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(Original Research)

Traditional Molecular Study of *Eimeria* Species (Eucoccidiorida, Eimeriidae) from Ostriches (*Struthio camelus* Linnaeus, 1758) in Middle of Iraq

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Abstract

Background: Coccidiosis in birds, including ostriches, is a major concern for animal health and productivity worldwide. There is limited data on *Eimeria* infections in farmed ostriches (*Struthio camelus*). Therefore, this research aims to investigate the species of *Eimeria* in ostriches using molecular methods. It is considered the first detailed molecular study of *Eimeria* species in Iraqi ostrich farms. **Methods:** A total of 150 ostrich feces samples from farms in three regions of Iraq using both microscopy and molecular methods. Statistical analysis of the positive samples was performed using the chi-square test, with the significance level set at a p-value < 0.05. **Results:** Initial testing showed a high prevalence rate of 51.33%. Five samples, out of a total of 77 suspected cases, underwent polymerase chain reaction (PCR) for confirmation followed by sequencing of the 18S rRNA gene. Sequencing results revealed two species of *Eimeria*: *E. praecox* and *E. tenella*. Notably, this is the first report of *E. tenella* infection in Iraqi ostriches. Genetic analysis of their sequences was performed and deposited in GenBank under accessions NO PV078121.1 to PV078125.1). **Conclusions:** These results have important implications, including understanding the host specificity of *Eimeria* and the parasite's transmission between other hosts. They also contribute to identifying coccidiosis control strategies in Iraqi ostrich farms. Furthermore, our molecular findings provide a foundation for future comparative research on the epidemiology of *Eimeria* in birds.

Keywords

Avian coccidiosis, *Eimeria* spp., Iraq, Ostrich farming, 18S rRNA

1. Introduction

Ostriches are among the hardiest birds and can be raised in North and South Africa. However, the climate presents a significant obstacle to profitable ostrich farming. Ostrich production is a multi-faceted industry, yielding a number of goods such as feathers, hides, and meat [1]. Ostriches primarily inhabit arid desert environments, forcing them to adapt their diet. They eat almost anything, including plants, lizards, seeds, and locusts, making them susceptible to various diseases, including parasitic infections [2]. Ostriches can be infected with external and internal parasites that also infect other birds [3].

Protozoa of the phylum Apicomplexa, family Eimeriidae, are responsible for coccidiosis; many of these species are members of the genus *Eimeria* and infect different parts of the intestine.

The parasite replicates in the host cell and causes significant intestinal mucosal damage during the quick (4–7 day) infectious process [4]. According to Daugschies and Najdrowski [5], *Eimeria* spp. infections are prevalent, particularly in young animals. These unicellular parasites can cause clinical or subclinical disease, such as coccidiosis, which can have serious economic repercussions. Multiple species are known to specifically infect domestic chickens [6]. Infection by Eimeria can lead to considerable harm to mucosal cells, resulting in heightened cell permeability, leakage of plasma proteins, and digestive issues [7]. Therefore, *Eimeria* infection negatively affects the microbial balance in the digestive system, which may lead to microbial imbalance [8]. These alterations encourage the colonization and growth of other pathogens, including *Salmonella enterica* and *Clostridium perfringens* [9]. *Eimeria necatrix*, which causes hemorrhagic enteric coccidiosis; *E. maxima*, which causes chronic enteric coccidiosis; and *E. tenella*, which causes hemorrhagic or cecal coccidiosis, are the most prevalent species [10]. Each of the four sporangia found in the Eimeria oocyst contains two infectious spores. By escaping from the sporangia and infecting a host intestinal epithelial cell, these spores are spread through feces. After a few cycles of asexual reproduction, sexual reproduction takes place to create an uninfected oocyst [11]. *Eimeria*'s clinical manifestations include reduced weight gain and feed intake, diarrhea (from mucous and watery to hemorrhagic), and, in extreme situations, death [12]. Although they can occasionally affect adults, clinical symptoms usually manifest in young animals [13]. The foundation of vaccination strategies is the ability of challenges with low levels of *Eimeria* to elicit the protective host immune response [14]. According to some, the illness only manifests when an animal is exposed to a high dose of the infectious agent or when the host's immunity is relatively low [13]. In systems that are widely raised, coccidiosis is rarely an issue because of the life cycle's self-limiting characteristics and increased resistance to reinfection. However, it becomes crucial in tightly enclosed and highly intensive production systems. The methods for managing the disease encompass the utilization of vaccinations and medications for preventive measures [15]. The importance of this study lies in understanding the specificity of the *Eimeria* parasite, as it infects a specific animal host and causes coccidiosis, as well as identifying its types and its transmission through contaminated food, water, or environmental contamination with feces.

