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Original Article

# Diversity of Intestinal Parasites in Ostriches *Struthio camelus* Linnaeus, 1758 by Using Routine Parasitological Techniques and PCR in Iraq

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**Abstract**

**Introduction:** Ostrich farming has emerged as a new livestock industry in Iraq, but scientists lack sufficient information on health concerns, including intestinal parasites that cause significant production losses and financial instability over extended periods.

**Methods:** Researchers collected 150 fecal samples from ostriches that dwelled in central and southern Iraq for microscopic examination of intestinal parasite occurrence.

**Results:** The six parasite species included *Entamoeba* sp., which made up 26.66% of the population, and *Cryptosporidium* sp. at 11.33%, *Ascaridia galli* at 10%, *Giardia* sp. at 4.6%, *Raillietina* sp. at 2%, and *Trichostrongyl*. Molecular analysis was performed on a subset of positive samples because Entamoeba sp. is highly prevalent. PCR amplification of the 18S rRNA gene revealed fragments of approximately 579 bp for *Entamoeba struthionis* (IDs: PV019353.1, PV019354.1), *Entamoeba polecki* (IDs: PV019355.1, PV019356.1), and *Entamoeba* sp. (ID: PV019357.1), the first time in Iraq. The NCBI database now has these sequences.

**Conclusion:** The current study concluded that molecular diagnostics in ostrich health management are crucial for early detection, precise treatment, and improved productivity. Regular monitoring is recommended to promote sustainable ostrich farming in Iraq.

**Keywords:** Intestinal parasite, Iraq, PCR reaction, Ostriches, 18S rRNA

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## Introduction

Ostriches, one of the world's strongest and most resilient birds, can be raised outside of Africa in both northern and southern climates. Climate-related issues, however, may affect the profitability of ostrich farming. Feathers, leather, and meat are just a few of the goods produced by the ostrich industry (1). Organisms that reside within or on another species are known as parasites (2). In addition to being essential for maintaining ecological balance, wild animals are valuable human companions. In addition to their crucial role in sustaining ecological balance, wild animals serve as significant companions to humans.

Over the past six decades, considerable ecological changes and habitat degradation have influenced the ostrich industry (3). Birds serve as hosts for numerous parasite species, which have adapted to live within them. The consumption of parasite eggs or oocysts does not always result in infection, and parasitism may not always present as a clinical disease (4). Birds serve as essential hosts for generalist parasites because they inhabit various environments and function as reservoirs for these parasites (5). The proper use of anthelmintics requires veterinary guidance because incorrect application can lead to drug resistance and persistent parasite infections (6). The ostrich is particularly susceptible to protozoan parasites, including *Entamoeba struthionis* (7). The prevalence of animal parasites depends on multiple factors, which include host immune defenses, environmental conditions, habitat characteristics, and species traits (8,9). Wildlife species require monitoring systems for parasitic infections to prevent disease transmission among species and protect their survival. The presence of *Strongyloides* and *Toxocara* parasites in animal habitats near human settlements increases the risk of zoonotic disease (10–12).

The parasites that cause cestode infections, which lead to tissue necrosis and vascular obstruction, pose a risk to animals living near populated areas. Parasites harm host tissues by physically entering host tissues when their larvae penetrate during the reproductive or establishment phases (13). Wild bird species are essential components of ecosystem and biodiversity protection. Wild birds perform essential functions as natural pest controllers, seed dispersers, and pollinators (14, 15). The reduction of biodiversity accelerates disease spread, creating more opportunities for zoonotic diseases to affect both domestic animals and people while simultaneously leading to the emergence of new disease outbreaks. This is especially relevant for species that regulate disease and pest populations. The established factors create a destructive feedback loop that threatens worldwide

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health protection systems (16, 17). Wildlife conservation initiatives should establish new populations and protect existing populations through habitat conservation to safeguard the genetic diversity of animal populations and endangered species. The practice of captivity helps protect birds from specific dangers, but it creates new stressful conditions that increase their susceptibility to parasites and health problems (18). Regular parasitological testing, together with advanced diagnostic methods, is essential for reducing adverse outcomes associated with parasitic infections. The techniques enable doctors to detect diseases in their early stages, thereby allowing them to develop specific treatment strategies (19).

The research focused on the scientific study of intestinal parasite infections affecting ostriches. The particular objectives of the study were:

1. Through microscopic examination and molecular analysis, researchers will identify all parasite species that affect ostriches.
2. To evaluate the effectiveness of advanced diagnostic techniques for diagnosing parasitic infections in ostriches.

