

Morphological and Molecular Identification of Eimeria Species in Broiler Chickens from Basra Governorate, South Iraq

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ABSTRACT

Background: Eimeria species are significant pathogens in poultry, causing coccidiosis and resulting in substantial economic losses. Accurate identification and characterization of Eimeria species are essential for effective control strategies. **Objective:** This study aimed to characterize and identify Eimeria species infecting broiler chickens in Basra Governorate, South Iraq, using both morphological and molecular diagnostic methods. **Methods:** The study involved the morphological identification of Eimeria species based on oocyst morphology and molecular confirmation using nested PCR targeting the internal transcribed spacer 1 (ITS-1) region. **Results:** Morphological analysis identified four Eimeria species in the intestinal samples, with infection rates of 13% for E. acervulina, 5% for E. praecox, 1% for E. necatrix, and 1% for E. tenella. Molecular analysis confirmed the presence of these species, with specific amplicon sizes: E. acervulina (321 bp), E. necatrix (285 bp), E. tenella (278 bp), and E. praecox (368 bp). **Conclusion:** This study provides robust morphological and molecular evidence of Eimeria species infecting broiler chickens in Basra Governorate. The findings highlight the importance of integrating molecular tools for precise species identification, which is crucial for developing targeted coccidiosis control measures.

Keywords: Coccidiosis, Broilers, Eimeria Spp

INTRODUCTION

Eimeria spp.'s composition and dynamics, due to being an etiological agent, are essential in determining the disease stringency [1]. The most typical and economically important poultry disease is coccidiosis, and annual losses attributed to this disease are estimated to be approximately \$13.6 billion in hens alone [2]. As well as clinically infecting birds, it can also cause subclinical infection. In its clinical form, coccidiosis causes bloody feces, diarrhea, morbidity, and death, while in its subclinical form, it causes impaired weight gain and declined feed conversion efficiency [3]. Chickens can be infected with seven species types of Eimeria with different levels of pathogenicity: *E. praecox*, *E. maxima*, *E. tenella*, *E. mitis*, *E. acervulina*, *E. brunetti*, and *E. necatrix* [4], when sporulated Oocysts are ingested or inhaled, this parasite can be acquired by ingestion of polluted feed or water [5, 6]. Most species of *Eimeria* impact birds in the ages of 3-18 weeks, which increases rates of mortality in young chicks [7]. Consequently, diagnostic laboratories are increasing their dependency on DNA-based approaches to identify the species of *Eimeria*. So far, all seven *Eimeria* species have been specified utilizing methods based on PCR [2, 8]. Precise identification and genotyping of the unique species of *Eimeria* are essential in the prevention of coccidiosis, management, and surveillance. Although well-conserved intraspecies, the gene sequences for ITS1 in eukaryotic cells can be very different among species [8]. It is therefore possible to identify a variety of diseases, including *Eimeria* infections, using ITS1 sequences. It has been proven that ITS1 primers

can successfully identify chicken species of *Eimeria* using polymerase chain reactions (PCR) [9].

MATERIALS AND METHODS

Samples Collection and Oocyst Isolation

From different areas in Basra Governorate, including houses for poultry in Shatt al-Arab, Safwan and Al Zubair, clinics of veterinary, and Al-Noor veterinary laboratory, 100 broiler chicken intestine samples of different ages and genders were gathered randomly. This study period began on December 1, 2021 and ended on October 31, 2022. The samples of poultry intestine were dispatched into the following categories: cecum, ileum, jejunum, and the duodenum. The scissors were used to open each portion of the intestine and its contents were gathered in a beaker. Intestinal content was assayed for Eimeria spp. oocysts via straight smear and standard floatation techniques [10]. The identification of *Eimeria* spp. was based on oocyst morphology, involving color, size, micropyle presence or absence, and cap [11]. The oocysts were gathered, kept at 40°C, and conserved in 2.5% K2Cr2O7 [12]. The confirmation of previously diagnosed *Eimeria* species according to the morphology of oocysts was performed by PCR using the primers. ITS1-PCR was then used to amplify the gene [13]. Table (1) contains a list of primers used in the current study.