2. Materials and Methods

2.1 Collection sample

A total of 150 fecal samples were gathered from individual ostriches of both sexes across

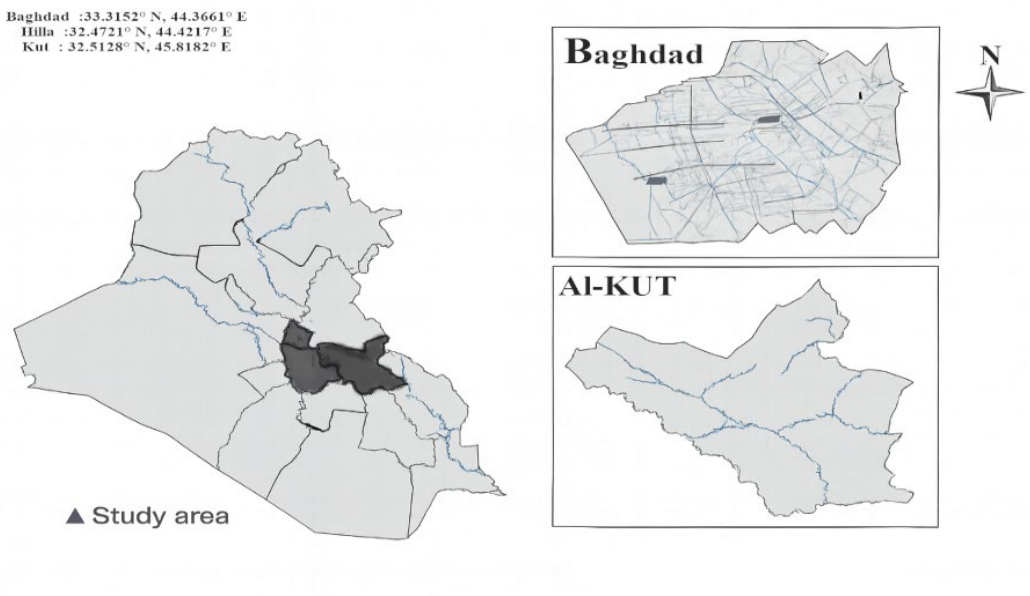

Figure 1. Study area and sampling locations. Ostrich (*Struthio camelus*) samples were collected from farms in Baghdad, Hillah and Al-Kut provinces in central Iraq.

three different regions of Iraq: Hilla, Baghdad, and Wasit (Fig. 1). The sampling took place from October 2024 to July 2025 to capture seasonal differences. Using the direct collection method, 10–20 grams of feces were collected from each ostrich at different times to address possible variations in parasite shedding patterns.

All samples were immediately placed in sterile, pre-labelled containers after collection. Each container was labelled with a unique identifier matching the ostrich. The samples were rapidly transported on ice to the laboratory and stored at -20°C until processing to preserve the parasites and prevent genetic material degradation. The frozen samples were subsequently processed for sporophyte examination and molecular analysis, with the preservation chain documented throughout the study period. In accordance with the ethics Number INHRCM-1 of the study, no harm was done to the animal because sample collection was limited to feces only.

2.2 Microscopic examination

After thawing, the samples were passed through two layers of gauze to remove impurities, then centrifuged to separate the fecal samples, and finally folded in a sugar solution [16]. The following staining techniques were then used: 2% Lugol's iodine solution and oocyst positivity staining, diluted with 2.5% potassium dichromate ($K_2Cr_2O_7$) solution. The parasite was identified using a digital camera mounted on a binocular microscope, which was used to image the parasites at 40x magnification using Kudu [17] as the reference.

