Original research Article

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

Abstract

Background: Many countries rely heavily on Old World camels for their economies, utilizing them in transportation, food production (meat and milk), and racing.*Eimeria* affects a wide variety of domestics including camels and wild animals,.The aimof this research was to study the distribution and histological effectsof*Eimeria spp*. camels (*Camelus dromedaries*) using molecular diagnosis andhistological studies. **Methods:** A total of one hundred (100) fecal samples **from** camels (*Camelus dromedaries*) were examined by conventional PCR from different reignsat AL-Najaf AL-Ashraf province of Iraq, from Novmber 2023upto April 2024. A total of 20 samples collected from small intestine of slaughtered camels were collected.**Results:**A total infections rate with *Eimeria*spp was 57% (57/100). The male camels recorded higher than female 63.2%(24/38).The age group of male (<5 years) show the higher rate of infection 76.7%(23/30) while the female (10 years ≥)recorded 80.8%(9/11) in age as a higher infection ratedistrictAl- Manathera was recorded 71.4% (12/29) with significant differences at($P \leq 0.05$) compared with district of AL-Najaf AL-Ashraf provinces.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, This is the first molecular study in Iraq to identify and characterize *Eimeria species* in camels, three species *of Eimeria* have been detected using traditionalmethods (morphological characterization) *E. cameli, E. dromedarii* and *Eimeriarajasthani* ,*Eimeria rajasthani* was the most common species identification incamles.

Keywords:*Camelus dromedarie, Eimeria*, Food,Histopathological, Infection, Molecular diagnosis

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's milk.*Eimeria*sppis one of the parasitic infections, particularly gastrointestinal which have had impact on camels 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 coccidian can transmit infection through water and food sources (Jilo*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).

Materials and Methods

A total of (2) grams fecal samples were taken from (100) camels (38 male, 62 female) of various ages from distinctregionsin Al-Najaf, Al-Ashraf, between November 2023 to 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 and gender werereported. Samples thensent in an ice box to a laboratory of Parasitology / College of Veterinary Medicine- University Al-Qasim Green University / Iraq for Molecular diagnosis.Twenty (20) 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.

GenomicDNAExtraction

Genomic DNA from fecal samples were extracted using Presto stool DNAExtraction Kit, (Geneaid Biotech /China) and done according to company instructions**.**

Polymerase chain reaction (PCR)

The PCR technique was performed for detection of *Eimeria spp.* in fecal samples for camels based on 18S small subunit ribosomal RNA gene, This method was carried out according to the method described by (Zainab*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 microscopicanalysis 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 infectionwith *Eimeria* spp. in camels (*Camelus dromedaries)*by Molecular diagnosisfrom different districtat AL-Najaf AL-Ashraf province of Iraq, during Novmber 2023to April 2024was 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

Infection ratewith *Eimeria spp*. in camels according to sex byMolecular diagnosis:-

According to sex (male and female))infections were recordedas63.2% (24/38) and 53.2% (33/62) respectively with no significant differences ($p \leq 0.05$) (Table 2).

Table (2):Infection ratewith *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
X2	0.948229		
P value	0.330171		

Nosignificant differences at (P≤0.05).

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

Infection with *Eimeria* spp. was recording the highest rate in camels group 76.7% (23/30), whilerecording in groups (10 years ≥) 0% (0/2) with significant differences (p≤0.05) (Table 3).

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

	exam.samples	No.	%oftotal
5years≤	30	23	76.7
5-10years	6	1	16.7
10years≥	2	0	0
Total	38	**24**	63.2
X^2	**11.354762**		
Pvalue	**0.003423***		

***: significant differences at (P≤0.05).**

Rate of infection with *Eimeria* spp. in femalecamels 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≤) 10 % (2/20) with significant differences (p≤0.05)(Table 4).

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

Age(year)	No.ofthe exam.samples	Positivesamples	
		No.	%oftotal
5years≤	20	2	10
5-10years	31	22	71
10years≥	11	9	81.8
Total	62	**33**	53.2
X^2	**22.541921**		
Pvalue	**0.000013****		

**** : significant differences at (P≤0.05).**

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

The rate of infection with *Eimeria*sppincamel samples at three district as following AL-Manatherawas71.4%(25/35), Slaughter housewas 55.6% (20/36),) and AL-Meshkabwas41.4% (12/29) respectively with significant differences (p≤0.05) (Table 5) .

