Epidemiological and Molecular Study of Eimeria Species in Calves from Holy Karbala, Iraq

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***Abstract*— Coccidiosis is one of the economically most important protozoan diseases of young calves, that is primarily caused by host-specific Eimeria species targeting the intestinal epithelium. This study investigated the prevalence, species identification and epidemiological determinants of Eimeria infection in calves from Holy Karbala, Iraq, using combined parasitological and molecular approaches. Between May 2024 and January 2025, a total of 296 faecal samples were collected from calves of different age groups, that were filtered using the flotation technique, followed by PCR amplification of the 1st internal spacer (ITS-1) region for the molecular detection of Eimeria bovis. The general prevalence reached 70%, the infection rate of calves younger than one year is significantly higher. Seasonal variation was clear, with peak infections recorded in December and January. PCR successfully amplified the expected 238 bp product of E. bovis, confirming its dominance among the detected species. The results support the epidemiological importance of coccidiosis in Karbala calves and highlight the advantage of molecular diagnosis over traditional microscopy for accurate species identification. The integration of PCR-based assays with better management and hygiene practices is essential for the effective control and prevention of coccidiosis in livestock.**

Key words: **Coccidiosis, Eimeria spp., Eimeria bovis, calves, ITS-1, PCR epidemiology, Karbala.**

INTRODUCTION

Coccidiosis is a commercially important disease that affects a wide range of animal species and can have significant long-term consequences for future generations (1). Although severe clinical manifestations are uncommon, affected animals may show fatigue, marked gastrointestinal disturbances or, in extreme cases, cardiovascular complications (2, 3). The disease is particularly severe in calves reared in closed breeding rooms, especially in newborns kept in small barns or grazed on irrigated pastures in winter.

Eimeria spp. life cycle It consists of two main stages: an extrinsic stage that occurs outside the host in the environment under favorable conditions and an intrinsic stage in that the parasite undergoes multiple asexual and asexual reproduction within the intestinal epithelial cells ultimately leading to the release of sporozoites into the gastrointestinal tract through stool (4).

Infection occurs primarily by ingesting feed or water contaminated with sporozoites. Clinical signs usually begin with diarrhea that can lead to bleeding dehydration reduced feed intake fatigue poor growth poor coat quality and in severe cases death (5). Infection can result in a morbidity rate of 10% to 40% and a mortality rate approaching 10% (6,7).

Eimeria species are highly host-specific and infection can affect one species or multiple species simultaneously (8). a bunch of morphologically similar Eimeria species can coexist in the gastrointestinal tract, that makes species-level diagnosis difficult during natural disease outbreaks (9). Diagnostic methods usually include clinical observation, examination of stool by flotation or direct swab and postmortem evaluation. Symptoms usually appear about 17 days after ingestion of the sporozoites, often after significant damage to the intestinal tract. its noteworthy that coccidiosis is often detected in the feces of clinically healthy young calves. The differential diagnosis should consider and exclude other conditions such as bovine viral diarrhea virus (BVDV), salmonellosis and neurotoxic syndromes (10, 11).

Therapeutic management involves supportive care, including fluid therapy and anticoccidial medications, to prevent recurrence. Isolation of infected animals is essential to reduce environmental contamination. Severely affected calves should be treated separately, and administration of medication via feed or water must be carefully managed to avoid exposure of healthy livestock.

Previous epidemiological studies on gastrointestinal parasitic infections in cattle and other livestock in Iraq have reported varying prevalence rates of Eimeria spp.: approximately 4% in Mosul, 10.96% in Baghdad, 6.81% in Al-Diwaniyah, and 10.71% in other regions (12–16).

The present study aimed to investigate the molecular detection of Eimeria spp., with particular focus on Eimeria bovis, in calves from Holy Kerbala, Iraq, using polymerase

chain reaction (PCR) targeting the ITS-1 gene. Additionally, the study evaluated epidemiological factors associated with infection, including age and seasonal variation, to provide insights into the distribution, prevalence, and risk factors of coccidiosis in this region.

Materials and Methods

A total of 296 fecal samples were collected for the present study due to the high prevalence of *Eimeria* infections in calves, as reported in previous investigations. The young calves were divided into two age groups: those younger than an entire year and those older than a period of time. To guarantee collection authenticity, samples of stool were collected straight into each calf's intestines. Pending further investigation, every sample was immediately stored in a freezer at four degrees Celsius in the Department of Veterinary Medicine's Diagnostic Laboratory at the University of Kerbala. To determine whether there are risk factors for Eimeria spp. infection in dairy calves, a uniform survey was conducted. Farm owners' sex, age, occupation, degree of training, and locality were recorded in the questionnaire. Enclosed included details about the calves themselves, such as age, vaccination background and overall health.