## Materials and methods

### *Collection sample*

The researchers collected fresh ostrich feces samples from 64 ( 42.66%) male and 86 (57.33%) female who lived in cages and reserves across different Iraqi locations which included Al-Mahawil (Babil), Al-Zawraa Park, Al-Adamiyah (Baghdad), and Azizia (Wasit) as shown in Figure 1 from November 2023 to July 2024, their ages ranged from one year to over three years old. The ostrich received four samples, each weighing between 10 and 20 grams, collected every three days. The samples were stored in a sterile container in a freezer until laboratory testing. The animals were assigned identification numbers when scientists collected their samples.

### *Microscopic examination*

At the Vertebrate Laboratory, INHM University of Baghdad, faecal samples were processed according to established coprological protocols (20-21). The process of detecting intestinal parasites involved centrifugation to concentrate samples, followed by flotation in Sheather's sugar solution and staining with 2% Lugol's Iodine to make cysts, oocysts, and eggs more visible. A calibrated ocular micrometer was used to obtain morphometric measurements, and high-resolution digital photographs were used to document the parasitic structures. Standard diagnostic methods reach their full potential when combined with PCR-based molecular tests, which identify parasite species at low concentrations and in complex fecal sample matrices.

### *Molecular study*

#### *PCR Amplification and DNA Extraction*

A commercial DNA extraction kit (Intron Biotechnology, Korea) was used to extract genomic DNA from 50 Entamoeba sp.- positive fecal samples that had previously been collected and stored at -20 °C in accordance with the manufacturer's instructions. The purified DNA samples were stored at -20 °C before use.

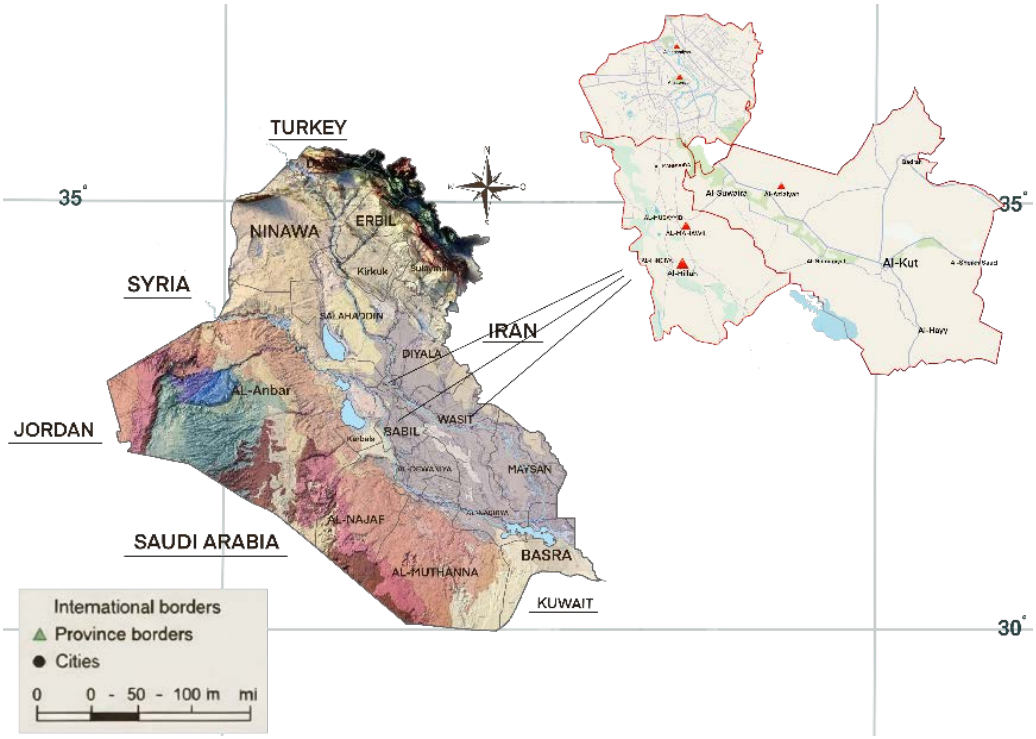


**Figure 1.** Geographical distribution of ostrich farming sites sampled in central Iraq

We amplified the 18S rRNA gene for molecular characterization using the Verweij *et al.* protocol (22). With the primer pair: Forward 5′-GTTGATCCTGCCAGTATTATATG-3′ and Reverse 5′-CACTATTGGAGCTGGAATTAC-3′. Ten representative samples were selected from the extracted DNA and subjected to PCR amplification. The 25 µL total volume of the PCR reaction was composed of 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 water. The mixture was gently vortexed and briefly centrifuged, then subjected to PCR amplification.