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

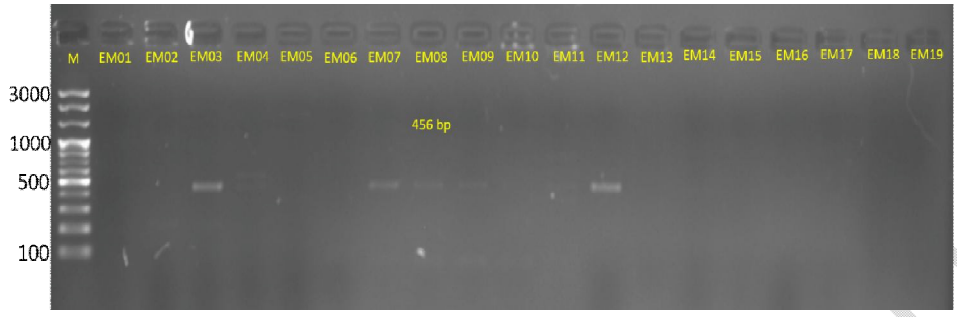
District	No.ofthe exam. samples	Positivesamples	
		No.	%oftotal
Slaughterhouse	36	20	55.6
AL-Manathera	35	25	71.4
AL-Meshkab	29	12	41.4
Total	100	**57**	57
X^2	**5.890539**		
Pvalue	**0.05***		

***: significant differences at (P≤0.05).**

ConventionalPCRproductanalysis:

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

RNA gene. Specificprimerswereusedtoidentifythespeciesof*Eimeria*.PCRof100sampleswas employed in the study exhibited a distinct band of (456 bp) from the PCR product on the agarose gel (Figur1 1 and Table 6).



Figure(1) PCR products of amplification of 18S RNA gene for detection of *Eimeria* species. Size of PCR product: 456 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea) , positive samples at lanes (3,7,8,9, and 12) with 456pb. Electrophoresis conditions: V: 95, Time: 45 minutes. M: DNA ladder

Table 6: set of primers used in PCR

Target gene		Sequence (5'-3')	Ta (°C)	Product size	Reference
18S RNA	F	5'-CGCGCAAATTACCCAATGAA-3'	60	456 bp	Hinsu et al., 2018
	R	5'-ATGCCCCCAACTGTCCCTAT-3'			

PCR Thermo Cycler Conditions

PCR thermocycler conditions were listed in table (Table 7) by using conventional PCR thermocycler system:

Table 7: PCR thermocycler conditions

Phase	Temp.(ºC)	Time	cycles
Initial Denaturation	94ºC	5min.	1x
Denaturation	94 ºC	30sec.	35x
Annealing	56 ºC	30sec	
Extension	72 ºC	60sec.	

Final extension	72 ºC	5min.	1x
Hold	4 ºC	Forever	-

DNA sequencing results

Seven of 57 samples were positive by using conventional PCR.Haplotypes of Sequencing were deposited toNCBI Genbank data base and DDBJ to get accession number codes (PQ100675, PQ111747, PQ129311,PQ129315, PQ129400. PQ120694 and PQ113350) for of local *Eimeria* species 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).