2.3 Molecular Study

2.3.1 DNA Extraction

Out of the 77 suspected samples examined microscopically, five samples were selected for genomic DNA extraction to detect *Eimeria* spp. DNA extraction was performed using a commercial DNA extraction kit (Intron Biotechnology, Korea) following the manufacturer's instructions. The DNA samples were then stored at −20 °C until further molecular analysis.

PCR amplification was performed using primers targeting the 18S rRNA gene of *Eimeria* spp., as previously described by Han *et al.* [18]. The primer sequences were as follows Forward primer 5′-TGTAGTGGAGTCTTGGTGATTC-3′ and Reverse primer (R): 5′-CCTGCTGCCTTCCTTAGATG-3′. The expected amplicon size was 207 bp. PCR reactions were carried out in a total volume of 25 µL containing 12.5 µL of GoTaq® Green Master Mix (Promega, USA), 1 µL of each forward and reverse primer (10 pmol/µL), 1.5 µL of template DNA, and 9 µL of nuclease-free distilled water. The conditions thermocycler was an initial denaturation for 5 min at 95 °C, 35 cycles of 45 sec at 95 °C (denaturation), followed by 45 sec at 52 °C (annealing), then followed by a final extension of one minute at 72 °C and a last run for five minutes at the same temperature. Following PCR, DNA fragments were separated on a 1.5% agarose gel and visualized with UV transilluminator.

2.3.2 DNA Sequencing and Phylogenetic Analysis

To confirm the molecular identity of *Eimeria* spp., 18S rRNA gene sequencing was performed for the five selected samples. Before sequencing, the amplified PCR products were purified using the StrataPrep PCR Purification Kit (Include here the manufacturer and the city). The South Korean company Macrogen carried out the DNA sequencing.

Clustal W, which is a feature of the Molecular Evolutionary Genetics Analysis program (MEGA version 6), was used to edit and align the acquired sequences. The Unweighted Pair Group Method with Arithmetic Mean, or UPGMA, approach was used to infer phylogenetic relationships. Reference sequences were retrieved from the NCBI GenBank database and included in the phylogenetic analysis for species identification.

The verified sequences were then uploaded to the NCBI GenBank database, where the local *Eimeria* isolates from Iraqi ostriches were given accession numbers.

2.4 Statistical Analysis

Observed prevalence of microscopy positive samples was calculated as number of positive samples divided by total examined and expressed as a percentage. The prevalence recorded here should therefore be regarded as the observed prevalence in the study group as the samples were collected from the selected farms during the study period and not from a randomly selected statistically representative sample of all Iraqi ostriches. The proportion of positive samples in different provinces was analyzed by the Chi-square test with a significance level *of* $p < 0.05$.

3. Results

This research was carried out in different area in middle of Iraq include Hilla, Baghdad Wasit of Iraq, from October 2024 to July 2025. *Eimeria* spp. were found to be present in 77 (51.33%) of the 150 fecal samples that were examined (Table 1 and Fig. 2). Based on their morphological traits, several unique *Eimeria* species were identified from ostrich fecal matter. The Chi-square analysis indicated no statistically significant differences in prevalence among provinces ($\chi^2 = 0.62$, df = 2, $p = 0.73$) (Table 2 and Fig. 3).

Table 1. Total infection with of *Eimeria* spp in ostriches.