The PCR products were analyzed by electrophoresis on a 1.5% agarose gel prepared with 1×TBE buffer. A GelDocTM XR+imaging system (Bio-Rad, USA) was used to visualize the gel under UV light after staining with ethidium bromide (0.5 µg/ml). The size marker was a 100-bp DNA ladder (Promega, USA). According to Verweij *et al.* (22), the *Entamoeba* 18S rRNA fragment was expected to be about 585 bp in size. In this study, the PCR products migrated near 579 bp, consistent with the anticipated size. Slight deviations in length (±5–10 bp) were due to variations in gel concentration and electrophoretic conditions.

### *Sequencing of DNA and phylogenetic tree*

To ascertain the molecular identity of *Entamoeba* species, DNA sequencing was performed on 10 positive samples using PCR-amplified Entamoeba spp. DNA. The amplified segment of the 18S rRNA gene was purified using the Strata Prep PCR Purification Kit. The UPGMA [unweighted pair group method with arithmetic mean] method was used to infer the evolutionary history. A phylogenetic tree and the GenBank database were used to obtain accession numbers for local *Entamoeba* species in Iraq, and bioinformatics for Ostrich Entamoeba.

The purified 18S rRNA PCR amplicons were sent to Macrogen Inc. for Sanger sequencing. To acquire accession numbers, the resulting *Entamoeba spp.* nucleotide sequences were entered into the NCBI GenBank database. ClustalW was used to align the sequences with reference sequences obtained from GenBank for subsequent analysis. MEGA version 6 was used to infer phylogenetic relationships using conventional evolutionary models. *Species identification of Entamoeba isolates was verified by BLAST against reference sequences* in the NCBI database.

### *Statistical Analysis*

A statistical analysis was performed to assess the relationship between geographical location, and the results showed a slight variation in the spread. However, it was not statistically significant at the 0.05 level ($\chi^2 = 6.47$, df = 3, $P = 0.09$).

## Results

This study was conducted at multiple sites across central Iraq, including Al-Mahawil (Hilla), Al-Zawraa Park, Al-Adamiyah (Baghdad), and Azizia (Wasit), from November 2023 to July 2024. Of 150 fecal samples, 85 (56.66%) were positive for intestinal parasites; the difference was not statistically significant at the 0.05 level (Table 1). Several different intestinal parasites were identified using the feces of ostriches based on their morphological traits and size included six species of intestinal parasite: *Entamoeba* sp (26.66%), *Cryptosporidium* sp. (11.33%), *Ascaridia galli* (10%), *Giardia* sp (4.6%), *Raillietina* sp (2%) and *Trichostrongylus tenuis* (2%), (Figure 2, Tables 2 and 3).

### *Molecular results*

Gel electrophoresis on 1.5% agarose showed clear, individual amplicon bands approximately 579 bp in length in all positive samples, consistent with the expected 585 bp fragment of the *Entamoeba* 18S rRNA gene (Figure 3).

This confirms the successful molecular identification of *Entamoeba* sp. Minor size variation (520–585 bp) across runs was attributable to electrophoresis conditions and marker calibration, rather than sequence differences.

### *DNA sequencing results*

The current study identified 50 samples suspected of containing *Entamoeba* sp. Ten were subjected to PCR, and five were sent for phylogenetic analysis and sequencing. MEGAX software was used to analyse the sequenced samples (23). The similarity percentage between the positive isolates and the NCBI isolates was calculated. The accession numbers for the local ostrich Entamoeba species were obtained by uploading the sequences to NCBI GenBank.

Three species were identified based on the 18S rRNA gene, as shown in Table 4: *Entamoeba struthionis* (ID PV019353.1, ID: PV019354.1), *Entamoeba polecki* (ID: PV019355.1, ID: PV019356.1), and *Entamoeba* sp (ID: PV019357.1).

### *Phylogenetic evaluation*

*A phylogenetic tree of Entamoeba species, constructed from* previously identified 18S ribosomal RNA sequences and illustrated with colored triangles and corresponding accession numbers, showed that the Iraqi isolates clustered closely together. Specifically, *Entamoeba struthionis* was closely related to isolates from Spain and Cameroon, while *Entamoeba polecki* clustered with isolates from China and Vietnam. In contrast, the *Entamoeba* sp. isolate displayed the highest similarity to a Chinese strain (Diagram 1). The PCR amplification results, confirmed by electrophoresis, revealed the expected band size (Figure 3).