Table 1: ITS1 Primer Sequences Utilized to Amplify Genes

Gene	Primer Sequences (5'-3')	Product Size	Reference
ET	F*: AATTTAGTCATCGCAACCT R*: CGAGCGCTCTGCATACGACA	278bp	[13]
EN	F: TACATCGCAATCTTTGAATCG R: GGCATACTAGCTTCGAGCAAC	285bp	[13]
EA	F: GGCTTGGATGATGTTTGCTG R: CGAACGCAATAACACGCT	321bp	[13]
EP	F: CATCATCGGAATGGCTTTTGA R: AATAAATAGCGCAAAATTAAGCA	368bp	[13]

*F: forward, *R: reverse, A: adenine, C: cytosine, G: guanine, T: thymine

Genomic DNA Extraction

For the extraction of genomic DNA, the gSYNC™ DNA Extraction Kit was utilized to extract DNA from each of the *Eimeria* spp. isolates within the protocols of the manufacturers.

PCR Detection of Genes

Table (2) lists the thermocycling programs of the entire genes. The annealing temperature for *ET*, *EP*, *EN* and *EA* genes was (55,50,50,52.5, and 54), respectively.

Table 2: Program of All Genes [13]

Step	Temperature, °C	Time	Cycle
Preliminary Denaturation	95	10 min	1
Denaturation	94	60 sec	
Annealing	50-54	90 sec	35
Stretching	72	60 sec	
Final Stretching	72	5 min	1

C. Agarose Gel Electrophoresis

DNA samples that isolated or PCR products were electrophoresed by mixing their 5µl with 2µl of loading dye (bromothymol blue) and loading in the suitable wells, then exposing it to an electric field (70 V for 45-60 min). Lastly, PCR products or nucleic acids were inspected using UV light (300 nm) passing through the gel in accordance with [14].

Results

Identify the *Eimeria* spp. according to Microscopic Examination

The current study also showed oocysts morphological characteristics for *Eimeria* species. Oocyst of *E. necatrix* distinguished by ovoid and oblong in shape, colorless with walls with two layers, micropyle absent and polar cap present Fig (1). Oocyst of *E. praecox* distinguished by oval to spherical oocyst, colorless with double-layered walls, polar cap present, micropyle absent Fig (2). Oocyst of *E.*

acervulina distinguished by ellipsoidal shape, yellowish color, double-layered walls with thin in the narrow end, Polar cap present and Micropyle not clear Fig (3). Oocyst of *E. tenella* distinguished by ovoid and broad shape, colorless draped with walls with double-layers, micropyle absent and polar cap present Fig (4).

Infection Rate of *Eimeria* spp. According to Microscopic Examination

The *Eimeria* species infection rate in broiler chickens was as follows: 13% of *E. acervulina*, 5% of *E. Praecox*, 1% for each of *E. necatrix* and *E. tenella* as revealed in fig (5) and (6).

PCR Results

The Internal Transcribed Spacer 1 (ITS-1) regions from *E. acervulina*, *E. necatrix*, *E. tenella* and *E. praecox* were amplified successfully and comprised 321, 285, 278 and 368 bp DNA, respectively as in fig (7).

