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All communications should be addressed to:

The Editor-in-Chief
Philippine Journal of Veterinary Medicine
College of Veterinary Medicine
University of the Philippines Los Baños
Laguna, Philippines 4031
Telefax No. +63-49-536-2727
Email: pjvm1964@gmail.com, pjvm.uplb@up.edu.ph

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(Original Article)

Cross-sectional Study of *Eimeria* Species in Local Rabbits in Baghdad, Iraq

Dalia Ahmed Kalef[1], Mohenned Alsaadawi[2], Maab Salah Jamel[1], Mustafa Adnan[1], and Omar Abdul Karim[1]

[1] College of Veterinary Medicine, Baghdad University

[2] College of Veterinary Medicine, AL-Muthanna University

*Correspondence: dalia.a.@covm.uobaghdad.edu.iq; mohenned.hemza@mu.edu.iq;

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Abstract

Background: Coccidiosis is caused by *Eimeria* resulting to diarrhea and weight loss in rabbits. This study aimed to investigate the prevalence of *Eimeria* species in local rabbits in Baghdad /Iraq. **Methods:** A total of 294 fecal samples were obtained from rabbits in local marketplaces and farms between November 2021 to October 2022. Data were recorded regarding the sex, age, and location of rabbits. The samples were transported to the Protozoology Laboratory /College of Veterinary Medicine/ Baghdad University, analyzing direct wet smear, and flotation techniques to determine the presence of coccidia. **Results:** The results found a 42.17% (124/294) infection rate of *Eimeria* species with highest rate observed in March (16.1%), and the lowest in October (1.6%). Male rabbits had a slightly higher incidence (51%) than females (49 %). Geographically, the highest rate was in Suq Alghazil and the lowest in AlGerma. Interestingly, rabbits under six months of age were more susceptible to *Eimeria* infection. **Conclusion:** This study showed presence of six species of *Eimeria* included: *E. intestinalis, E. perforans, E. magna, E. exigua, E. stiedai,* and *E. media.* Further research is needed to explore novel *Eimeria* species using nucleic acid implications and their relevance for human and animal health.

Keywords: *Eimeria,* Rabbits, Microscopic examination, Baghdad, Iraq

1. Introduction

Recently, rabbits have played an important role as an additional source of protein for Iraqi families. Currently, there is a lack of information regarding breeding practices, farm management and disease resistance of rabbits in correlation to improving rabbit production. Therefore, epidemiological studies of veterinary important pathogens in Iraq [1-5] can provide information on disease control programs to improve rabbit production. Coccidia has widespread distribution in different parts of the world and can infect a wide variety of animals including rabbits [6-8]. Intracellular protozoon parasites belonging to the genus of *Eimeria* are known to cause significant mortality rates in domestic rabbits [9]. This parasite is an obligatory intracellular parasite belonging to apicomplexan protozoa [10]. Twelve *Eimeria* species are known to cause coccidiosis in rabbits [11]. Coccidiosis, one of the most prevalent illnesses in rabbits, causes significant financial losses in the rabbit industry every year [12]. Coccidia may infect any domestic and wild rabbits, although it is more common in young rabbits less than 4 months than in old rabbits [13]. According to Meng *et al.* [14], morbidity and mortality rates can reach up to 90% in young rabbits. There are

twelve species of *Eimeria* that infect rabbits. These are *Eimeria* stiedai, E. intestinalis, E. flavescens, E. irresidua, E. magna, E. media, E. piriformis, E. coecicola, E. exigua, E. perforans, and E. vejdovskyi. Two clinical forms of coccidiosis affect rabbits; the hepatic form is caused by *Eimeria* stiedae [15] and the intestinal form, caused by E.magna, E.irresidua, E.media, E. perforans, E. flavescent, E. intestinalis, or others *Eimeria* species [16]. Two species of *Eimeria* are considered the most pathogenic species: *Eimeria* magna and *Eimeria* irresidua. In Iraq, prevalence studies on rabbit coccidiosis have been difficult due to the variety of rabbit raising sites. Rabbits are sold in public markets and transported to different provinces of Iraq. Currently, there is little knowledge about *Eimeria* in rabbits in Iraq, especially in Baghdad. Therefore, this study aimed to detect *Eimeria* oocysts in local rabbits in Baghdad using morphological characterization and sporulation time.

