JWPR
Journal of World's Poultry Research
2025, Scienceline Publication
J. World Poult. Res. 15(3): 332-337, 2025
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2025.32
PII: S2322455X2400032-15
ISSN: 2322-455X

Isolation and Identification of *Eimeria* spp. From Domestic Pigeons (*Columba livia domestica*) in Basrah, Iraq

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Received: July 08, 2025, Revised: August 12, 2025, Accepted: September 04, 2025, Published: September 25, 2025

ABSTRACT

Coccidiosis is a widespread disease among pigeons worldwide, resulting in significant economic losses due to mortality, morbidity, and reduced feed efficiency. The present study aimed to isolate a field strain of *Eimeria* spp. in domestic pigeons (*Columba livia domestica*) in Basra, Iraq. To ensure precise species identification, light microscopy techniques were combined with the advanced molecular method of polymerase chain reaction (PCR). Sixty fresh fecal samples were collected from pigeon lofts, ensuring minimal environmental disturbance and contamination. The flotation method was employed to analyze fecal samples. The present results indicated that 36 out of 60 samples contained coccidian oocysts, which represented an overall prevalence of 60%. The PCR technique was employed to amplify the *18S rRNA* genes, which were subsequently utilized for sample detection. Six representative sequences were selected, registered, and deposited in the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). The present findings were validated through molecular analysis, and six representative gene sequences were submitted to the National Centre for Biotechnology Information (NCBI) database. The current study revealed a high prevalence of coccidiosis, affecting 60% of domestic pigeons in Basra, highlighting its impact on their health. Moreover, this is the first report of *Eimeria chalcoptereae* in pigeons from Basra, Iraq.

Keywords: *Eimeria chalcoptereae*, Molecular technique, Phylogeny, Pigeon

INTRODUCTION

Coccidiosis is a prevalent disease among pigeons globally and results in considerable economic losses due to mortality, illness, and diminished feed efficiency (Santos et al., 2020; Aboelhadid et al., 2021). The performance of poultry is adversely affected by this condition as it induces acute disease and impairs nutritional utilization (Al-Agouri et al., 2021). Coccidiosis is a significant veterinary disease with a notable economic impact on the pigeon industry (He et al., 2024).

The signs of coccidiosis include watery diarrhoea accompanied by mucus (Mohammed et al., 2017). Watery diarrhea with mucus is a common sign of mucoid or bloody diarrhea, often associated with clinical features such as dysentery, enteritis, emaciation, decreased feed conversion, drooping wings, poor growth, and even death. This condition serves as one of the early diagnostic

indicators for avian coccidiosis (Ola-Fadunsin et al., 2017). Pigeons are infected by 21 species of intracellular apicomplexan protozoan parasites from the genus *Eimeria* (Albasyouni et al., 2024). To reduce the risk of parasite spread, regularly remove feces and clean cages and floors. Since coccidiosis spreads rapidly in crowded environments, preventing overcrowding in barns is essential, along with ensuring proper ventilation and humidity control in breeding areas. Additionally, a balanced diet supplemented with vitamins and immune-boosting nutrients such as A, E, and C is recommended (Alsayeqh and Abbas, 2023). Although management and biosecurity measures may potentially prevent the introduction of *Eimeria* spp. to a farm, in practice, these measures are insufficient to prevent outbreaks of coccidiosis. However, extensive and prolonged use of anticoccidial medicine has resulted in the development of resistance worldwide against all such medications (Peek and Landman, 2011). In Basra, Iraq, where pigeon breeding holds considerable cultural and economic importance, *Eimeria* spp. presents both health and economic difficulties. The present study aimed to isolate a field strain of *Eimeria* spp. in domestic pigeons (*Columba livia domestica*) of Basra, Iraq.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Committee approved the experimental procedures and animal care. Research Ethics Committee No. 83/37/2025 from the University of Basrah-College of Veterinary Medicine confirms that all protocols were followed, and appropriate measures were taken to minimise discomfort.