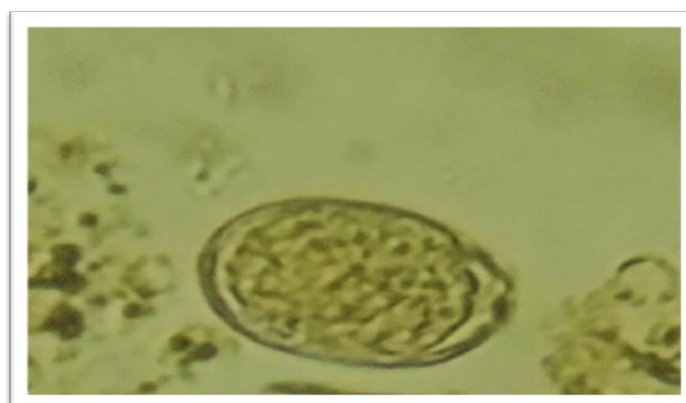


FIGURE 1: Unsporulated oocyst of *E. necatrix* 40X

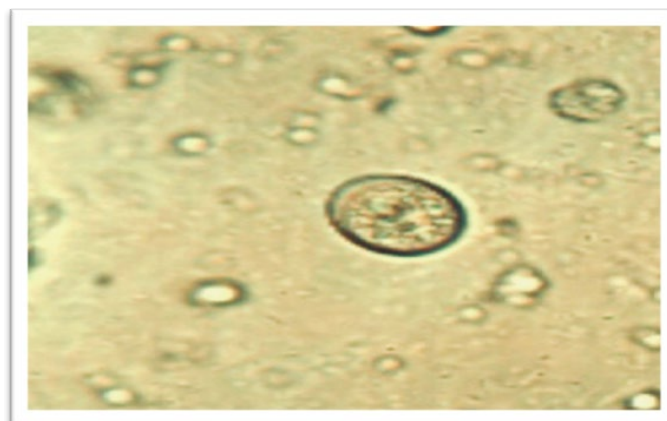


FIGURE 2: Unsporulated oocyst of *E. praecox* 40X

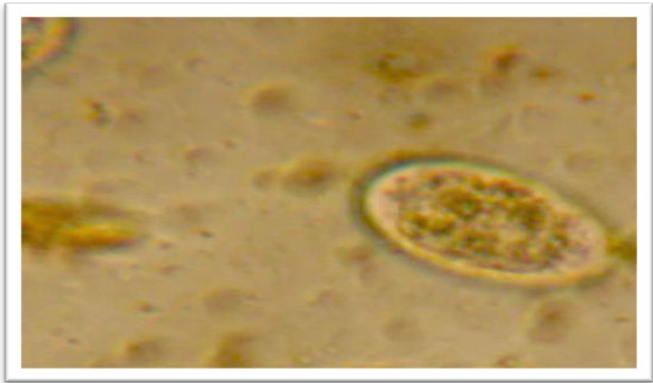


Figure 3: Unsporulated oocyst of *E. acervulina* 40X

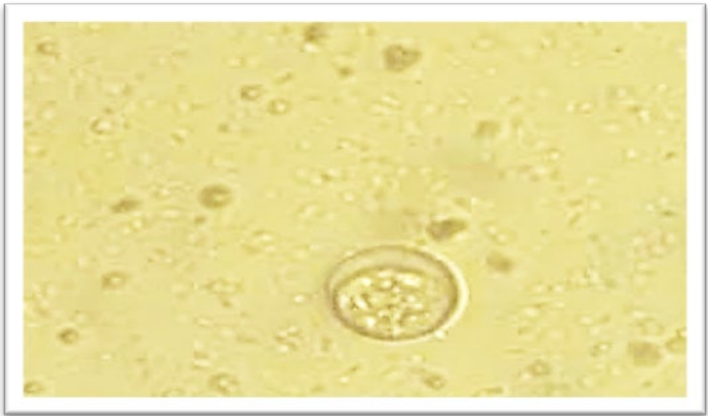


Figure 4: Unsporulated oocyst of *E. tenella* 40X



Figure 5: coccidiosis in broiler chicken

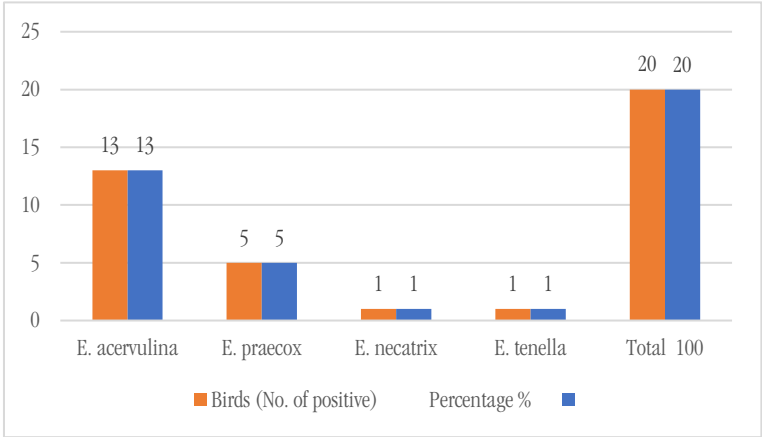


