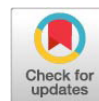


Research Article



Clinical and Molecular Detection of Different *Eimeria* Species in Goats at Babylon Governorate

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Abstract | Coccidiosis, a disease affecting cattle and especially goats, is caused by *Eimeria*, a genus of apicomplexan parasites. The current study aimed to identify the incidence and infection rate of *Eimeria* species in goats, in the Babylon Governorate of Iraq. Microscopic and molecular techniques were used to examine 88 fecal samples collected from goats between January and October 2020 from various areas in Babylon Governorate. Clinical observations revealed that diarrhea, fever, weight loss, and weakness were the main symptoms associated with the infection. The results showed infection rates of *Eimeria* species in goats to be 42.04% and 43.24% using microscopic and molecular methods, respectively. The infection rate in females was 41.17%, while in males it was 43.24%. Furthermore, the results indicated that goats older than two years had the highest infection rates compared to other age groups, with no significant differences observed between age groups. This study highlights the occurrence of *Eimeria* species infection in goats in Babylon Governorate, Iraq. Further molecular studies, such as DNA sequencing and phylogenetic analysis, may be necessary to identify and understand the distribution and genotypes of this protozoan species in the region.

Keywords | Coccidian, *Eimeria*, Goats, Microscopic, PCR

Received | November 04, 2024; **Accepted** | January 29, 2025; **Published** | April 13, 2025

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Citation | Rabeea AHMH, Marza AD, Al-Hasnawy MH, Abdul-Ameer BG, Chassab MK (2025). Clinical and molecular detection of different *Eimeria* species in goats at babylon governorate. J. Anim. Health Prod. 13(2): 295-300.

DOI | <https://dx.doi.org/10.17582/journal.jahp/2025/13.2.295.300>

ISSN (Online) | 2308-2801

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INTRODUCTION

The single-celled parasitic species belonging to the genus *Eimeria* (Apicomplexa: Eimeriidae) is an obligatory intra-cellular protozoan parasite (Yakhchali and Rezaei, 2010). *Eimeria* parasites are found in the gastrointestinal tract's epithelium as well as other bodily parts including the kidney and liver (Dubey *et al.*, 2019). Most domestic and wild ruminants, including cattle (Bangoura *et al.*, 2011), sheep and goats (Fthenakis *et al.*, 2012), and deer (Davidson *et al.*, 2014), are generally infected with

these parasites. Most of the time, asymptomatic adult carrier animals that transfer disease through their feces and oral secretions use food and water to spread *Eimeria* spp. The majority of infections are caused by the environment, particularly during births due to crowding, newborns being licked by their dams, and others migrating from an infected farm (Diao *et al.*, 2022; Mohamaden *et al.*, 2018). The total number of ingested sporulation oocysts determines when clinical symptoms can appear (Peek, 2010). The underlying mucosa's connective tissue and the intestinal epithelium are often destroyed in cases of coccidio-

sis, resulting in clinical symptoms. Diarrhea, inflammation of the catarrhal cavities, and bleeding into the intestinal lumen may accompany this. Tenesmus, dehydration, and the release of blood or tissue are possible symptoms. Only seriously injured animals exhibit changes in hemoglobin or packed cell volume, however there may be noticeable alterations in serum protein and electrolyte contents (usually hyponatremia) (Yusof and Isa, 2016). *Eimeria* species are generally considered to be host-specific parasites, at least down to the host genus level (Bangoura et al., 2022). Some *Eimeria* species, such as *E. bovis*, *E. brasiliensis*, *E. bukidnonensis*, *E. canadensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis*, and *E. zuerinii* within the bovine, can, nonetheless, infect hosts from many closely related genera (2–4 host) (Bangoura and Dauschies, 2018). Various host groups can be infected by various species. For example, *E. dispersa* and *E. innocua* can infect gallinaceous birds belonging to the Odontophoridae and Phasianidae families (Vrba and Michal, 2015). Investigating novel *Eimeria* species that infect several hosts is crucial, as noted by Turner et al. (2012). *Eimeria* species are common in small ruminants, and the degree of tissue damage varies according to the species, age, body condition, immunity, and inherited vulnerability. It was found that the infection by *Eimeria* species causes pathological alterations in epithelial layers of infected young animals, leading to modifications in their host’s immune system (Khodakaram and Hashemnia, 2017). The taxonomy of *Eimeria* was centered on the morphological features of the sporulated oocysts (Ogedengbe et al., 2015). Therefore, diagnosis of *Eimeria* oocysts by morphological characteristics requires a skillful technique (Bawm et al., 2020). Some traditional macroscopic and microscopic fecal examinations (such as; direct smear, flotation methods and sheather’s or Nacl solutions) were commonly carried out for investigation of *Eimeria* oocysts according to Verocai et al. (2020). According to current local data, molecular studies on *Eimeria* species in some animals, including goats are quite inadequate (Majeed et al., 2020). Thus, the present study aims to diagnose *Eimeria* species in Babylon province, Iraq, using a PCR-based molecular technique.