Study area and sample collection

The present study was conducted from April to December 2024. A total of 60 adult domestic pigeons (*Columba livia domestica*) from different lofts located in several areas of Basrah, Iraq, were included. All of the pigeons exhibited clinical signs of coccidiosis, including weight loss (Emaciation) and the presence of bloody droppings. There were no pigeons found without signs of coccidiosis. The number of pigeons per loft differed from 20 to 80, with some lofts experiencing substandard living conditions and inadequate provisions and sanitation.

Fecal examination

Fecal samples of approximately 1 g were collected from each pigeon and placed in individual screw-capped plastic containers labelled appropriately. These samples were subsequently transported to the Department of Veterinary Parasitology at the College of Veterinary Medicine, University of Basrah, Basrah, Iraq. Initially, the samples were examined to determine color, consistency, and the presence of blood, mucus, or other contaminants. The presence or absence of coccidia oocysts was checked using an Olympus microscope (Japan) with Sheather's sucrose solution. In positive samples, oocysts were concentrated via the flotation technique described by Alasadiy et al. (2022). Oocyst morphology and sporulation time were used to identify the species of *Eimeria* spp., following the guidelines outlined by Silva et al. (2022).

Polymerase chain reaction amplification, DNA extraction, and sequencing

The DNA was obtained from the purified oocysts for molecular identification using a commercial DNA extraction kit. The *Eimeria* spp. *18S rRNA* gene was targeted by a polymerase chain reaction (PCR). The QIAamp PowerFecal Pro DNA Kit (Cat. No/ID: 51804, QIAGEN, Australia) was used to extract total DNA from 350 mg of each fecal sample, following the manufacturer's instructions. A nested PCR was conducted using the methods outlined by Yang et al. (2016a;b). An expected PCR product of approximately 1510 bp was anticipated. The primers EiGTF1 5′-TTCACAGGACCCTCCGATC and EIGTR1 5′- AACCATGGTAATTCTATGG were employed for the external amplification of the *18S rRNA* gene.

Sequence and bioinformatics analysis

The positive PCR results were sequenced forward at the Macrogen® sequencing facility (Seoul, South Korea). The obtained sequences were verified using a BLAST search and a sequence identity for further analysis. Sequences were aligned with relevant reference sequences using the CLUSTAL-X approach (Thompson et al., 1997). The Tamura-Nei model was used for a comprehensive evaluation of taxonomic relationships based on nucleotide analysis, and phylogenetic trees were constructed using the maximum likelihood approach (Tamura et al., 2011).

RESULTS

The total population of domestic pigeons was estimated at 60, with a 60% prevalence of coccidian infection, which was detected using the flotation technique. The oocyst was sub-spherical to spherical in shape and lacked a micropyle. The wall was thick, smooth, and consisted of two layers. Sporulation occurred within 24 to 48 hours. An example of a non-sporulated oocyst is shown in Figure 1.

Detection of DNA by the polymerase chain reaction

Polymerase chain reaction was employed to amplify the DNA extracted from *E. chalcoptereae* samples utilizing both forward and reverse primers. Subsequently, eleven samples were identified using PCR (Figure 2). The DNA bands, approximately 1510 base pairs in length, were observed after examination on an agarose gel.

Phylogenetic analysis

According to the sequence analysis results, all six positive strains exhibited a proximate relationship. Six representative sequences were selected and uploaded to the NCBI database. The new records are labelled as PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1. Figure 3 presents the phylogenetic trees, which depict the evolutionary links between pigeon *Eimeria* spp. from the NCBI database and strains isolated from pigeons in Basra, Iraq, marked by the red square.

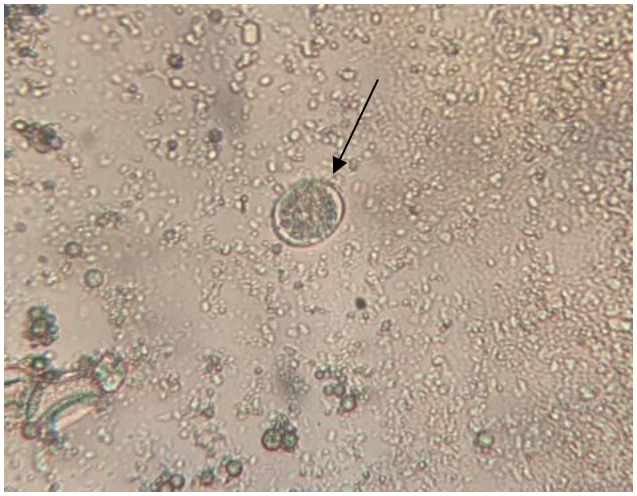

Figure 1. The *Eimeria chalcoptereae* oocyst that is not sporulated (black arrow, ×40) isolated from a pigeon in Basrah, Iraq.