2. Methodology

2.1. Location

The samples were collected from eight selected regions in Baghdad that have been identified with large populations of rabbits. These locations are: Abu ghraib, Al-Amiriya, AlBayaa, AlGazaliyah, Suq Alghazil, AlTajea, Animal House-College of Veterinary Medicine, Baghdad University, and AlGerma.

2.2. Sample Collection

A total of 294 fecal samples were collected from *Oryctolagus cuniculus* rabbits bought from local markets and farms from different regions in Baghdad city. According to age, the examined animals were classified into two groups; less or more than six months old. Fresh fecal samples were transferred in sterile containers to Protozoology Laboratory at the College of Veterinary Medicine, Baghdad University, for isolation and identification of *Eimeria* oocysts. The study period was twelve months from November 2021 to October 2022. Data including rabbit age, sex and date of collection were recorded on the containers of the samples.

2.3. Isolation and Sporulation of *Eimeria* Oocysts.

Fecal samples were processed and *Eimeria* species oocysts were identified by keeping them in a 2.5 % potassium dichromate solution to induce sporulation [17]. Samples were microscopically examined using saturated salt concentration technique for isolation of oocysts for sample examination. A volume of 9 ml of distilled water was added to 1 g of feces, and centrifuged at 800× g for 5 min. Then, the sediment was diluted with 10 mL of salt solution and centrifuged for 5 min at 800x g. Finally, the floatant was examined under light microscopy [18].

2.4. Floatation Method Using NaCl

The flotation solution should have a higher specific gravity than the *Eimeria* oocysts in order for them to float [19, 20]. Five grams of the samples were diluted in distilled water (20 mL) and filtered using four layers of clean gauze to remove the fecal debris. Then, 10 mL of NaCl solution (357 grams of salt in 1L H2O) was mixed with the sediment that was poured from the beaker/fecal cup into a 15 mL centrifuge tube for centrifugation. The rest of the tube was filled with a flotation solution to a slightly positive meniscus and covered with a coverslip. The coverslip was removed from the tubes and placed on slides that were labeled with for identification. The entire coverslip was examined under the microscope at 10X and then 40X to identify *Eimeria* oocysts and the result was recorded [16].

2.5. Isolation of Oocysts for Measurement

Identification of *Eimeria* species in rabbit fecal samples was based on morphology during the sporulation phase. *Eimeria* oocysts were detected and scanned under the light microscope at 40x magnification. The length and width of the oocysts were measured using an ocular micrometer to detect the species of intestinal and hepatic *Eimeria* spp. [21]. In addition, the presence or absence of micropyle was also recorded.

2.6. Data analysis

The obtained results of *Eimeria* spp. in relation to risk factors was statistically analyzed using the IBM SPSS Statistics version 28.0. Statistical significance was evaluated using Chi Square and t-test.3.

3. Results

The total infection rate of rabbit coccidiosis in all investigated areas was 42.17% (124/294). Different criteria were investigated like: the location, period of sample collection, sex and age of the rabbits.

3.1. Infection rates of *Eimeria* species according to the area of study

The results found that the incidence of *Eimeria* species varied across Baghdad. Suq alghazal recorded the highest positive results of *Eimeria* (35.5%), followed by AlBayaa and Al-Amiriyah with (16.1%) and (14.5%), respectively. Abu Greab and AlGerma were recorded with the lowest rates of (3.2%) and (4%), respectively (Figure 1)

The randomly collected data were recorded, arranged according to the collecting region, and analyzed using the SPSS program, Chi-square test, P≤0.05 (A and B). Negative (–) and positive (+) results indicate the absence or presence of coccidia. The highest rates of infection were in Suq Alghazel while the lowest rate was recorded in Abu Greab (C). ****= P≤0.0001.

