

Molecular and histological diagnosis of *Eimeria* spp. (Camelus dromedaries) at AL-Najaf AL-Ashraf provinces , Iraq

Molecular diagnosis with histological study of *Eimeria* spp. (Camelus dromedaries)atAL-Najaf AL-Ashraf provinces

Abstract

Many nations rely heavily on the export of Old World camels for their economic needs. They are employed in camel transportation, the production of food such as meat and milk, and racing. Many countries rely heavily on Old World camels for their economies, utilizing them in transportation, food production (meat and milk), and racing.

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Eimeria affects a wide variety of domestic including camels and wild animals,. The aims of this study research was to study the distribution and histological effect of *Eimeria* spp. camels (*Camelus dromedaries*) using Molecular diagnosis with and histological studies. A total of 100 fecal samples of 100 camels (*Camelus dromedaries*) were examined by conventional PCR from different regions at AL-Najaf AL-Ashraf province of Iraq, from November 2023 up to end of April 2024. A total infection rate with *Eimeria* spp was 57% (57/100) , male camels recorded higher infections than females. 63.2%(24/38) .The age group of males at (<5years) show the higher rate of infection 76.7%(23/30) while the females (10 years >) recorded 80.8%(9/11) in age (10 years >) as a higher infection rate. District Al- Manatherawas recorded 71.4% (12/29) with significant differences at (P<0.05) compared with another district of AL-Najaf AL-Ashraf provinces.

A total of 20 samples collected from small intestine of slaughtered camels were collected. Histopathological analysis of the infected tissues revealed granulomatous, hemorrhagic, and chronic enteritis; in some cases, the intestinal glands have a rose-shaped appearance, while in other cases, there is a severe infiltration of chronic inflammatory cells with no intestinal gland structure; hypereosinophilia and various developmental stages of *Eimeria* are also present. In conclusion.....;

Keywords: Food, *Eimeria*, infection, *Camelus dromedaries* molecular diagnosis , Histopathologica

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Introduction

The camel is a significant and unusual mammal that has adapted to survive and procreate in the intense heat and dryness of the desert. a significant food source, transit for African and Asian desert nomads. One of the best kinds of milk is camel's milk, because it has more iron and vitamin C than cow milk. from cows. *Eimeria* spp is one of the parasitic infections, particularly gastrointestinal which have had impact on camel's development and output. The economic harm they cause, including the mortality of animals and the diminution of livestock products, is significant, particularly in young animals (Kamal et al., 2024) .The feces of both symptomatic and carrier animals contain oocysts shed by host-specific protozoan parasites called coccidia. These carriers, can transmit infection through water and food sources

(Jilou et al., 2022). Coccidiosis is an obligatory illness that causes hypertrophy and death in parasitized cells (Hassan et al., 2024) Coccidia can cause injury to host's intestinal cells cause anemia and a loss of Electrolytes (Murshed et al., 2024)

When animals consume contaminated feed or water, the parasite injures intestinal cells, causing diarrhea and hematochezia (Utebaeva *et al.*, 2021). The great resilience of oocysts to environmental conditions is a crucial aspect of the infection's epidemiology (Gao *et al.*, 2024).

Eimeria's classification was based on the morphological features of both sporulated and non-sporulated oocysts (Hegazi *et al.*, 2023).

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Materials and Methods

A total of (2) grams fecal samples were taken from (100) camels (38 male, 62 female) of various ages from ~~47~~ certain distinct regions in Al-Najaf, Al-Ashraf, between November 2023 and the end of April 2024. Fecal samples obtained in sterile plastic containers were firmly sealed, and safety precautions such as disposable gloves were removed owing to consecutive numbers. All sample information, including age, gender, and date, were reported. Samples then sent in an ice cooling box to a the laboratory of Parasitology / College of Veterinary Medicine- University Al-Qasim Green University / Iraq for Molecular diagnosis.

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Twenty samples were collected from the intestine of slaughtered one humped camels at Al-Najaf, Al-Ashraf. Tissue samples were performed within 1 to 5 hours after death. Samples were taken from intestines which showed gross lesion and used for histopathological examination.

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Genomic DNA Extraction

Genomic DNA from feces-fecal samples were extracted by using Presto stool DNA Extraction Kit, (Geneaid Biotech /China) and done according to the company instructions.

Polymerase chain reaction (PCR)

The PCR technique was performed for detection of *Eimeria spp.* in feces-fecal samples in for camels based on 18S small subunit ribosomal RNA gene, This method was carried out according to the method described by (Zainabet *et al.*, 2016)

Preparation of histological sections:

Samples from various regions of the small intestine (duodenum, colon, and jejunum) were collected and fixed in 10% neutral buffered formalin solution, then washed, dehydrated in various grades of ethyl alcohol, clarified in methyl benzoate, and embedded in paraffin wax. Blocks were handled according to conventional processes. Used hematoxylin-eosin to stain 5-µm thick sections for microscopy-microscopic analysis according to (Spencer *et al.*, 2012).