The survey also addressed a number of ecological and managerial aspects, including the number of animals in the livestock group, the design of living quarters, where the water came from, the type of flooring in the barns, and the regularity of cleansing both the feeding and water apparatuses. This thorough method evaluated potential correlations between these variables and the prevalence of Eimeria spp. infections.

Sample collection and study design

During May 2024 and January 2025, 296 of stool samples were taken from calves on different farms throughout Holy Kerbala. Two age groups—one year and older—were created from the calves. Fresh fecal samples were collected directly from the rectum using disposable gloves. and placed in plastic containers with labels. Before being processed more thoroughly, every sample was kept at 4°C and transferred to ice.

Information on livestock demographics (age, sex, and breed), herd size, housing circumstances, watering and nourishment systems, and cleaning procedures was gathered via a questionnaire that was organized. The possible risk associated with Eimeria contamination was subsequently evaluated via these parameters.

Parasitological examination

To identify Eimeria oocysts, the stool samples were tested by the flotation method with a saturated sodium chloride mixture. Eimeria oocysts were found and identified using a combination of sedimentation and flotation methods. Seriously, in order to distinguish the fetal cells based on their specific gravity among the intestinal lesions, the stool samples were first washed with a saturated sodium chloride (NaCl) solution. In order to retrieve oocysts that would not have floated because of weight changes, a deposition operation was then carried out. The suspended matter removed for microscopic analysis at 10× and 40× magnifications after the fragments had been centrifuged for five minutes at 1500 rpm.

According to Genevet et al. (2011), unique morphological properties such as shape, size, color, wall thickness, and the presence or absence of a micropyle were used to identify oocysts.

Both formed and uncontaminated oocysts were noted and categorized. Throughout a range of stages of growth, this dual approach increased both sensitivity and reliability. In our investigation, primers were utilized.

PCR amplification of the ITS-1 region

Conventional polymerase chain reaction was used to amplify the ITS-1 gene of Eimeria bovis via specific primers:
Forward primer: 5′-tcataaaacatcacctccaa-3′
Reverse primer: 5′-ataattgcgataagggagaca-3′
Expected product size: 238 bp,

Table1. Primer sequences used for PCR amplification of *Eimeria bovis* ITS-1 region.

Parasites	Forward	Reverse	PCR product
Eimeria bovis	5-tcataaaacatcacctccaa-3	5-ataattgcgataagggagaca-3	238

DNA Extraction

The goal of the StormTM Faeces Molecular DNA Extraction Machine is to swiftly extract bacterial and fungal genomic material from faecal samples.

The techniques' components are comparable:

1. For this procedure, between 180 and 220 mg of excrement should be put into a pottery pellet-filled bead-breaking tube. With species with exceptionally tough or fibre-packed intestinal tracts, such as lambs, rabbits and even mice, the amount of stool produced can be lowered to 60–80 mg so that the intestinal feces can absorb the enzyme-based lysis solution. Natural food, such as fruits or agricultural stalks, may be found in single excrement.

It is not recommended to transport these kinds of particles. The rats received an injection of 800 µl of ST1 Buffer. Following brief vortex motion, the mixture was incubated at seventy degrees Celsius for five seconds. The bead -baiting tubes are attached diagonally onto standard cyclones via an adhesive or an adapter that fits. The vortex reportedly reached its maximum speed for ten minutes at ambient temperature. The bead beating tube was turned at 8,000 × g for a minute and a half at a comfortable temperature to eliminate the foam coating that the surfactant in the ST1 solution had produced. A new 1.5 ml microcentrifuge tube was filled with 500 µl of residue. PrestoTM's objective The Stool gDNA Extraction Kit works quickly.

