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Molecular diagnosis of naturally infection with *Eimeria stiedae* in domestic rabbits in Baghdad city-Iraq

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Abstract
This study was designed for parasitological and molecular diagnosis of hepatic coccidiosis in rabbits. The ten hindered of local breed rabbits (*Oryctolagus cuniculus*) of both sexes were used. The animals were brought from the local markets in Baghdad city during the period from the beginning of January 2016 to 30th May 2016. The total infection rate was 19%, in which 18% males and 20% in females respectively with no significant difference at the level of (P>0.01). The high infection rate in young rabbits were (30%) and in adult (8%). The DNA amplification was confirmed by the electrophoresis analysis depending on DNA marker (976 bp). The results of this study confirmed high infection rate and more efficient monitoring of infection by using polymerase chain reaction as a the modern molecular diagnostic techniques of hepatic coccidiosis.

Keywords: *Eimeria stiedae*, hepatic coccidiosis, Rabbit, PCR, Baghdad city

1. Introduction
Rabbit were reared for fur production, medical and biological purposes [1]. Domesticated rabbits infected by up to eleven species of *Eimeria* sp. Including *E. stiedae* [2], which is one of the primary parasites with severe infection (hepatic coccidiosis) [3],which infects the epithelial cells of the bile ducts and causes massive liver damage [4,5]. Also affects the both body weight and feeding rates that can result in the death of infected animal [6]. Many sensitive diagnostic methods were used for this parasite such as ELISA and molecular assays and the later based on genomic marker that identify the 11 *Eimeria* species of the domestic rabbit [7]. Also, the coccidian ribosomal RNA internal transcribed spacer 1 (ITS-1) genomic region has been used as a target for PCR amplification in chicken species [8,9]. The present study was designed for parasitological and molecular diagnosis of hepatic coccidiosis in rabbits (*Oryctolagus cuniculus*) in Baghdad city-Iraq.

2. Materials and Methods
2.1 Samples collection
A total of 100 local breed rabbits (*Oryctolagus cuniculus*) of both sexes were used. Animals were brought from the local markets in Baghdad city during the period from the beginning of January 2016 to 30th May 2016. After slaughtered of the animals, the oocysts of *E. stiedae* were collected from gall bladders then transferred to clean sterile Petri-dish containing, potassium dichromate solution (2.5%) till used [10] and their measurements (dimensions) by ocular micrometer [11].

2.2 DNA extraction and Purification:
Isolated oocysts were washed with distilled water and several centrifugation (2500/ min.) for 5 minutes. DNA extraction was done according to the protocol described by Fernandez *et al* [12]with some modification by treated the oocysts with SDS(0.5%) and proteinase K (100µg/ml) in extraction buffer (Tris–Hcl 10mµ, PH O.8; EDTA 50mµ,PH8.0)for 2H AT 50 ºC, which facilitate the subsequent oocysts disruption and increase the final DNA yield.
The extracted DNA was used in PCR with *E. stiedae* specific primer set Es1F and Es1R (forward primer 5º -ACCATGGGTCGGTTCGGTC-º3 and reverse primer 5º -ATGCGCGCGCCAACAAGCTAC- º3), 976 bp [13]. PCR amplicons were electrophoresed through a 1.5% agarose gel in tris acetate.

2.3 Statistical analysis
Statistically analyzed of the data were done by using Chi-square tests with significance difference (P<0.01) (SPSS).

3. Results
The total infection rates of *Eimeria stiedae* in local breed rabbits were 19%, in which 18% male and 20% female, with no significant difference (P<0.01)(Table1). Microscopic examination of oocysts showed that *E. stiedae* oocysts was oval shape with a small cap, smooth wall and no residual body with average dimensions measuring 30.15×18.33 µm (Fig. 1).

Table 1: *Eimeria stiedae* infection rate according to sexes in local breed rabbits.