Animal	No. of samples examined	No. of positive	Percentage (%)
Ostriches	150	77	51.33

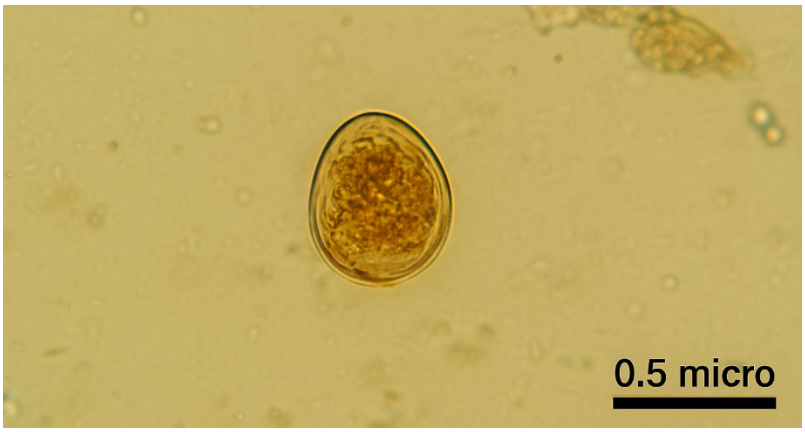

Figure 2. Light micrograph of an unsporulated *Eimeria* spp. oocyst.

Table 2. Positivity of *Eimeria* in ostriches by province.

Province	Examined (n)	Positive (n)	Prevalence (%)
Baghdad	55	30	54.50
Babylon	50	26	52.00
Wasit	45	21	46.70
Total	150	77	51.33

*No statistically significant differences

3.1 Molecular Results

3.1.1 PCR analysis

Conventional polymerase chain reaction (PCR) was performed to amplify and isolate 18S RNA genes from five selected samples. Each of these samples produced amplification products of the expected size, confirming the presence of Eimeria DNA in all of them.

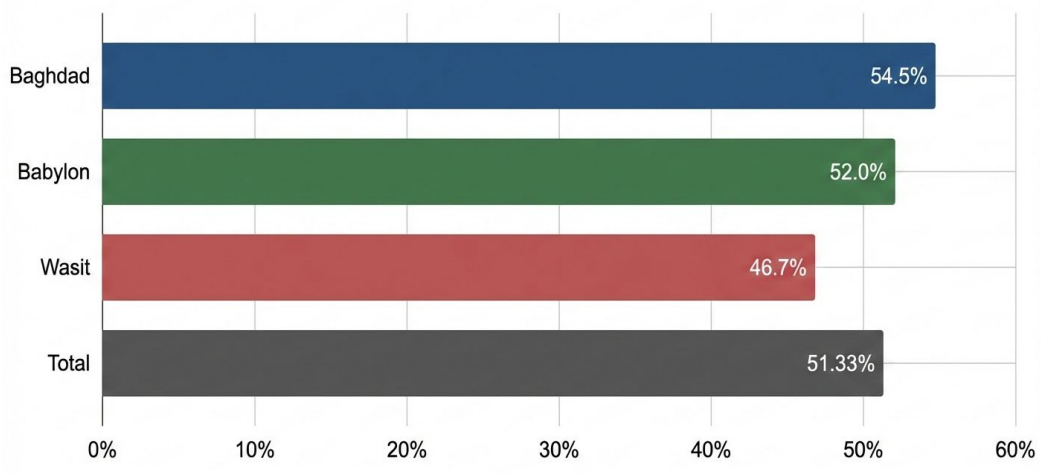

Figure 3. Prevalence of *Eimeria* infection in ostriches across Baghdad, Babylon, and Wasit provinces.

3.1.2 DNA sequencing results

The five samples underwent DNA sequencing. Sequences were aligned and analyzed using MEGA version 6 software, and similarity percentages were determined via BLAST analysis against reference sequences in the NCBI database. The confirmed sequences were submitted to NCBI GenBank, with accession numbers assigned to the local ostrich-derived Eimeria isolates. According to 18S rRNA gene sequencing and BLAST homology, two *Eimeria* spp. and *E. tenella* (accession numbers PV078121.1 and PV078122.1), *E. praecox* (accession numbers PV078123.1 and PV078124.1) and *Eimeria* spp. (accession numbers PV078125.1) were identified, as shown in Table 3.

3.1.3 Phylogenetic analysis

Colored circles and their matching accession numbers are used to illustrate the phylogenetic tree analysis of *Eimeria* spp. based on the already recognized sequences (18S ribosomal RNA). As is evident, the Iraqi strains mostly appeared to be closely related. Targeting the 18S ribosomal RNA, however, revealed that *E. tenella* and an isolate from India were closely related, whereas *E. praecox* and an isolate from the USA were closely linked (Fig. 4).