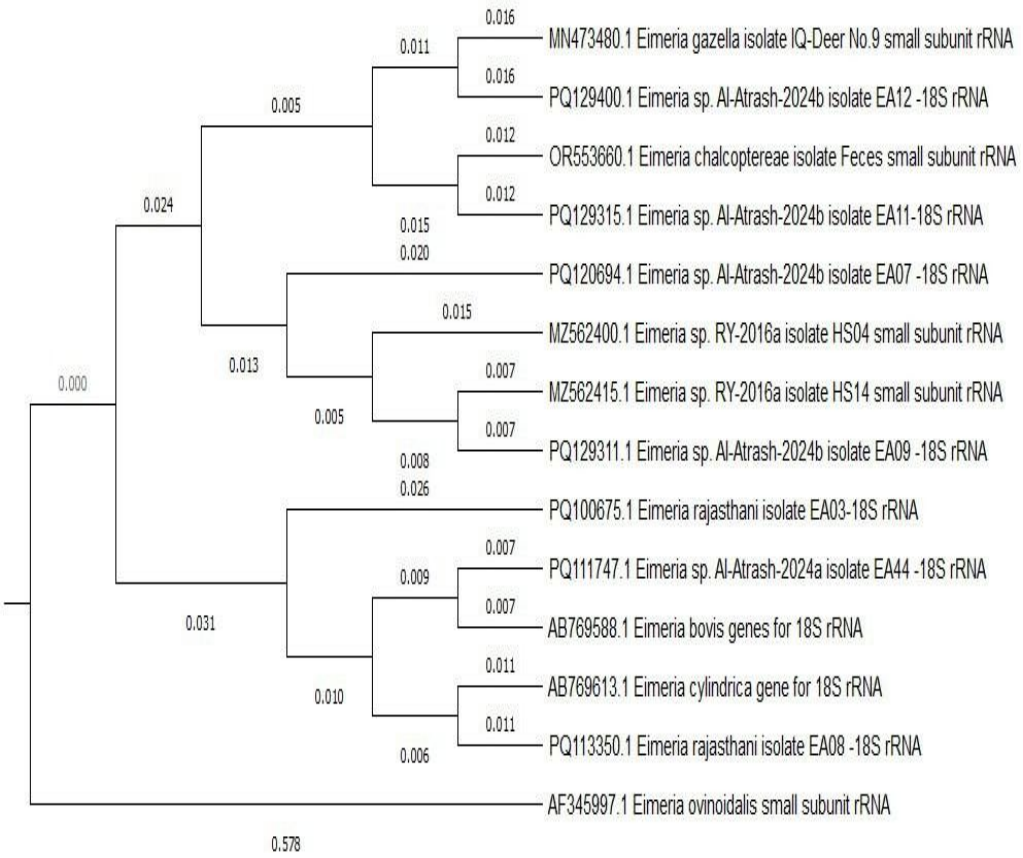


Figure (2): Specific phylogenetic confirmative detection of local *Eimeria* species

Histological change results

They 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 ccidian 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 (figure3).

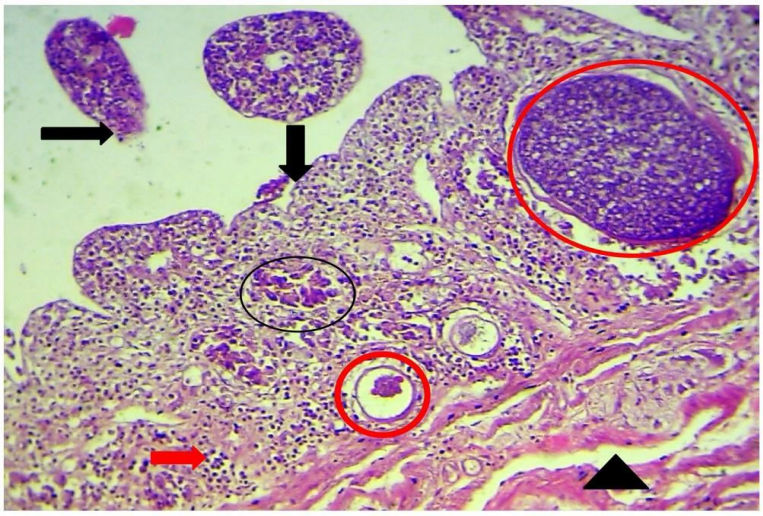


Figure (3): Histopathological section of camel intestine infected with *Eimeria*sppshowing different developmental stagesof Eimeria (red circles) with inflammatory cells infiltration (red arrow), also atrophy and sloughing of intestinal villi (black arrow)necrosis in intestinal gland (black circle), with edema among the muscularis layer(headarrow)(H &E stains, 200x).