## Discussion

This study gathered 150 ostrich droppings. Ostriches

**Table 1.** Total infection with intestinal parasites in Ostriches according to the location study

| Location | Total Samples (n) | Positive Samples (n) | Negative Samples (n) | Prevalence (%) |
|---|---|---|---|---|
| Al-Mahawil (Babil) | 35 | 17 | 18 | 50.0 |
| Al-Zawraa Park (Baghdad) | 45 | 32 | 13 | 70.0 |
| Al-Adhamiyah (Baghdad) | 40 | 24 | 16 | 60.0 |
| Al-Azizia (Wasit) | 30 | 12 | 18 | 40.0 |
| Total | 150 | 85 | 65 | 56.66 |

Not statistically significant at the 0.05 level ($\chi^2 = 6.47$, df = 3, $P = 0.09$).

**Table 2.** Prevalence of intestinal parasites according to family parasite in this research

| Parasite | Family parasite | Stage | Parasite Group |
|---|---|---|---|
| *Entamoeba* sp | *Entamoebidae* | Cyst | Protozoa |
| *Cryptosporidium* sp | *Cryptospordiidae* | Oocyst | Protozoa |
| *Ascaridia galli* | *Ascarididae* | Egg | Nematode |
| *Giardia* sp | *Hexamitidae* | Cyst | Protozoa |
| *Raillietina* sp | *Davaineidae* | Egg | Cestode |
| *Trichostrongylus tenuis* | *Trichostrongylidae* | Egg | Nematode |

**Table 3.** Frequency distribution of intestinal parasite species among 150 clinical samples

| Parasite | No. of positive | Percentage (%) |
|---|---|---|
| *Entamoeba* sp | 40 | 26.66 |
| *Cryptosporidium* sp | 17 | 11.33 |
| *Ascaridia galli* | 15 | 10 |
| *Giardia* sp | 7 | 4.6 |
| *Raillietina* sp | 3 | 2 |
| *Trichostrongylus tenuis* | 3 | 2 |

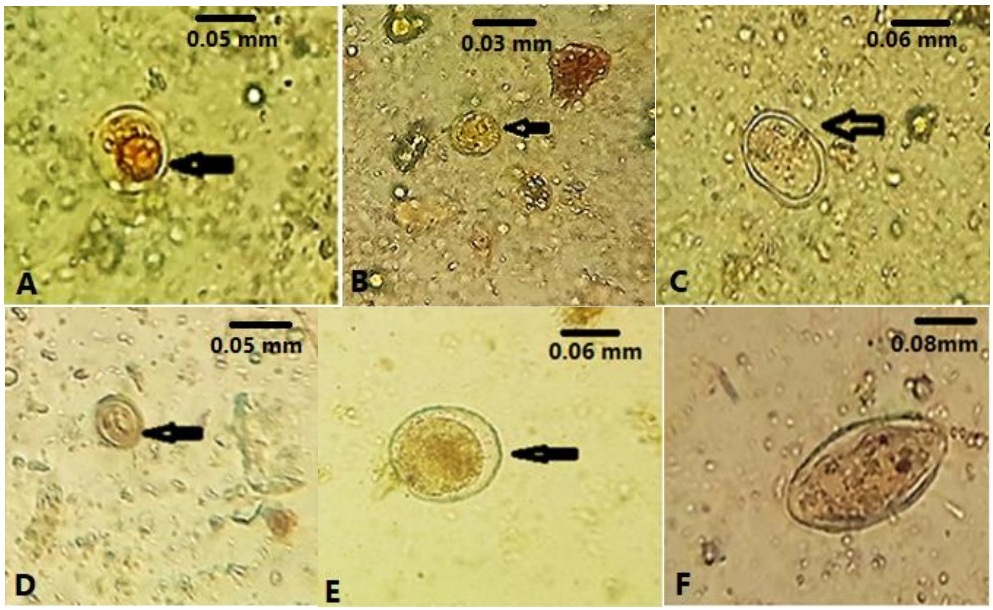


**Figure 2.** A. Cyst of *Entamoeba* sp, B. *Cryptosporidium* sp *oocyst*, C. *Ascaridia galli* egg, *D.* Cyst of *Giardia* sp, E. *Raillietina* sp egg, F. *Trichostrongylus tenuis* egg