4. Discussion

This study was conducted between October 2024 to July 2025, from different areas in the

Table 3. The homology sequence identity (%) between NCBI-BLAST deposited strains and local *Eimeria* sp isolates from ostrich feces that were deposited in the 18S rRNA gene bank.

No	Accession No	Identical to	Genbank accession No.	Country	Identity %
1	ID: PV078121.1	*Eimeria tenella*	ID: EF210326.1	India	99%
2	ID: PV078122.1	*Eimeria tenella*	ID: EF210326.1	India	99%
3	ID: PV078123.1	*Eimeria praecox*	ID: FJ236364.1	USA	100%
4	ID: PV078124.1	*Eimeria praecox*	ID: FJ236364.1	USA	99%
5	ID: PV078125.1	*Eimeria* sp	ID: KU160242.1	USA	99%

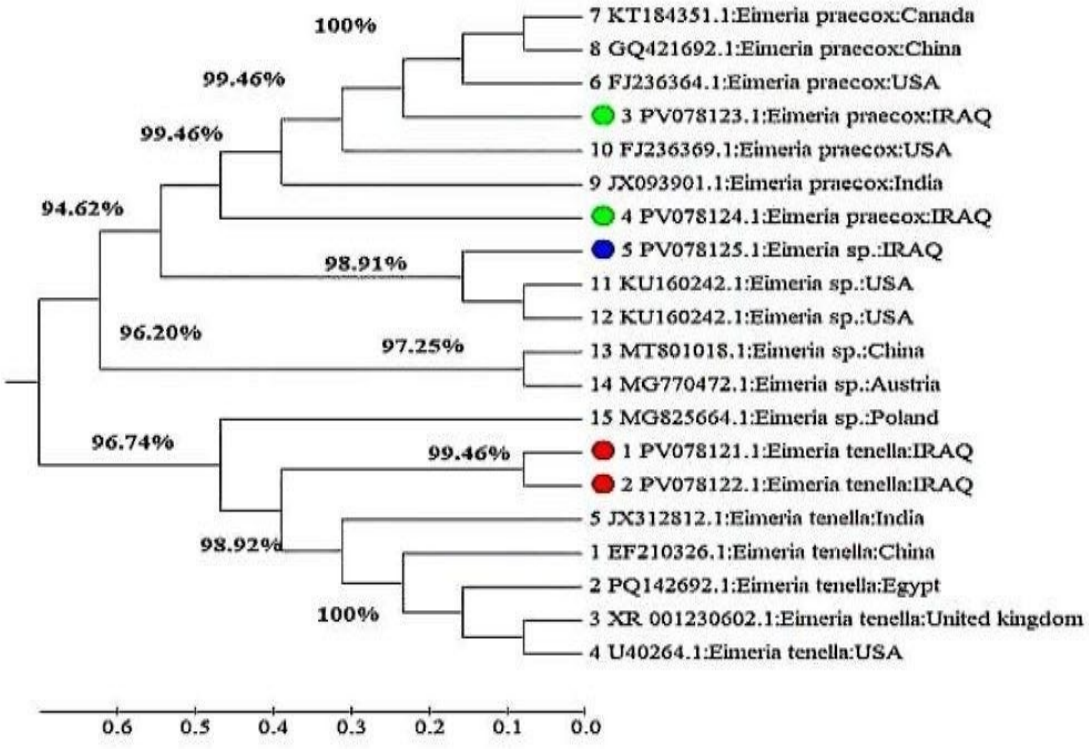

Figure 4. Phylogenetic tree analysis of *Eimeria* species focusing on the 18S rRNA gene.