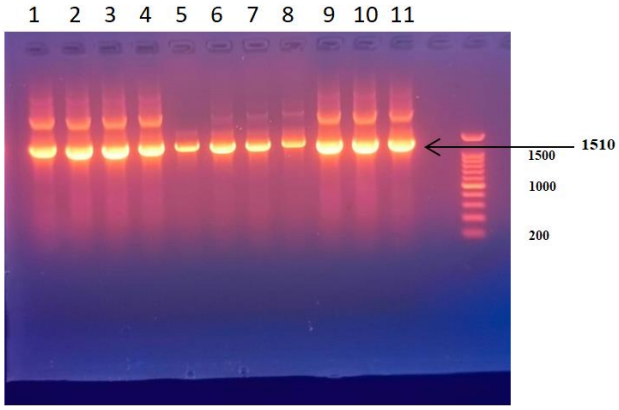

Figure 2. Polymerase chain reaction analysis of *Eimeria chalcoptereae* isolated from a pigeon in Basrah, Iraq, using agarose gel electrophoresis. M: 1510 bp molecular Ladder, Lane: Samples 1-11 were examined.

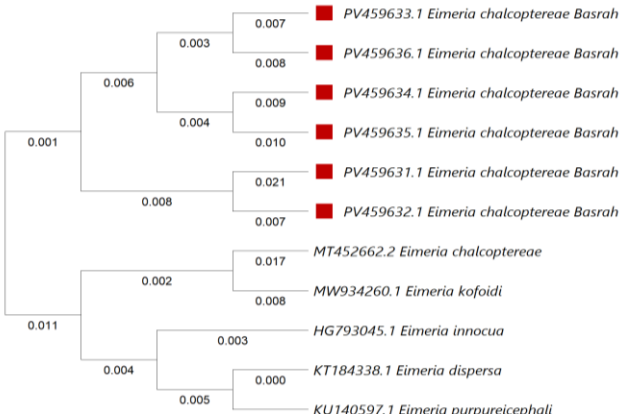

Figure 3. Phylogenetic tree analysis of six *Eimeria chalcoptereae* isolated from pigeons in Basrah, Iraq. It is constructed based on the *Eimeria chalcoptereae* small subunit ribosomal RNA gene, partial sequence, displaying the evolutionary relationships between closely related pigeon *Eimeria* spp. from the NCBI database and strains isolated from pigeons (red square). Utilizing MEGAX11 software, the neighbor-joining approach was used to create the tree and perform evolutionary studies.

DISCUSSION

Based on the present results, numerous breeds of pigeons were bred in Basra, Iraq, for different purposes. According to the present study, 36 out of 60 samples tested positive for coccidian oocysts, indicating an overall incidence of 60%. This prevalence aligns with data from other countries, including 75% as reported by Ramesh et al. (2018) in India, 67.58% as documented by Gül et al. (2009) in Turkey, 58.2% as observed by Elseify et al. (2018) in Egypt, 56.2% as noted by Joseph et al. (2017) in Nigeria, and 52% as reported by Huang et al. (2018) in China. A reduction in food consumption, weakness, and greenish watery diarrhoea were the clinical manifestations of pigeon intestinal coccidiosis. Several studies have reported that coccidiosis causes significant losses in domestic pigeons (Dalloul and Lillehoj, 2005; Bandyopadhyay et al., 2006; Quiroz-Castañeda et al., 2015; Gadelhaq and Abdelaty, 2019). One of the limitations of microscopy is its inability to reliably discriminate across species. For increased sensitivity and species identification, previous studies have emphasized the importance of incorporating molecular diagnostics, such as PCR (Haug et al., 2008). In Al-Diwaniyah province, Iraq, Jawad and Jasim (2025) demonstrated that PCR-based diagnostics significantly enhanced the accuracy of identifying *E. tenella*, indicating its prevalence in broiler farms. The current findings highlighted the crucial role of molecular surveillance in poultry