2. PCR Inhibitor Removal was used following the addition of 50 L of ST2 Solution and overworking for five seconds. The mixture was allowed to sit at 0 - 4°C for five minutes. Inhibiting agents for PCR and soluble particles were separated by centrifugation at 16,000 × g for three minutes at room temperature. A substance eradication barrier (violet circle) was placed into a 2 mL centrifuge jar. The substance that blocks the removal filter should be filled with 500 µl of clear

residual material. The mixture was centrifuged at 16,000 × g for a minute at ambient temperature. To bind DNA, keep the route in the 2 mL centrifuge jar. Removal Transfer a dehydrated GD segment to a one brand-new 1.5 ml microcentrifuge container. Removal buffer 1 (30–100 µl) was used. fluid 3 or TE2 into the central columnar framework. The sample was centrifuged at 16,000 × g for two minutes at room temperature to extract pure information. 1. If more DNA was needed a 30-µl extraction mixture (10 mM Tris-HCl, pH 8.5) was used. The same 30 µl of additional elution solution (2) was used to continue this process. Since EDTA retains DNA over extended preservation, it is beneficial to elute it via TEs (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). However, EDTA will affect later, extremely sensitive applications such as PCR. TE must be added to the center section of the GD column vector, to ensure that it is fully incorporated. All three rinse water samples were verified to have a pH between 7.0 and 8.5. ddH2O should be fresh since ambient atmospheric carbon dioxide can quickly cause acidity. All of the substances were properly poured before being incorporated into the structural core section of the GD columns. DNA deterioration can be prevented by keeping water-rinsed DNA at -20 degrees Celsius.

3. To aid in movement, 800 µl of ST3 Buffer was added to attach the DNA, and everything was quickly mixed by shaking vigorously for five seconds. A GD column (green ring) should be present in a 2 mL collection tube. The portion that passed through was extracted following centrifugation for one minute at room temperature at 16,000 ×g. The gastrointestinal columns were reinstalled into 2mL collection tubes. The remaining specimen combination was added to the GD column. After the opening was removed, the GD column was returned to 2 mL collection tubes.

4. Each concentrator was filled with 400 µl of ST3 buffer. The mixture was centrifuged at 16,000 × g for 30 seconds at room temperature. Following the removal of the tunnel, the columns of blood were returned to 2 ml collection tubes. The column of water was previously filled with 600 µl of purified buffer. The mixture was centrifuged at 16,000 ×g after 30 seconds at room temperature. Following the removal of the tunnel, the gas chromatography column was returned to a 2 ml collection tube. To ensure that only genuine ethanol was added, 600 µl of wash buffer was added to the GD segment. The mixture was centrifuged at 16,000 × g for 30 seconds in the context of the surroundings. The following settings were applied to the PCR thermostat (Table 2):

Table 2. PCR thermocycler conditions: these conditions were determined via a kit.

PCR cycling		
Initial denaturation	95°C, 10 minted	1 cycle
Denatured state	95°C, 15 s	40 cycle
Annealing state	60°C, 30 s	
elongation	72 °C, 30 s	
The final extension state	72°C , five minted	

STATISTICAL ANALYSIS

Data were analyzed using the statistical package for the social sciences (spss), version 25.0. descriptive statistics were applied to summarize the dataset, and chi-square tests were performed to assess the associations between infection status and potential risk factors. a p-value < 0.05 was considered statistically significant.

RESULT & DISCUSSION

Among the 296 fecal samples initially collected from calves, only 200 were selected for molecular and parasitological analyses. The remaining 96 samples were excluded because of insufficient quantity, poor preservation, or contamination during collection.

Based on flotation and PCR results, 140 (70%) of the analyzed samples tested positive for Eimeria spp., whereas 60 (30%) were negative. A significantly higher infection rate was recorded among calves aged ≤1 year (69.2%) compared with those older than 1 year (30.8%) ($p < 0.05$). Although 13 Eimeria species are known, not all of them are pathogenic. Microscopic examination showed that 100% of the positive samples contained Eimeria oocysts with varying contamination levels (Figure 3). For DNA extraction, 97 samples were selected based on the intensity of infection. Amplification of the ITS-1 region using conventional PCR produced fragments ranging from 348 to 546 bp in 45 samples (46.39%) (Figure 3).

Table 3. The percentage of calves infected with the *Eimeria* parasite according to age (over 1 year and under 1 year).