3.2. Infection rates of *Eimeria* species based on time of collection

According to the time of sample collection, the highest rate of infection with *Eimeria* species was in March followed by April at a rate of (16.1%), and (14.5%), respectively. December recorded (8.1%) prevalence from the total incidence. The lowest rate was in October (1.6%), January (3.2%), and September (3.2%), as shown in Figure 2.

The collected data were categorized for each month. – and + in the figure refer to the samples that showed a negative or positive result when they were tested for parasitic existence. The data were analyzed using Chi-square (SPSS),

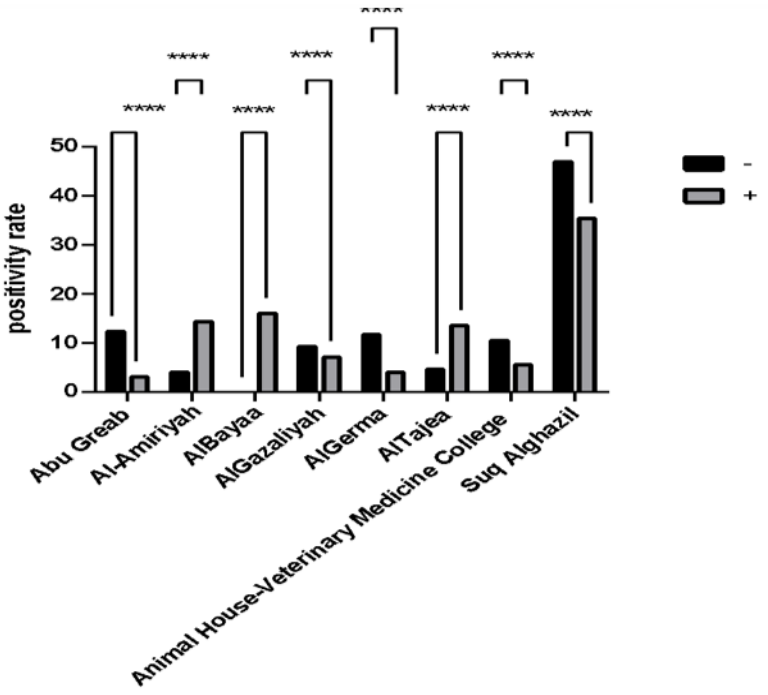

Fig. 1. The positive results of *Eimeria* species based on region.

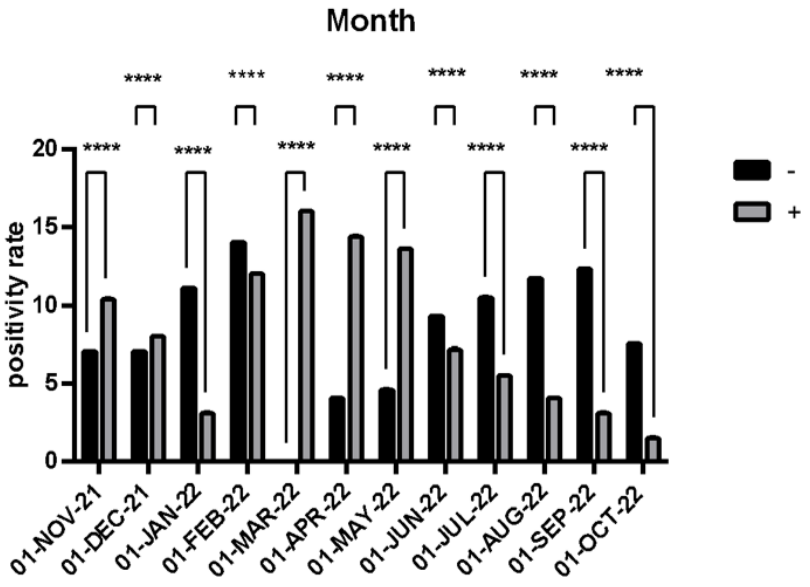

Fig. 2. The positive infection rates of *Eimeria* species infection according to the months of the study.