MATERIALS AND METHODS

STUDY DESIGN

From January 2020 till the end of October 2020, 88 fecal samples (10-15 g/sample) were collected from goats in various regions of the Babylon Governorate, representing both sexes and ages (51 females and 37 males). Each sample was put in a screw cap container, marked with the number and date of collection and transferred to a laboratory; all information about the samples and animals including the clinical signs, age, sex, state, location, number of suspected cases and animal’s description was recorded in a special note book for this purpose.

MICROSCOPIC EXAMINATION

Direct smear and floatation techniques based on Sheather’s or Zinc Sulphate solutions were used in the current study for detecting the oocyte of *Eimeria* spp. in fecal samples of goats. Using the direct smear method, a little amount of faeces was obtained, well mixed on a glass slide, and then covered with a coverslip in order to examine under 40X and 100X magnification (Cañal et al., 2024). For the floatation method, this was directed using Sheather’s and/or Zinc Sulphate solutions to examine *Eimeria* oocysts as advices by Verocai et al. (2020).

POLYMERASE CHAIN REACTION (PCR)

When assessing fecal samples, the development of PCR methods enables excellent levels of specificity and sensitivity (Sayın and Sari, 2024). Diagnosis of *Eimeria* parasite in animal has been accomplished using PCR, which amplifies DNA. Numerous methods for analyzing parasites present in tissue samples and clinical materials, as well as those produced in vitro, have shown to be highly sensitive and specific (Kawahara et al., 2010). Thus, using samples of goat feces and the 18S small subunit ribosomal RNA gene, PCR was used to detect *Eimeria* (Kawahara et al., 2010).

EXTRACTION OF GENOMIC DNA

To extract genomic DNA from feces samples, the Presto Stool DNA Extraction Kit was (STLD100; Geneaid Biotech, Taiwan) used as per the guidelines provided by the manufacturer. A Nanodrop spectrophotometer (ND-2000; THERMO, USA) with an emphasis on 260/280 nm absorbance measurement was used to investigate the genomic DNA extraction process from fecal samples.

PREPARATIONS OF PRIMERS

Specific primers for the 18S rRNA gene of *Eimeria* species were used, as per the NCBI GenBank database and Primer Plus. The primers were obtained from Macrogen, Korea.

THERMO-CYCLER PCR CONDITIONS

The thermocycler PCR conditions are documented in Table 1, using a conventional thermocycler PCR system.

Table 1: Conventional PCR conditions.

PCR steps	Temperature	Time	Repeat
Initial Denaturation	95°C	5min.	1
Denaturation	95 °C	30sec.	35 cycle
Annealing	58 °C	30sec	
Extension	72 °C	2min.	
Final extension	72 °C	5min.	1
Hold	4 °C	Forever	-

PCR PRODUCT ANALYSIS

The procedures for examining PCR results using agarose gel electrophoresis involved several steps. First, a 1.5% aga-

rose gel was prepared by dissolving it in a water bath with 1X TBE buffer at 100°C for 15 minutes, then allowing it to cool to 50°C. Ethidium bromide stain (3µ) was added to the agarose solution, and the mixture was thoroughly mixed. The solution was then poured into a gel tray and left to solidify at room temperature for 15 minutes. Afterward, the PCR product was carefully loaded into the comb wells along with a DNA ladder to ensure accurate electrophoresis. The electrophoresis chamber was filled with 1X TBE buffer, and the gel was subjected to an electric current of 100 volts and 80 mA for 1 hour. Finally, PCR products were visualized using a UV transilluminator.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Analysis System (SAS) software, and data were considered statistically significant at a level of $P < 0.05$. This means that any observed differences or relationships between variables were unlikely to have occurred by chance, indicating that the results are reliable and meaningful.