operations, particularly considering that microscopic detection may underestimate the true level of infection, especially in subclinical cases. In the present study, PCR was employed to validate the initial clinical diagnosis, representing the initial effort to identify and isolate *Eimeria* spp. in Basra, Iraq. Phylogenetic trees are frequently employed to analyze *Eimeria* spp. in domestic pigeons in Basra, Iraq. Six representative sequences were selected, documented, and uploaded to the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). In the current investigations, extremely small genetic differences were observed between the *E. chalcoptereae* isolates from Basra, Iraq, ranging from 0.000 to 0.004%, indicating a shared origin or local evolution, along with advantageous genetic conservation. The isolates from Basra and the reference strain of *E. chalcoptereae* (MT452662.2), as documented by Yang et al. (2020), exhibited pairwise genetic distances ranging from 0.007% to 0.009%, indicating a moderate level of divergence, likely due to regional or host-specific factors. It is inferred that the sequences identified in the present study and those documented by Yang et al. (2020) most likely correspond to the same species, as the present study and Yang et al. (2020) are currently the only sources of molecular data available. This is attributed to the role of Basra as a central hub for the illicit transportation of pigeons originating from Persian Gulf countries, despite these pigeons emanating from two distinct and geographically isolated regions (Al-Hasnawy and Rabee, 2023; Jaafar, 2023). Pairwise distances were notably greater, ranging from 0.008% to 0.021%, compared to other *Eimeria* spp., including *E. kofoidi* (MW934260.1), *E. innocua* (HG793045.1), *E. dispersa* (KT184338.1), and *E. purpureicephali* (KU140597.1). The current results confirmed the uniqueness of the *E. chalcoptereae* isolates found in Basra, Iraq, highlighting their differentiation at the species level. This phylogenetic analysis revealed that the isolates from Basra, Iraq, are genetically consistent and clearly distinct from other *Eimeria* spp. These findings have deepened the understanding of local parasite evolution and may guide future studies on host-pathogen interactions and control strategies.

CONCLUSION

The current study revealed a high prevalence of coccidiosis, exceeding 60%, among domestic pigeons in Basra, Iraq. Molecular analysis identified 11 samples that amplified the *18S rRNA* genes. *Eimeria chalcoptereae* was detected in *Columba livia domestica* pigeons, which presented the first record of *Eimeria chalcoptereae* in Basra, Iraq. Six representative sequences were selected, registered, and deposited in the NCBI database (PV459631.1, PV459632.1, PV459633.1, PV459634.1, PV459635.1, and PV459636.1). To understand the distribution and genetic diversity of *Eimeria* spp., future studies should concentrate on seasonal infection patterns, their effects on productivity, and possible medication resistance. Additionally, molecular surveillance should be expanded throughout bird flocks in Iraq.

DECLARATIONS

Acknowledgments

The authors wish to acknowledge the Laboratory of the Department of Veterinary Parasitology at the College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Authors' contributions

The study's conception and design, data collection, and analysis were all conducted by Alaa Ismail Saood, Isam Azeez Khaleefah, Sara Salim Mohammad, Abduljabbar Khadim Alkinani, Khawla Bedan Nassir Aljassim, and Harith Abdulla Najem. Abduljabbar Khadim Alkinani and Khawla Bedan Nassir Aljassim took part in sample processing and laboratory procedures. Harith Abdulla Najem and Sara Salim Mohammad assisted with the manuscript's development and critical review. Alaa Ismail Saood oversaw the study, created the graphical abstract, and completed the manuscript. The final draft of the manuscript was authorized for publication by all authors.

Funding

This study did not receive any financial support or funding.

Conflict of interests

The authors declared that they have no conflicts of interest.

Ethical considerations

The authors are the original authors of this paper, which has not been published anywhere. The authors verified that their original scientific findings served as the basis for the article's writing by running it through a plagiarism index.

Availability of data and materials

The corresponding author can provide the data supporting these findings upon reasonable request.

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