Statistical analysis

The Chi-square test was used to detect the association between parasite infection rate and each animal (camel). P-values < 0.05 were regarded statistically significant using SPSS (statistical package for social

sciences) version 26 (SPSS, 2020). User Guide Statistic Version, 26th Edition. SPSS, the statistical tool for social science, user handbook statistical version, sixth edition.

Results

The total rate of infection with *Eimeria* spp. in camels (*Camelus dromedaries*) by Molecular diagnosis from different districts in AL-Najaf AL-Ashraf province of Iraq, during November 2023 to end of April 2024. A total infection rate with *Eimeria* spp. was 57% (57/100) (Table 1).

Table (1): Rate of infection with *Eimeria* spp. in camels by using Molecular diagnosis.

Host	Fecal sample examined	Molecular diagnosis	
		NO. of positive	Percentage%
Camel	100	57	57

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Infection rate with *Eimeria* spp. in camels according to sex by Molecular diagnosis:-

According to sex (male and female) infections were recorded as 63.2% (24/38) and 53.2% (33/62) rate of infection respectively, with no significant differences ($p \leq 0.05$) (Table 2).

Table (2): Infection rate with *Eimeria* spp. in camels according to sex.

Sex	No. of samples examined	No. of positive	Percentage (%)
Male	38	24	63.2
Female	62	33	53.2
Total	100	57	57
X ²	0.948229		
P value	0.330171		

No significant differences at ($P \leq 0.05$).

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Rate of infection with *Eimeria* spp. in male camels according to age groups

Infection with *Eimeria* spp. was recording the highest rate in camels group (.....) 76.7% (23/30), while recording in groups (10 years \geq) 0% (0/2) with significant differences ($p \leq 0.05$) (Table 3). 92

Table (3): Rate of infection with *Eimeria* spp. in camels male according to age

exam.samples		No.	% of total
5 years \leq	30	23	76.7
5-10 years	6	1	16.7
10 years \geq	2	0	0
Total	38	24	63.2
χ^2	11.354762		
Pvalue	0.003423*		

*: significant differences at ($P \leq 0.05$).

Table (4): Rate of infection with *Eimeria* spp. in female camels according to age groups

Highest rate of infection with *Eimeria* spp. was recorded in female 81.8 % (9/11), while lowest recorded in groups (5 years \leq) 10 % (2/20) with significant differences ($p \leq 0.05$) (Table 4).

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Table (4): Rate of infection with *Eimeria* spp. in camels female according to age

Age(year)	No. of the exam.samples	Positive samples	
		No.	% of total
5 years \leq	20	2	10
5-10 years	31	22	71
10 years \geq	11	9	81.8
Total	62	33	53.2
χ^2	22.541921		
Pvalue	0.000013**		

** significant differences at ($P \leq 0.05$).

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Rate of infection with *Eimeria* spp. in camels according to the district in AL-Najaf AL-Ashraf

The rate of infection with *Eimeria* spp. in camel samples at three districts as follows: AL-Manathara was 71.4% (25/35), Slaughter house was 55.6% (20/36), and AL-Meshkab was 41.4% (12/29) respectively with no significant differences ($p \leq 0.05$) (Table 5).

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Table 5): Rate of infection with *Eimeria* spp. in camels according to the district in AL-Najaf AL-Ashraf

District	No. of the exam. samples	Positive samples	
		No.	% of total
Slaughterhouse	36	20	55.6
AL-Manathara	35	25	71.4
AL-Meshkab	29	12	41.4
Total	100	57	57
χ^2	5.890539		
Pvalue	0.05*		

*: significant differences at ($P \leq 0.05$).

Conventional PCR product analysis:

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114 Genomic DNA was obtained from camel fecal samples and subjected to molecular analysis by using PCR for detection of small subunit ribosomal RNA gene.

115 Specific primers were used to identify the species of *Eimeria*. PCR of 100 samples was employed in the study exhibited a distinct band of (456 bp) from the PCR product on the agarose gel.

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Figure 2D PCR products of amplification of 18S RNA gene for detection of *Eimeria* species. Size of PCR product: 456 bp. The gel was run at 128.5% and the DNA dye is RedSafe (Intron, Korea). Electrophoresis conditions: V: 95, Time: 45 minutes. M: DNA ladder

Explain positive and negative samples

DNA sequencing results

7-125 out of 57 samples from were positive by using Conventional PCR. and Haplotypes of sequencing sequences were deposited to in to NCBI Genbank data base and DDBJ to get accession number codes (PQ100675, PQ111747, PQ129311, PQ129315, PQ129400, PQ120694 and PQ113350) for of local *Eimeria* species at for the first time in Iraq.