Figure 6: *Eimeria* infection rate according to the species

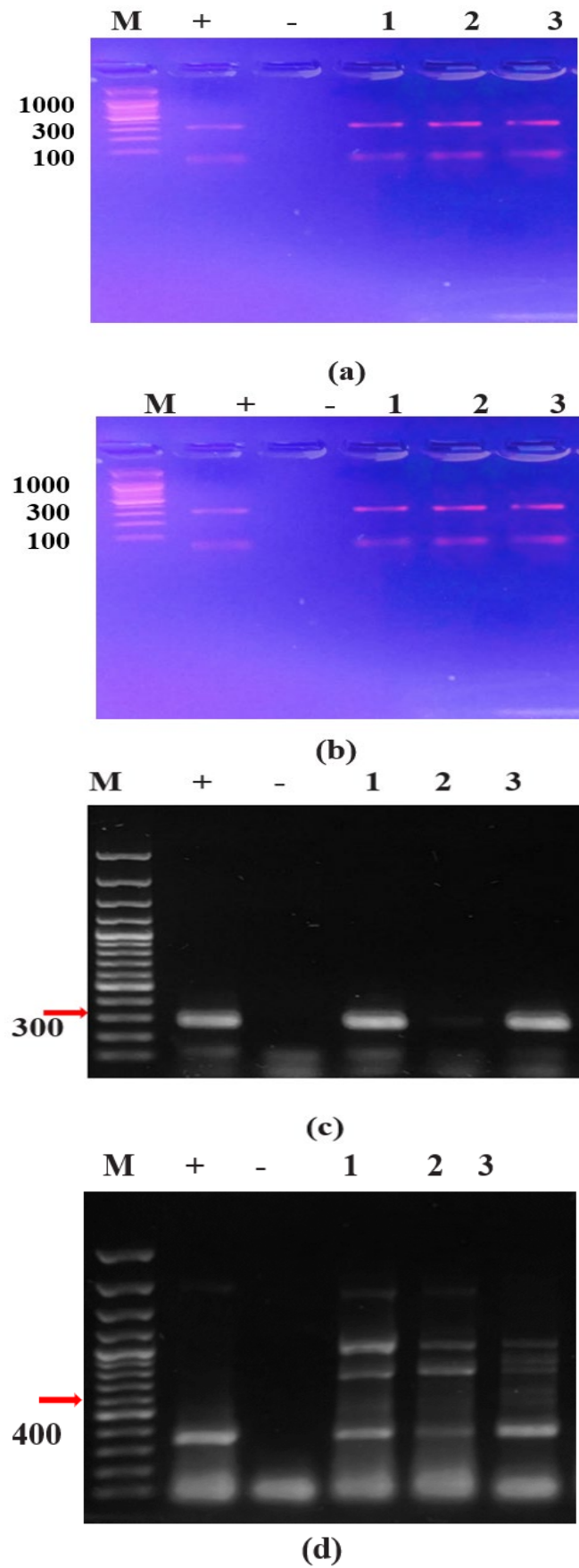


Figure 7: Images of ITS1-PCR yields from oocyst samples resolved on 1.5% of agarose gel. Boosting of PCR was detected utilizing primers specific for (a) *E. acervulinai* (~321 bp), (b) *E. necatrix* (~285 bp), (c) *E. tenella* (~278 bp) and (d) *E. praecox* (~368 bp). A 100-bp molecular weight DNA ladder was employed as a marker, + (Positive control), - (Negative control) and 1-3 samples.