were selected using a technique that ensured samples were drawn from multiple farms to account for variation in parasite prevalence. 85 (56.66%) of these samples contained intestinal parasites. This finding offers significant new information about the scant studies on parasites in ostriches. Most parasites in these birds are found in the digestive system. The examination of ostriches revealed no health problems despite the presence of intestinal parasites, including species that commonly harm ratites. This asymptomatic carriage could facilitate parasite spread between farms, particularly when animals are transported between facilities and countries (24). As a result, farmed ostriches may serve as reservoirs for a variety of intestinal parasites, posing a risk to humans, other animals, and farm laborers. Contaminated feed, water, insect vectors, and human contact are all routes by which parasites spread. Regular parasite screening, controlled animal movement, good sanitation, and staff hygiene training are examples of preventive measures (25). However, there was no subtype overlap for *Cryptosporidium spp.* between domestic animals, local villagers, and Giardia duodenalis, suggesting a limited role in direct zoonotic transmission. The discovery of zoonotic protozoa in domestic animals underscores the need for heightened caution when interacting with them.

Seasonal fluctuations, sample size, local methods of parasite control, and regional and climatic factors are likely to influence the prevalence of parasites in ostriches

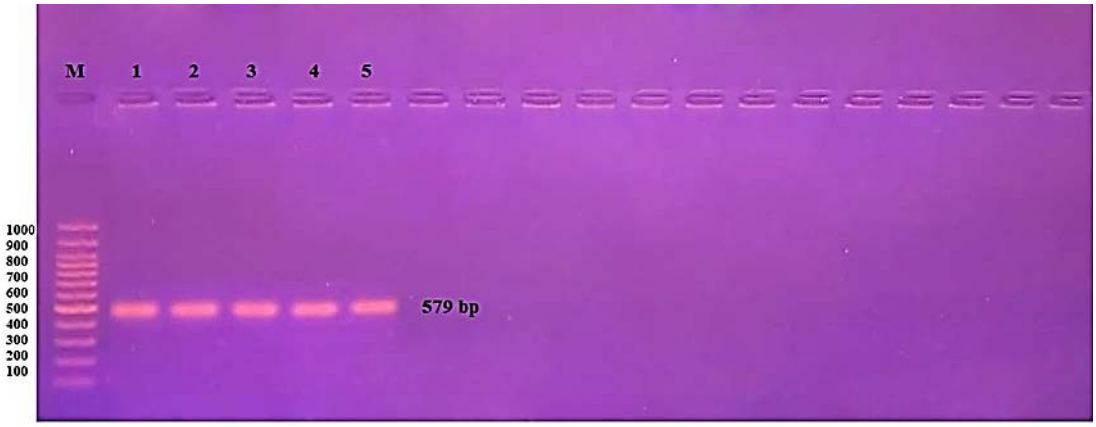


**Figure 3.** PCR amplification of the *Entamoeba* 18S rRNA gene (~579 bp) is demonstrated by agarose gel electrophoresis (1.5%). Lane M is a 100 bp DNA ladder; Lanes 1 through 5 are positive samples with clear single bands; the remaining lanes are negative controls