RESULTS

CLINICAL SIGNS

Several clinical signs as showed in Table 2 were recorded in infected animals, some of these animals had one or more signs.

Table 2: Clinical signs that were observed in infected goats during the study.

Signs	Number of animals	Percentage
Diarrhea	21	23.86
Fever	19	21.59
Anorexia	14	15.90
Weight loss	17	19.31
Dehydration	11	12.50
Weakness	17	19.31
Sub-clinical	4	4.54

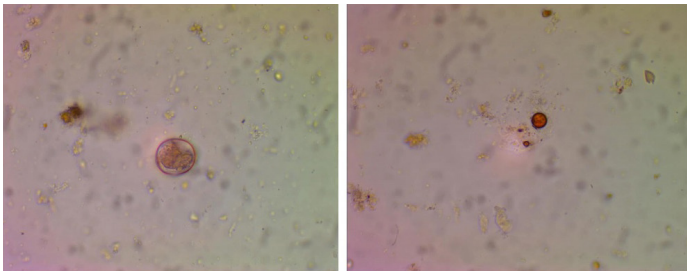


Figure 1: Oocysts of *Eimeria* in fecal samples of goat at 40X using the direct smear methods.

RATE OF INFECTION ACCORDING TO THE MICROSCOPIC EXAMINATION

The results from traditional techniques using a light microscope indicated that 37 out of 88 animals (42.04%) were infected with *Eimeria*, as shown in Figure 1.

According to the 18S rRNA gene, the PCR technique based on gel electrophoresis bands revealed that 39 out of 88 animals (44.31%) were infected with *Eimeria*, as shown in Figure 2.

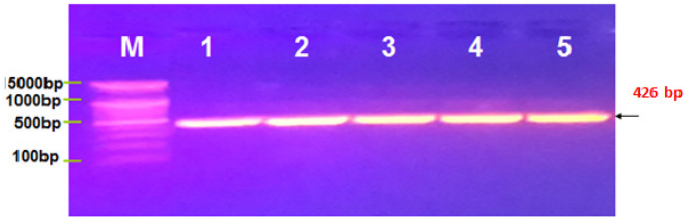


Figure 2: Agarose gel electrophoresis for the 18S small subunit ribosomal RNA gene of *Eimeria* species in goats was performed. The bands were separated on a 1% agarose gel and visualized under UV light after staining with a red stain. Lane M contains the 426 bp molecular weight ladder.

INFECTION RATE ACCORDING TO SEX

A total of 51 females, selected randomly from different age groups and breeds, were examined, with 21 (41.17%) showing infection. Additionally, 16 out of 37 randomly selected males from various age groups and breeds were examined, and the results revealed an infection rate of 43.24%, as shown in Table 3. Although males exhibited a slightly higher infection rate compared to females, no statistically significant difference ($P \geq 0.05$) was found between the two sexes.

Table 3: Infection rate according to sex.

Sex	No. of samples	No. of infected animals	Percentage
Female	51	21	41.17
Male	37	16	43.24
Total	88	37	42.04
X ²	0.998		
P value	2.221*		

*No significant differences ($P \geq 0.05$) were found between females and males.

INFECTION RATES ACCORDING TO AGE GROUPS

The infection rate of examined animals according to age group is presented in Table 4. The results indicated that animals older than two years had the highest infection rate, with 12 out of 23 samples testing positive (52.17%), compared to other age groups. However, no significant differences ($P \geq 0.05$) were observed between the age groups.

DISCUSSION

Coccidiosis, caused by *Eimeria* species, is a significant disease affecting the intestinal tracts of both wild and domestic animals. This group of apicomplexan parasites leads to economic losses in livestock, impacting both meat and milk

production, particularly in ruminants such as cattle, sheep, goats, and buffalo, as well as wild ruminants like deer and bison (Kenyon and Jackson, 2012). Infections with *Eimeria* can cause symptoms such as diarrhea, fever, weight loss, and weakness, and in severe cases, mortality. Previous studies have shown that *Eimeria* infections lead to economic damage, particularly in goat farms, where weight loss and even death in lambs can result from heavy infections (Diao *et al.*, 2022).

Table 4: Infection rates according to age groups.