P≤0.05 (A and B). March has the highest rates while October is the lowest (C). ****= P≤0.0001.

3.3 The positive results of *Eimeria* species infection according to the sex of the rabbits

According to the sex of the rabbits, the result of this study displayed that the infection was comparable in males (51%) and females (49%) with no significant differences (Figure 3).

The sex of each sample was documented and calculated for the statistical differences (A and B) using Chi-square (SPSS), P≤0.05 which showed no significant differences were found (C). – and + in the figure refer to the samples that showed a negative or positive result when they were tested for parasitic existence. ****= P≤0.0001.

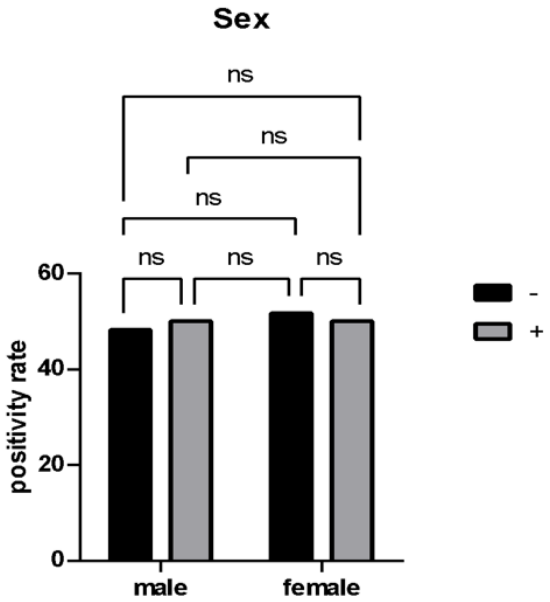

Fig. 3. The occurrence of *Eimeria* species according to the sex of the rabbits.

3.4. The infection rate of *Eimeria* species infection according to the age of the rabbits

Age-related findings revealed that rabbits less than 6 months had a higher infection rate (31.81%) than rabbits younger than 6 months of (7.27%) (Figure 4).

Ages of the samples were categorized into two groups, less than 6 months and more than 6 months. The data were analyzed using Chi-square (SPSS), P≤0.05. Younger rabbits of less than 6 months were more infected than older rabbits. ***= P≤0.001.

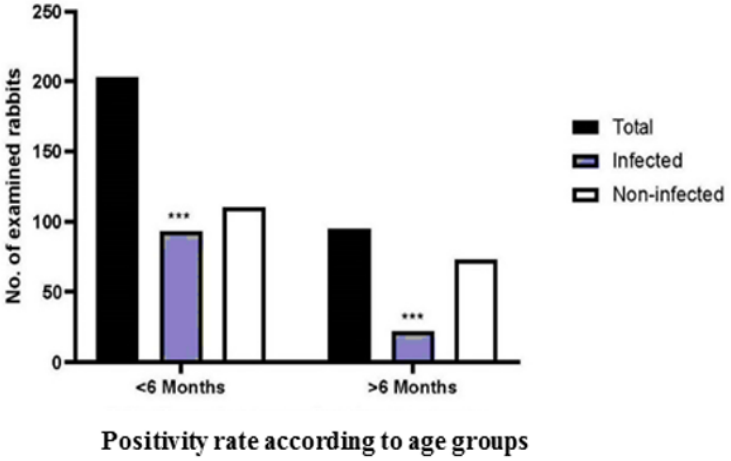

Fig. 4. The rate of infection of *Eimeri*a species infection according to the age of the rabbits