Phylogenetic confirmative detection

Specific phylogenetic confirmative detection of local *Eimeria* species was performed by using the phylogenetic tree analysis and compared with NCBI-BLAST *Eimeria* species (figure2).

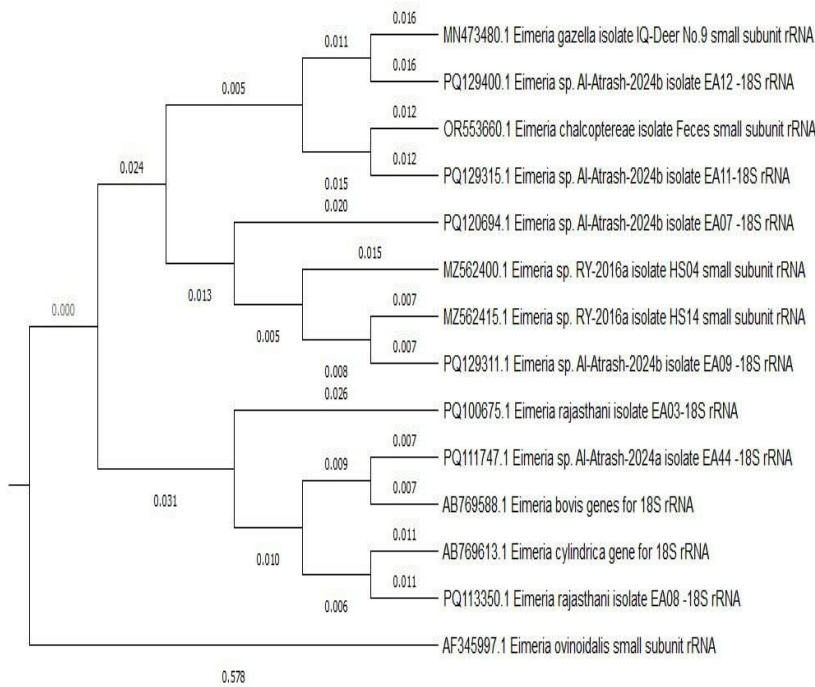


Figure (2): Specific phylogenetic confirmative detection of local *Eimeria* species

Which method you use for construction of the tree and what these numbers in the tree stands for you have to show

Histological change results

The demonstrated villous atrophy, degeneration, necrosis, and denudation. The mucosal glands in the afflicted gut were likewise found to be vacuolated, with epithelial cell disintegration. Occasionally, the

crypt epithelium detached from the basement membrane. Catarrhal colitis, goblet cell hyperplasia, and isolated chronic inflammation in the mucosa with large lymphocytic infiltration were clearly visible, as well as chronic enteritis, hemorrhagic enteritis, and granulomatous enteritis with goblet cell proliferation of the intestinal gland. Furthermore, the infiltration of chronic inflammatory cells between intestinal glands was found. Instead of the large intestine, the lamina propria of the small intestine had coccidian stages. Many nuclei grouped together in blastophores. The parasites are found intracellularly in sizable parasitophorous vacuoles; they are also found macrogamontly in the lamina propria, where they have completely destroyed the intestinal glands and infiltrated the cells responsible for chronic inflammation. microgamont with several nuclei (figure 3).

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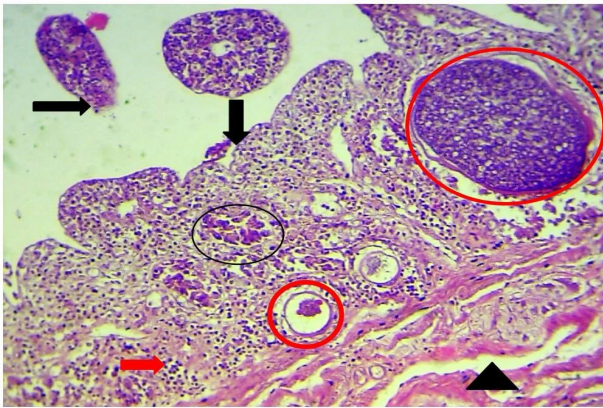


Figure 43): Histopathological section of camel intestine infected with *Eimeria* spp showing different developmental stages of *Eimeria* (red circles) with inflammatory cells infiltration (red arrow), also atrophy and sloughing of intestinal villi (black arrow) necrosis in intestinal gland (black circle), with edema among the muscularis layer (black arrow) (H & E stains, 200x).