middle of Iraq including Hilla, Baghdad, and Wasit province. A total of 150 fecal samples were collected and 77(51.33 %) of the samples are suspected to be infected with *Eimeria* spp. with no statistically significant differences in positivity between provinces. A study by Mushi *et al.* [19] in Botswana revealed that 34% of ostrich chicks that appeared healthy had coccidia oocysts. This contrasts with the study of Eslami *et al.* [3], wherein only a small number of *Eimeria* oocysts that have not yet finished sporulation were identified in fecal samples from farm ostriches in Garmsar Province of Iran. Mshelia *et al.* [20], on the other hand, observed a total distribution rate of 11.6% in ostrich farms for *Eimeria* oocysts in Nigeria. In Egypt, El-Shahawy, and Abou Elenien [21] observed that *Eimeria* spp. infected ostriches were at a rate of 25%. However, for farmed ostriches in Egypt, El-Sayed [22] documented that *Eimeria* sp. infected only 3.85% of sampled ostriches. The differences and spread of parasites between farms and countries can be explained by the movement of animals between countries [23]. Seasonal variations and quantity of animals used in various studies could also account for the variations in the dominant species of *Eimeria*. Local control strategies, and regional and agroclimatic diversity could also contribute to these differences.

It is noted that there is a paucity of molecular studies regarding the *Eimeria* parasite and its species in ostriches. Molecular analysis of *Eimeria* conducted recorded two species for the first time in Iraq namely: *E. praecox* and *E. tenella.* Due to this paucity, this study compared it result to different avian species. In chickens, Meireles *et al*. [24] also observed *Eimeria* from156 field samples subjected to PCR, wherein 70 samples tested positive for *E. praecox*. *E. praecox* is a coccidia parasite that infects and grows in the duodenum. Additionally, in commercial broiler farms in Thailand, Tongkamsai *et al.* [25] observed that the most common species (40%) were *E. tenella* and *E. praecox*.

For *E. tenella* infection, wherein ostrich samples tested positive despite not observing any clinical signs, was also observed in chicken embryos. Jiang *et al.* [26], used chicken embryos as a model for *E. tenella* infection and manifested no clinical signs. Birds with *E. tenella* may have been completely resistant to the parasites and do not undergo further development. This resistance may result in birds releasing the oocyst without

forming lesions or the birds to developing lesions and not manifesting clinical signs. [27]. The identification of *E. tenella*, may also suggest potential cross-transmission between domestic poultry and ostriches under local farming conditions. This type of farming conditions makes ostriches vulnerable to internal and external parasites that affect other birds. Under certain conditions in chicken coops, *Eimeria* eggs can multiply rapidly and contaminate environmental materials, including equipment, feed, soil, and bedding, thereby posing a continuous risk of disease transmission. Therefore, further detailed studies are needed to clarify different aspects of parasitic infection including its effects on infected ostriches [3,28]. One of the most frequent methods of spreading oocysts of coccidia is mechanical transmission, which includes the movement of people or equipment between farms, as well as the presence of rodents and insects like flies and beetles [29]. Anthropogenic transmission is probably favorable to parasite dispersal, e.g. through contaminated work tools and vehicles. Moreover, transmission events between wild and domestic populations of the same or closely related host species sharing *Eimeria* spp. cannot be excluded [30].

5. Conclusions

This is the first report on the molecular identification of *Eimeria* in ostriches. This paper will also improve knowledge about Eimeria in ostriches and how it infects birds through contamination and mechanical transfer. In this study, the 18S gene was used particularly as a basic and fixed reference step for diagnosis of *Eimeria* spp. and as a database. Thus, in the future, for the highest taxonomic level of strains, the COX1 gene and ITS1 will be used. As a result, future research may focus on the specificity of the parasite within the host and on biosecurity in the poultry farming system, especially in mixed systems.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available in the GenBank repository under the accession numbers PV078121.1 to PV078124.1. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization, Z.A.M.; Methodology, Z.A.M., and M.M.A.; Investigation, Z.A.M., M.M.A., and H.M.J.; Data Curation, M.M.A.; Writing – Original Draft Preparation, Z.A.M., and M.M.A.; Writing – Review & Editing, Z.A.M., M.M.A., and H.M.J.; Supervision, Z.A.M. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

Sample collection was carried out in accordance with ethical standards for animal research and approved by the relevant institutional guidelines.

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Conflict of Interest

The authors declare no conflict of interest.

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