Age	infected animals	Non –infected animals
1 year	90	40
more than 1 year	50	20
total	140	60

Table 4. Rate of infection with the *Eimeria* parasite in calves according to the months of the year

months	Number of animals examined	infected animal	Percentage %
may	50	35	70%
June	20	15	75 %
September	35	25	71 %
October	20	15	75 %
November	15	10	66.6 %
December	40	25	62.5 %
January	20	15	75% %
total	200	140	

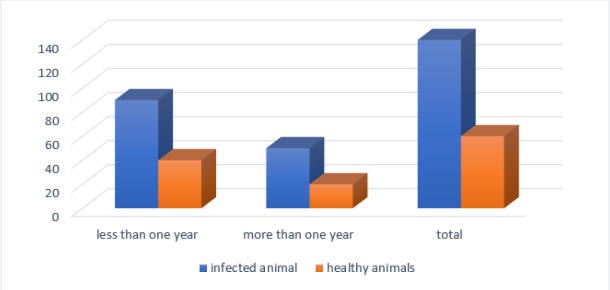

Figure 1. The percentage of calves infected with the *Eimeria* parasite according to age (over 1 year and under 1 year).

Monthly variation in infection -

The highest infection rates were recorded in March and January (75%), whereas the lowest was recorded in December (62.5%) .

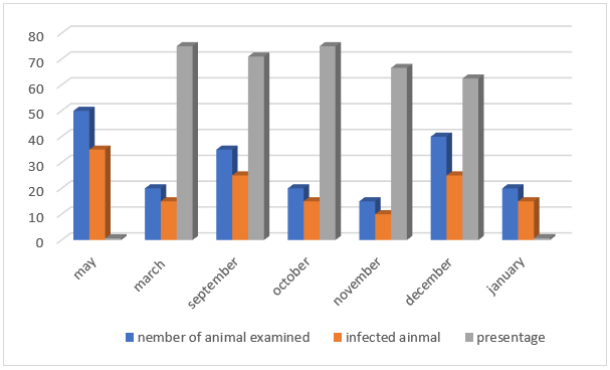

Figure 2 . Rate of infection with the Eimeria parasite in calves according to the month of the year.

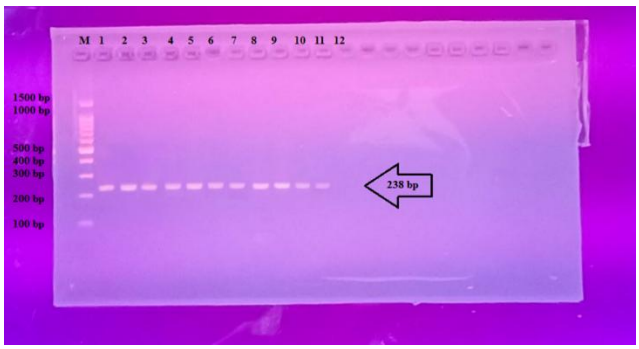

Figure 3 . Agarose gel electrophoresis results for samples positive for the genus *Eimeria*. The symbol M represents the molecular marker (100–2000 base pairs). Lanes 1–10 show PCR products with band sizes ranging from 348 to 546 base pairs

Eimeria, belonging to the phylum Apicomplexa, comprises essential intracellular protozoans, some of which are highly pathogenic (17,18). Eimeria species are frequently observed in water buffaloes (Bubalus bubalis) (19), and several genotypes have been reported to infect calves during their first year of life [20]. In many dairy production systems, large numbers of calves are raised in close proximity, while adult cattle continue to shed Eimeria oocysts asymptomatically. This continuous environmental contamination increases morbidity and mortality in young calves (21).

Although 13 *Eimeria species* have been identified in cattle, only a few are considered highly pathogenic. Among these, E. bovis and *E. zuernii* are the most important causes of bloody diarrhea, severe illness, and even mortality in young calves (22,23). Parasitic infections are also common among companion animals, where various coccidian parasites can infect hosts; however, infections remain host-specific (24).

The present study highlights the high incidence of Eimeria spp. infecting calves in Holy Kerbala, Iraq. Using both traditional flotation and PCR targeting the ITS-1 region, the overall infection rate was 70%. This finding aligns with previous studies conducted in Iraq and neighboring countries, which have similarly reported high prevalence rates of coccidiosis in young ruminants. The significantly higher infection rate in calves aged ≤1 year likely reflects their underdeveloped immune system and increased exposure to contaminated environments due to poor hygiene or close contact with infected animals.

Seasonal variation played an important role in infection dynamics. The highest prevalence was recorded during the cooler, more humid months of December and January. These conditions enhance oocyst survival and sporulation in the environment, increasing the risk of transmission. For example, sporulation rates of Eimeria acervulina are strongly influenced by high humidity and mild temperatures. Optimal environmental conditions for Eimeria reproduction occur at 25–30°C with humidity levels above 80%, which commonly correspond to late autumn and early winter.

