.3%) (Temim *et al.*, 2019). In Iraq, many reports were summarized, using standard diagnostic laboratory test of naturally occurring infection of (300) rabbits reared in Nenevah Province, Iraq, were investigated (17%). Coccidial infection were included *E. stiedae*, *E. magna*, *E. perforans*, *E. media*, *E. intestinalis* and *E. irresidua* (Al-Sadi and Al-Khafaji, 2000). In the same region was reported in Mosul, eleven species were: *E. exigua* 38.57% , *E. perforans* 32.85%, *E. nagpurensis* 25.71%, *E. elongata* 17.14%, *E. stiedae* 15.71%, *E. media* 14.28%, *E. coecicola* 14.28%, *E. irresidua* 10%, *E. matsubayashii* 8.57%, *E. magna* 5.71% and *E. intestinalis* 5.71%, with total infection 57.14%. Triple infection and more was most common in rabbits was 67.5% (Sulaiman,2005). The important role of rabbits in the dissemination of protozoan and transferring the disease to both humans and animals. The higher prevalence also observed among the wild rabbits *O. cuniculus* in Al-Diwaniyah province/Iraq, 41/55 (74.55%) were infected with eight different types of external and internal parasites (Marhoon *et al.*,2018).

CONCLUSION

In conclusion, the current study revealed seven species were infected of local rabbit and this consider the first report of intestinal coccidiosis in Karbala province, Iraq. The prevalence of rabbit coccidial infection is high. Knowledge and evaluate of current prevalence of coccidiosis will benefit to plan of control programs to minimize the economic losses.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally.

ETHICAL APPROVAL

During the writing process of the study titled "Occurrence of *Eimeria* species in Naturally Infected Domestic Rabbits (*Oryctolagus cuniculus*) in North of Karbala Province, Iraq", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. Ethics required are approved by the Ethical Committee of college of veterinary medicine/ university of Kerbala under acceptance number- UOK.VET. MI.2023.065.

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