Age group	No. of samples	No. of infected animals	Percentage
0- 12 months	22	10	45.45
1-2 years	43	17	39.53
More than 2 years	23	12	52.17
Total	88	39	44.31
X ²	3.019		
P value	0.193*		

*No significant difference (P≥0.05) were found between various age groups.

The present clinical observations corroborate these findings, as the main signs of infection observed in infected goats included diarrhea, fever, weight loss, and weakness, with these symptoms being the most prevalent in the infected animals. These findings align with previous studies (Chartier and Paraud, 2012; Sharma *et al.*, 2017). Our study revealed significant infection rates of *Eimeria* in goats from the Babylon province, with infection rates of 42.04% and 44.31% using microscopic and molecular techniques, respectively, confirming the importance of this infection as a major disease in ruminants. These results are comparable with those from other studies in Iraq, such as those conducted in Mosul (Cruvinel *et al.*, 2021) and Wasit (Al-Rubaie and Al-Saadon, 2018), although the infection rate in our study was lower than that found in other regions of Iraq, including Baghdad (69.6%) (Kalef *et al.*, 2013), Al-Muthana (67.5%) (Mohammed, 2013), Diyala (86.09%) (Minnat, 2014), and Al-Diwaniyah (80.48%) (Khader and Jarad, 2022), as well as in Egypt (60%) (Mohamaden *et al.*, 2018) and southeastern Iran (89.91%) (Kheirandish *et al.*, 2014). These variations in infection rates are often attributed to factors such as management practices, hygiene conditions, agroecological factors, climate, host immune status, sampling methods, and breed susceptibility (Altaf and Hidayatu, 2014).

In our study, the infection rate was found to be higher in males than in females, although the difference was not statistically significant. This observation is consistent with a previous study on sheep in Sudan, which reported no significant difference between male and female infection rates

(Hamid *et al.*, 2024). Our findings also align with studies from Mosul, where male goats had a slightly higher infection rate (95.7%) compared to females (92.3%) (Khader and Jarad, 2022), and a similar trend was observed by Cruvinel *et al.* (2021). However, these results differed from those of a recent study in Al-Diwaniyah province, which showed a different infection pattern (Khader and Jarad, 2022). The discrepancy may be attributed to the higher number of male goats slaughtered in abattoirs compared to females, as males are generally more abundant.

Regarding age, the study revealed that goats older than two years had the highest infection rates, although the differences compared to younger goats were not statistically significant. Interestingly, young goats under one year old showed a relatively high infection rate of 45.45%, suggesting that even younger animals are susceptible to infection. These findings are somewhat different from previous studies in sheep and goats, which generally found higher infection severity in younger animals (Etsay *et al.*, 2020). Young ruminants are more vulnerable to *Eimeria* infections due to their underdeveloped immune systems and lack of previous exposure to the parasite, whereas adult animals tend to build immunity through repeated low-dose exposures (Yu *et al.*, 2011; Dawid *et al.*, 2012).

Molecular results obtained via PCR using the 18S small subunit ribosomal RNA gene showed infection rates comparable to those obtained with the microscopic method. This finding confirms the accuracy and reliability of the microscopic examination. PCR is widely recognized as a critical molecular diagnostic tool for detecting *Eimeria* spp. (Al-Rubaie and Al-Saadon, 2018), and other molecular techniques, including DNA sequencing and phylogenetic analysis, have been used to identify and classify *Eimeria* species (Trejo-Huitron *et al.*, 2020).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study highlights the significant prevalence of *Eimeria* spp. infection in goats in Babylon Governorate, Iraq. It underscores the need for further molecular research, including DNA sequencing and phylogenetic analysis, to better understand the genotypes of *Eimeria* affecting small ruminants in the area and to provide updated data on the spread and impact of this protozoan infection.

ACKNOWLEDGEMENTS

The authors express gratitude to the College of Veterinary Medicine at Al-Qasim Green University for their helpful provision and evidence in carrying out this research.

This study highlights the occurrence of *Eimeria* species infection in goats in Babylon Governorate, Iraq. Further molecular studies, such as DNA sequencing and phylogenetic analysis, may be necessary to identify and understand the distribution and genotypes of this protozoan species in the region.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the manuscript.

FINANCIAL DISCLOSURE STATEMENT

The study was self-funded.

ANIMAL RIGHTS STATEMENT

Not applicable.

CONFLICT OF INTEREST

The author asserts that there is no conflict of attention in the publication of this object.

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