Stressors such as early weaning also compromise immunity in young calves, increasing susceptibility to infection. Similar patterns have been reported in other bovine parasitic diseases.
To target the ITS-1 region of rDNA, PCR assays were used to identify the five predominant Eimeria species infecting buffaloes. Consistent with previous reports (25), sequencing analysis showed clear interspecies divergence but minimal intraspecies variation, confirming the suitability of the ITS-1 region for species-level identification and molecular epidemiology in bovines.

The infection rate of 70% reported here is lower than the rates documented in Mosul (26), Baghdad (27), and the north-western Czech Republic (28). In the United States, prevalence varies widely by region, with reported rates of 40.7%, 16.9%, 85%, and 64.9% (29). In the eastern region of Saudi Arabia, Eimeria infections are also widespread (30).

Similarly, studies from Germany have reported prevalence rates ranging from 2% to 48%, with higher rates occurring during specific environmental conditions (31). Differences in infection rates may be attributed to host factors such as immunity, age, sex, management systems, and exposure to stressors including weaning and nutritional deficiencies. Environmental factors such as humidity, temperature, and seasonal changes also greatly influence oocyst survival and transmission (32).

Grasslands contaminated with oocysts provide ideal conditions for infection so proper treatment is essential. In this study the highest infection rate was observed in March while the lowest was observed in September. This variation

may be related to environmental immunity fluctuations in animal age groups and seasonal changes in temperature and water availability. The increased infections during the calving period are also due to the fact that ewes empty too a bunch of oocysts before calving pollute the environment and expose newborn lambs to infection during grazing (34,35). Lower infection rates in autumn (September–November) are consistent with the findings of (36) consistent with moderate temperatures despite low precipitation and humidity.

Previous studies have shown that all age groups are susceptible to infections, but the severity of the disease varies by age. Most serious infections usually occur in animals younger than 6 months of age. This may be due to immature immunity, stress caused by early weaning, transportation, high environmental humidity and insufficient colostrum intake. In contrast, adults usually develop partial immunity, that reduces the rate and severity of infections (37).

Conventional morphological identification of oocysts is inexpensive and does not require advanced laboratory equipment; However, its sensitivity is limited and it can give false positive or ambiguous results due to similarities between oocysts from different Eimeria species. In this study, molecular identification by species-specific PCR provided greater accuracy and reliability, highlighting its value for epidemiological studies and field diagnostics. Because some Eimeria species share similar morphological characteristics, precise species identification requires molecular confirmation (38). Our results confirmed the genetic variation among Eimeria species in the ITS-1 region, that is consistent with oocysts morphological differences.

Overall, PCR showed excellent accuracy, speed and reliability compared to conventional microscopy, making it an effective diagnostic tool for Eimeria spp. In number of animals (39,40).

CONCLUSION

The findings of the present study confirm that coccidiosis caused by Eimeria spp., particularly Eimeria bovis, remains a significant parasitic threat to calf health and productivity in Holy Karbala, Iraq. The overall prevalence of 70% highlights the widespread environmental contamination and the continuous exposure of calves to infective oocysts. Younger calves (≤1 year) demonstrated a markedly higher susceptibility, emphasizing the critical role of immature immunity and management-related stressors in shaping infection dynamics. The pronounced seasonal pattern, with infection peaks during December and January, further underscores the influence of climatic conditions—especially humidity and moderate temperatures—on the sporulation and survival of oocysts. Molecular detection using ITS-1 PCR proved superior to morphological identification, enabling precise species-level confirmation of E. bovis and validating the necessity of integrating molecular tools into routine diagnostic practices. Overall, the study reinforces the multifactorial nature of coccidiosis epidemiology and the need for comprehensive control strategies tailored to local environmental and management conditions.

RECOMMENDATIONS

1. Perform routine PCR-based molecular screening for early and accurate detection of Eimeria infection.
2. Promote hygiene and biosecurity in the calf rearing environment especially in spring and early winter.
3. Separate young calves from older animals to reduce exposure and spread of eggs.
4. Educating farmers and livestock breeders about early colostrum feeding stress management and regular disinfection of feeding and watering systems.
5. Seriously Carry out further tests for Eimeria spp. to evaluate its genetic diversity. Seriously in Iraq and investigating possible drug resistance patterns.

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