Sexes	No. of animal examined	Infected	%
Males	50	9	18
Females	50	10	20
Total	100	19	19

P<0.01

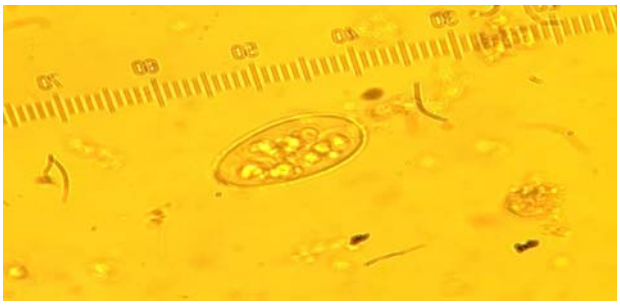

Fig 1: sporulation oocyst of *Eimeria stiedae* by flotation method, Eosin stain (X100)

A significant difference (P<0.01) was showed among different age groups. The high infection rate was recorded in young rabbits (30%) and lowest infection rate in the adult rabbits 8% (Table 2).

Table 2: *Eimeria stiedae* infection rate according to age in local breed rabbits.

Age	No. of animal examined	Infected	%
Young	50	15	30
Adult	50	4	8
Total	100	19	19

P<0.01

The PCR amplification was performed on the DNA extracted from all isolates were confirmed by the electrophoresis analysis depending on the DNA marker (976 bp DNA) (Fig. 2).

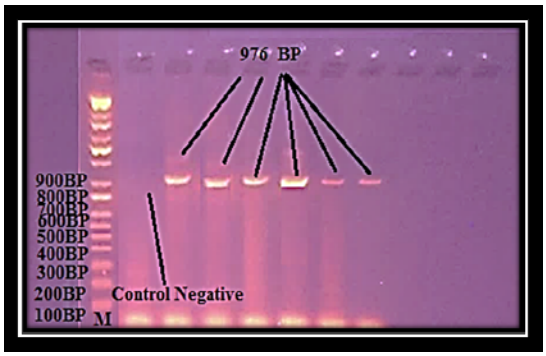

Fig 2: Amplification of *Eimeria stiedae* RNA ITS-1 by PCR. Lane 1 was negative and Lanes 2, 3, 4, 5, 6, 7 positive (976 bp band).

3. Discussion
Till now, more than 10 *Eimeria* species were infected rabbits. All of them are parasites of the intestinal tract, except the *E. stiedae*, invades the liver [14]. The results of present study showed that rate of infection of *E. stiedae* in local rabbits in Baghdad city was 19%. These results were in agreement with Al-Mathal [15] in Saudi Arabia who showed that the prevalence of *E. stiedae* in rabbits were 32.24%. In Iran Tehranin *et al* [16] were indicating that the most important species of rabbits coccidiosis was *E. stiedae* (26.87%).While Khider *et al* [17] found the infection with *E. stiedae* compared to other species of *Eimeria* where it approached (3.75%) in Baghdad. This variation of the infection rates may be due to a difference of examined animals or climatic and geographical condition. Morphological features, such as oocyst size, shape and micropyle (conspicuous) were compatible in the results with [18]. The results of our study show that, there are a closely infection rate between males and females, this results was agreement with [16, 19]. This could be due to both sexes exposed to same environmental condition. Also the present study may be indicated that the age may affect the infection rates. That was confirmed by the significant high prevalence of *E. stiedae* in the young than in adult. Similar observations were reported in rabbit [20]. This could be attributed to lower resistance in the young compared to older animals. In for our experience, it is difficult to distinguish between oocysts species by using microscopically alone, but the molecular assays, which are specific and highly sensitive, may be able to supplement the classical methods for diagnosis [9]. Also, identification of *Eimeria stiedae* in rabbits, were based on a set of morphological features which is time-consuming and inefficient [21]. In the same way, molecular assays have been introduced to supplement morphological identification, and some of them are based on 18S rRNA gene [22, 23, 24]. In this study, the RNA ITS-1 genomic region was too highly conserved to be used to identify rabbit *E. stiedae*.

4. Conclusion
In present study, it was investigated that the *E. stiedae* is only contribute for rabbit hepatic coccidiosis infection while other *Eimeria* species invade the intestinal tract. The infection rate is highly in young rabbits than adult, and this is the first molecular diagnosis done in Iraq for identification of *E. stiedae* in rabbits as pathogenic species.

5. Acknowledgements
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