Discussion

Identification *Eimeria* spp. According to Microscopic Examination

This study explained that the *E. necatrix* distinguished by ovoid and oblong in outline, colorless with walls that have double-layers, micropyle absent and polar cap present, while the *E. tenella* was distinguished by broad ellipsoidal shape, colorless draped with walls with double-layers, micropyle absent and polar cap present. This result agreed with [15] who explained similar thoughts about the *E. necatrix* distinguished by an ellipsoidal shape, colorless with walls with double-layers, micropyle absent and polar cap present, while the *E. tenella* broad and shaped like an ovoid, colorless draped with walls with double-layers, micropyle absent and polar cap present.

The *E. acervulina* distinguished by an ellipsoidal shape, yellowish color, double-layered walls with thin in the narrow end, polar cap present and micropyle not clear; this result completely agreement with [16] who explained the morphological characterization of the *E. acervulina* is as follow ellipsoidal shape, yellowish color, two smooth layers with thin in the narrow end, polar cap present and inconspicuous micropyle. The *E. praecox* is distinguished by ellipsoidal to subspherical oocyst, uncolored with double-layered walls, presence of polar cap and absence of micropyle. The result was in concordance with the investigation done by Waqar et al. [16], where it was reported that oocysts of *E. praecox* were ellipsoidal, colorless, possessing two smooth layers, polar cap presence, and micropyle absence. Thus, these species of *Eimeria* have a wide distribution in the study area.

Infection Rate of *Eimeria* spp. According to Microscopic Examination

Based on the present study, *E. acervulina* predominated with 13%, next by *E. praecox* with 5%. In line with these findings, Al Se'adawy Mohenned [17] reported similar in Al-Muthanna province and Nematollahi et al. [18] in Iran. These reports showed the predominance of *E. acervulina* as the most prevalent species for

Eimeria. Thus, most likely, these species of *Eimeria* are widespread in several countries. Nevertheless, an earlier investigation performed in the province of Basra by Waqar et al., [16] proved that the most abundant species was *E. praecox*. The discrepancy is probably due to the different *Eimeria* species virulence under various management systems and/or owing to the prophylaxis utilization of anticoccidial drugs in water and feed, or probably owing to disparities in breed. Most recent anticoccidial medications have developed resistance [19]; *Eimeria* of all species is susceptible to only a small number of medications [20].

Molecular Study

Because of the highly conserved sequence within the species, ITS-1 has been shown to be a very specific genetic indicator for chicken *Eimeriaviva* PCR [8]. In addition, ITS1-PCR's excellent accuracy level, suggests that it has applicative value as an affirmative field diagnostic approach for coccidiosis in chickens. This study explained PCR results showed were positive for the four *Eimeria* species analyzed *E. acervulina*, *E. praecox*, *E. necatrix* and *E. tenella*. *Eimeria* species were identified using the PCR method in this study, as in those by Alam et al., [21] and Moraes et al., [22].

In the present work, the technique of PCR enhances the diagnosis of the *Eimeria* species, which was not easy to pinpoint due to the morphological features of the oocysts. Because of this, the technique of PCR is the procedure that has earned more pertinence due to its precise and fast diagnosis [4].

Conclusions

The identified species of coccidia were *E. tenella*, *E. necatrix*, *E. praecox*, and *E. acervulina*, where the most prevalent species were *E. acervulina* and *E. praecox*. The percentage of infections found in this study could be a sign of circumstances that were especially conducive to the development and spread of acidosis. This finding has also indicated that ITS1-based PCR can be employed for the *Eimeria* species' effective recognition in the field.

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