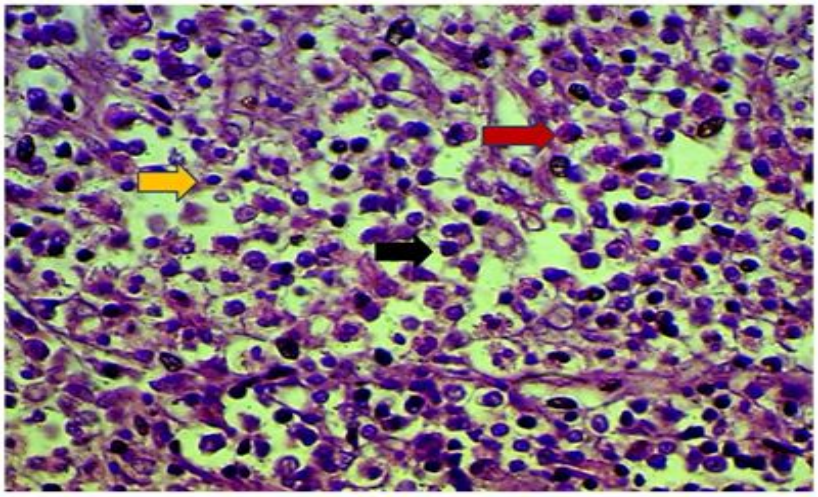


Figure (4): High magnification for inflammatory cells infiltration showing presences of eosinophile (red arrow), macrophage(black arrow) and lymphocyte(yellow arrow) H &E stains, 400x.

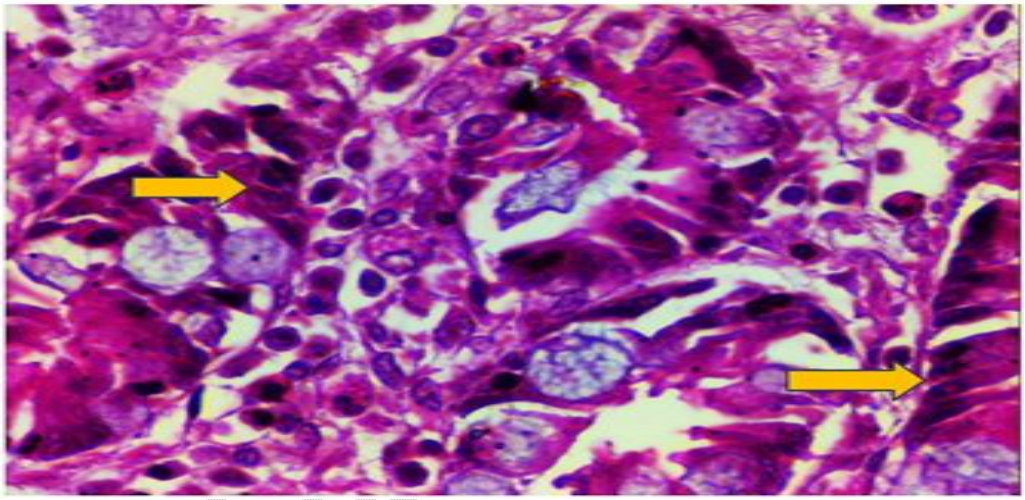


Figure (5): Histopathological section of camel intestine infected with Eimeria sppshowing presence of mature macrogamonts inside the intestinal glands (yellow arrow) (H &E stains, 400x).

Discussion

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-Bahy*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 (Hussein et al 1987). The age and sex of the camels in the current investigation for camel coccidiosis did not significantly affect the prevalence. These results disagreed with those of Yakhchali and Cheraghi (2007). Due to their greater sensitivity to infection, their breeding,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).

The prevalence and distribution of coccidiosis may be due to variations in management and hygienic conditions, temperature, agro ecology, environment conditions, weather, host immunity, sample size, sampling period and breed susceptibility to coccidia in different areas

The ileum, colon, and jejunum were the most frequently afflictedtissues 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(Kheirandish*et al.*,2012)Large parasitophorous vacuoles containing the parasites are seen inside cells. Finding 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, he formation of *Eimeria*spp oocyte wall is unclear. In. 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 are 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:

This is the first molecular study in Iraq to identify and characterize *Eimeriaspecies* in camels, three species *of Eimeria* have been detected using traditionalmethods (morphological characterization) *E. cameli*, *E. dromedarii* and *Eimeriarajasthani* ,*Eimeria rajasthani* was the most common species identification incamles. Inmolecular diagnostic method was more precise and confirm *Eimeria sp.*diagnosis by microscopic examination, Histopathological examination of infected tissues showed chronic, hemorrhagic ,and granulomatous enteritis.

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