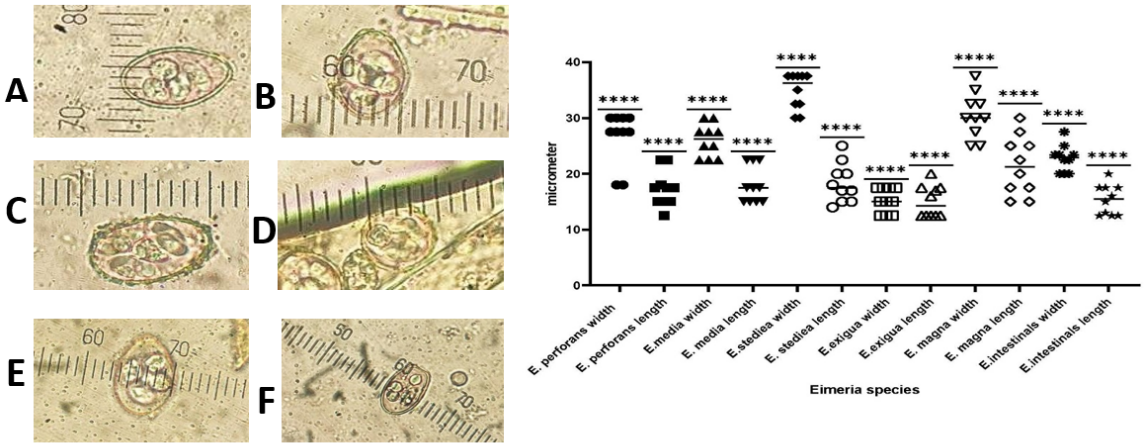

Fig. 5. The characteristic features of *Eimeria* species in Rabbits
Right Panel: spreading of oocysts measurements of *Eimeria* species in Rabbits. The measurements of each species have a statistical difference of P≤0.05 using simple t-test analysis; **Left Panel:** The *Eimeria* species detected in the fecal samples of the rabbits in this study at a magnification of (×400). | *A, E. intestinalis; B, E. perforans; C, E. magna; D, E. exigua; E, E. stiedai; F, E. media.* ****= P≤0.0001.

3.5. *Eimeria* species were recorded in the study according to morphological characteristics.

The species found during this research were verified in the fecal samples of infected rabbits according to the measurements of the sporulated oocysts. Six species were detected under the light microscope at a magnification of 400x. They are *E. intestinalis, E. perforans, E. magna, E. exigua, E. stiedai,* and *E. media* as illustrated in Figure 5.