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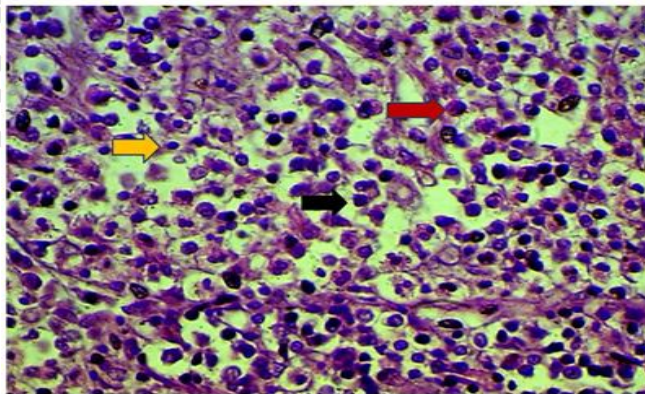
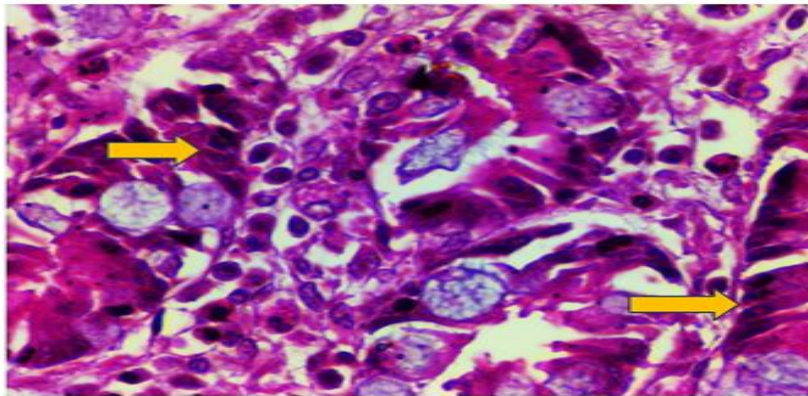


Figure 34: High magnification for inflammatory cells infiltration showing presences of eosinophile (red arrow), macrophage(black arrow) and lymphocyte(yellow arrow) H &E stains, 400x.

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Figure 33: Histopathological section of camel intestine infected with *Eimeria* spp showing presence of mature macrogamonts inside intestinal glands (yellow arrow) (H &E stains, 400x).

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Discussion

The large class of parasites known as coccidia has to be present within the cell. Numerous cattle species are impacted by the economically significant sickness known as coccidiosis.

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(Ashfaq *et al.*, 2023). The symptoms of coccidiosis vary from indifference and mild, transient diarrhea to severe instances that include large amounts of black, bloody diarrhea and, in the worst situations, fatality (Ashfaq *et al.*, 2023). In order to handle coccidiosis in camels in an economical and efficient manner, a thorough understanding of the *Eimeria* species involved is necessary. (El-Bahyet *et al.*, 2023).

Thus identifying the frequency and variety of *Eimeria* species was the goal of the current study. Prior to this investigation, *Eimeria* species were thought to be harmful to newborn camel calves.

(Hussain *et al.* 1987). The age and sex of the camels in the current investigation on-for camel coccidiosis did not significantly affect the prevalence. These results disagreed with those of Yakhali and Cheraghi (2007), how stated..... Due to their greater sensitivity to infection, their breeding, and overcrowding systems seen in their various qualities, and their immature immune system development as compared to older ruminants, young ruminants are more prone to illness than older ones- (Safaa and May ,2020).

~~Contributing to a few thinks about the variability in~~ The prevalence and distribution of coccidiosis may be due to variations in management and hygienic conditions, temperature, agro ecology, environmental conditions, weather ~~conditions~~, host immunity status, sample size, sampling period and breed susceptibility to coccidia in different areas.

The rum, colon, and jejunum were the most frequently affected tissues in this research with chronic enteritis; the discovery of gigantic schizonts in the lamina propria with total destruction of intestinal glands and infiltration of chronic inflammatory cells was consistent with ~~the~~ Heirandish et al., 2012. Large parasitophorous vacuoles containing the parasites are seen inside cells surrounding the macrogamont within the lamina propria and total annihilation of gut glands containing cells involved in chronic inflammation invasion, multi-nucleated microgamont; There are debris strands in the parasitophorous vacuole. was comparable to the outcomes of Dubey et al., 2018.

The intestinal mucosa included *E. cameli* oocytes, meronts, and macrochizonts, which are various developmental stages. These phases belonged to *Eimeria* spp, as Immature oocysts of *Eimeria* spp were discovered within the Intestinal epithelial mucosa. To our knowledge, the formation of *Eimeria* spp oocyte wall is unclear. In *Eimeria* species, the oocyst wall is initiated by the secretions of WFB. There are two types of WFB in most *Eimeria* species, type 1 and type 2 (Ferguson et al., 2003)

As the macrogamont matures, the WFB develops the oocyst wall (Ferguson et al., 2003). Furthermore, there is the other veil-forming (VFB); in certain coccidian parasites, they generate a veil-like outer layer on the oocyst wall; once the Oocysts are expelled in feces, and the wall has vanished.

Conclusion

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