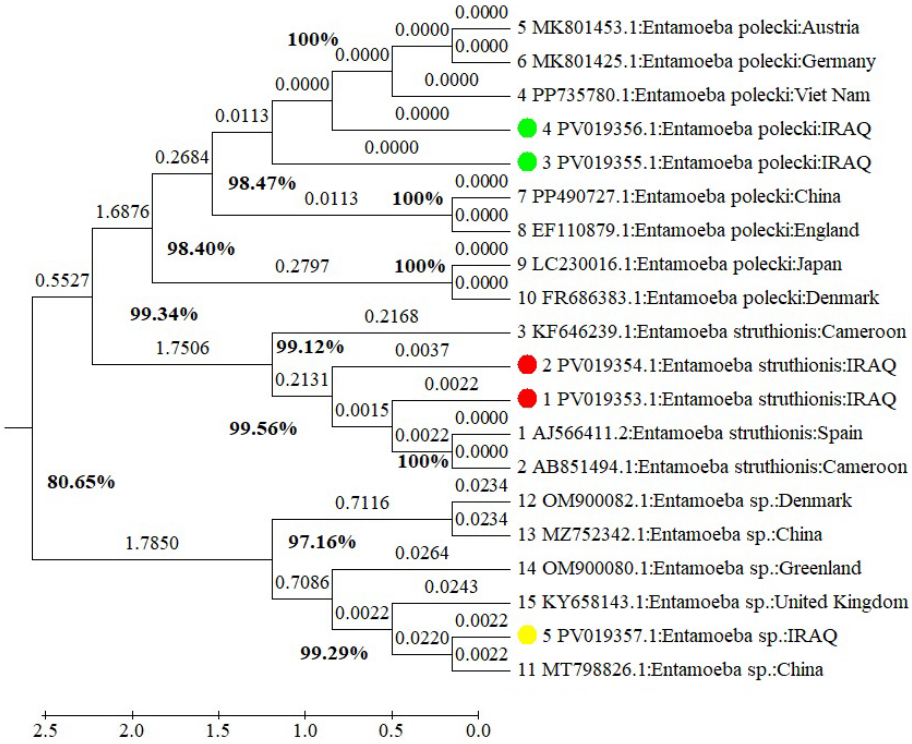


**Diagram 1.** Phylogenetic tree analysis of *Entamoeba* species with a focus on the 18S rRNA gene

**Table 4.** Homology of Local *Entamoeba* sp. Isolates with GenBank References

| No. | Local Isolate ID | Closest Species (NCBI) | GenBank Accession No. | Country | % Identity | Bootstrap Value |
|---|---|---|---|---|---|---|
| 1 | PV019353.1 | *Entamoeba struthionis* | AB851494.1 | Spain | 99% | 99.56% |
| 2 | PV019354.1 | *Entamoeba struthionis* | KF646239.1 | Cameroon | 99% | 99.12% |
| 3 | PV019355.1 | *Entamoeba polecki* | PP735780.1 | China | 100% | 100% |
| 4 | PV019356.1 | *Entamoeba polecki* | PP735780.1 | Vietnam | 99% | 100% |
| 5 | PV019357.1 | *Entamoeba sp.* | MT798826.1 | China | 99% | 99.29% |

(26). In this study, two *Entamoeba* species—*Entamoeba struthionis* and *Entamoeba polecki*—previously identified in ostriches were reported for the first time in Iraq. The researchers documented the initial observation of Entamoeba with a single-nucleus mature cyst in an ostrich through their study (27), which confirmed results from Ponce-Gordo *et al.* (28) who identified Entamoeba species in *Struthio camelus*. Analysis of full-length short ribosomal RNA gene sequences revealed substantial genetic differences between this species and other

*Entamoeba* species. Simonidou *et al.* (29-31) reported that *Balantidium coli,* along with *E. struthionis,* appeared for the first time in ostriches, which Greece had never seen before. Scientists used molecular amplification techniques on the ITS region and partial 16S rRNA gene sequences to study different types of *Entamoeba* strains, which tend to form clusters. The research findings indicate that ostriches experience protozoan infections more frequently than helminth infections because their environment contains silt contaminated with fecal matter.

Previous research has shown that parasite prevalence depends on the sampling site, living environment, geographical conditions, and sanitation practices (32, 33). The genus *Entamoeba* exhibits considerable adaptability, as it can establish itself in the digestive tracts of diverse animal species (34-37).

## Conclusion

The study focused on identifying intestinal parasites affecting ostriches (*Struthio camelus*). The researchers identified two new *Entamoeba* species that had not been detected in Iraq. The study shows that these parasites can infect people as they spread into areas where they were not previously observed. The study indicates that ostriches function as disease vectors, transmitting infections to other species through contact, even when they appear healthy. Several *Entamoeba* species pose a threat to human health. Assessing the risk of human infection from ostrich-derived species requires particular attention, given that people interact with these animals. Research on transmission methods, host range, and disease transmission to humans in Iraq requires additional epidemiological and molecular studies to be conducted by public health officials and researchers.

### Authors' Contribution

Conceptualization, Data curation, Investigation, Project administration, Software, and Supervision of the research idea for this study originated with Zainab A. Makawi, together with Maryam M. Al-Khaiat and Hiba Mohammed Jihad. Zainab A. Makawi conducted the formal analysis, methodology, validation, visualization, microscopic diagnosis, genetic analysis, and writing–original draft of the manuscript. All authors approved the final manuscript for submission after completing their individual contributions to the paper.

### Competing Interests

The publication of this article receives full disclosure for potential conflicts of interest from its authors.

### Ethical Approval

Not relevant.

### Funding

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