4. Discussion

Eimeria in rabbits is an important protozoan disease due to the ability of this parasite to infect vital organs, such as the liver and heart [22, 23]. It is crucial to consider the implications of these findings for both animal health and potential economic impacts on rabbit farming. Twelve species of *Eimeria* are the main protozoan parasites in rabbits [11], particularly E. stiedai, which uniquely occupies the bile ducts, which is of notable concern due to their potential to induce severe pathological changes [24]. The other *Eimeria* species that contaminate the alimentary tract and give gastrointestinal disturbances, malabsorption, and secondary infections that complicate the health status of infected rabbits. The findings of our study reveal a significant overall positive rate of *Eimeria* species among local rabbit populations in Baghdad, at 42.17%. From diverse geographical areas within Baghdad city, with a prominent 35.5% (44/124) positive rate observed in Suq Alghazil, a prominent market for pet animal trade, compared to a mere 3.2% (4/124) in the more controlled environment of Abu Greab. Factors contributing to the rates may include husbandry practices, the density of rabbit populations, inadequate biosecurity measures, and overcrowding. Previous research in Baghdad indicated a remarkably high prevalence of (72.5%), mainly intestinal coccidiosis [25]. In contrast to the 12.5% rate observed in Egypt [26] and the 32.24% prevalence of *E. stiedae* in Saudi Arabia in domestic rabbits [17]. These inconsistencies of coccidiosis epidemiology in these researches due to the variation of data collection numbers, seasonal variations and the genetic diversity of rabbit variations in susceptibility to coccidiosis. This research highlight significant temporal variations in the rate of coccidiosis infection, with a peak incidence of 16.1% (20/124), observed in March and a notable decline to 1.6% (2/124) in October. Wet seasons, characterized by increased humidity and rainfall, create an optimal environment for the sporulation of *Eimeria* oocysts, which are typically met in the early spring months. Our results indicated that there are no significant in positive rates between male and female, this outcome aligns with the results reported by Ola-Fadunsin *et al*. [27]. From a biological perspective, the absence of sex-based differences in infection rates suggest that the pathogen's does not discriminate between male and female hosts. The findings of this study indicate a significant difference in the positivity among rabbits based on age. Specifically, rabbits younger than six months exhibited a notably (46.5%) rate compared to their older counterparts, who showed a rate of only (23.4%). This inconsistency with previous research, which recognized no variations of age-related infection between adult rabbits and weaners [28]. The higher rates observed in younger rabbits can be largely attributed to their underdeveloped immune systems as documented in various parasitic infection [29]. The specific strains of *E. stiedai, E. intestinalis,* and *E. flavescens* infection are known to be highly severe in young rabbits [30]. The observed signs of these infected rabbits including weight loss and high mortality rates [11]. The identification of mixed infections across the collected samples indicates a both intestinal and hepatic in rabbits of Baghdad city. The identification of six *Eimeria* species, namely, *E. intestinalis, E. perforans, E. magna, E. exigua, E. stiedai,* and *E. media* underlines the importance of intestinal and hepatic forms of these parasites as aligned with previous study that recorded a total of ten *Eimeria* species in local breed rabbits [25]. The prevalence of this coccidian parasite in rabbit populations observed in different geographical regions. In Nigeria, a remarkable 78.6% prevalence was recorded, identifying seven *Eimeria* species [27]. In contrast, in China identified only eight species of *Eimeria* by using both morphological and PCR techniques [30]. In Indonesia, the identification of ten *Eimeria* species at a rate of 70.3% in rabbits [8]. This discrepancy

suggested that environmental, ecological, and management factors may influence *Eimeria* diversity and prevalence. Each species of *Eimeria* possesses distinct morphological characteristics that can be examined by microscope and significant differences in measurements of individual species were documented in this study. The variations in the size and shape of this protozoa is critical in identifying and managing infections. The implications of these findings extend to practical relevance in rabbit farmers and to improve effective management strategies.

5. Conclusions

This study conducted the incidence of rabbit coccidiosis in Baghdad city was 42.17% (124/294). It showed that the positivite rates of *Eimeria* species Suq alghazil had the highest rate of (35.5%), and Abu Greab had the lowest positivite results of 3.2%. According to the months of the study, the highest infection rates with *Eimeria* species was in March, a rate of (16.1%), and the lowest rate was in October (1.6%). The findings also indicated no significant in the infection rate of coccidiosis between male and female rabbits with rates of (51%) and (49%), respectively. Six *Eimeria* species namely *E. intestinalis, E. perforans, E. magna, E. exigua, E. stiedai,* and *E. media*, were identified in this study.

Author Contributions

Conceptualization, D.A.K. and M.S.J.; Data Curation, D.A.K. and M.S.J.; Investigation, D.A.K. and M.S.J.; Methodology, D.A.K. and M.A.; Formal analysis, D.A.K. and M.S.J.; Writing - original draft preparation, D.A.K. and M.A.; Visualization, M.A.; Writing - review and editing, M.A.; Project Administration, M.S.J.; Collection, recording and arranging of the samples, M.A. and O.A.K.

Ethics Approval and Consent to Participate

This study was a part of a bigger project that was technically approved by the Scientific Committee at the College of Veterinary Medicine at Baghdad University. The Ethical Form was numbered D.A. 671, dated 16/03/2022.

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Conflict of interest

The authors declare that they have no conflict of interest.

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