Acta Scientiae Veterinariae, 2026. 54: 2004.

RESEARCH ARTICLE
Pub. 2004

ISSN 1679-9216

# Intestinal Parasites in Ducks from Central Jiangsu, China - Epidemiological Survey

**Yu Feng*, Feiyan Wang*, Jinjun Xu, Jianping Tao & Dandan Liu**

**ABSTRACT**

***Background*:** Domestic ducks (*Anas platyrhynchos domesticus*) are essential to rural livelihoods, supplying meat and eggs. However, they are frequently affected by intestinal parasites, primarily helminths and protozoa, which reduce growth, egg yield, and survival. Coccidiosis severely impacts ducklings, with infection rates of 30-60%. As duck farming intensifies, parasite dynamics are shifting. While morphological identification is conventional, it lacks resolution for cryptic species. Molecular tools, particularly ribosomal markers, improve detection and phylogenetic accuracy. This study combines both approaches to assess parasite prevalence and diversity in central Jiangsu.

***Materials, Methods & Results*:** Between June and November 2023, fecal samples were collected from 36 duck farms employing diverse rearing systems across 6 districts in 3 cities. Samples were analyzed using morphological methods and PCR amplification of 18S rDNA, ITS, and SSU rRNA gene regions, followed by sequencing. Morphological identification revealed several parasites, including *Eimeria anatis*, *Isospora mandarin*, *Drepanidotaenia lanceolata*, *Capillaria* spp., and trematode eggs. Molecular data confirmed these findings and further identified associations with reference strains such as *Capillaria anatis*, *Echinostoma miyagawai*, and *Cryptosporidium baileyi*. The overall prevalence of intestinal parasites was 83.33%, with *Cryptosporidium* spp. most prevalent (58.33%), followed by coccidia (30.56%) and helminths (16.66%). Infection rates varied by region, duck age, and rearing system. Ducks aged 21-50 days exhibited the highest prevalence of *Cryptosporidium* spp. (71.43%), while older ducks showed higher *Eimeria* spp. infections. Intensive floor-rearing and water-based free-range systems had higher infection rates than courtyard pasture systems.

***Discussion*:** This study revealed a high prevalence and diversity of intestinal parasites in domestic ducks. A 100% infection rate with *Cryptosporidium* spp. was observed in ducks reared in multilayer cages, despite the absence of helminths or coccidia-likely reflecting hygienic and automated management, though the limited sample size warrants cautious interpretation. Helminth eggs, including *D. lanceolata*, *Capillaria* spp., and trematodes, were recovered via nylon-sieve washing and saturated salt flotation. *D. lanceolata* eggs were distinguished from *Fimbriaria fasciolaris* by the absence of polar filaments. PCR targeting ITS and 18S rDNA identified trematodes clustering with *E. miyagawai* and *Capillaria* spp. with isolates from *Gallus gallus domesticus*. Coccidian oocysts were preliminarily identified as *E. anatis* and *I. mandarin*. The former were ovoid with thick walls and micropyles; high temperature hindered sporulation, complicating differentiation from *Tyzzeria perniciosa* or *Wenyonella philiplevinei*. Phylogenetic analysis confirmed *E. anatis*, while *I. mandarin* oocysts, containing 2 sporocysts with 4 sporozoites and refractile bodies, showed high similarity (PV785220.1) to waterfowl *Eimeria*. Close phylogenetic clustering reflects shared Eimeriidae traits in sporozoite development and host invasion. Ecological and immunological characteristics of waterfowl may promote co-infection and niche overlap. Nested PCR targeting SSU rRNA confirmed *C. baileyi* infection, showing 100% identity with a *Passer montanus* isolate. While *C. baileyi* typically infects avian respiratory and intestinal tracts, molecular data on duck-derived isolates in China remain scarce. Overall, this study underscores the substantial burden and diversity of intestinal parasites in ducks from central Jiangsu, emphasizing the importance of integrating morphological and molecular diagnostics. Long-term, large-scale studies are essential for designing targeted control strategies in duck farming systems.

**Keywords:** ducks, intestinal parasites, molecular identification, epidemiology, rearing systems.

DOI: 10.22456/1679-9216.149327

Received: 30 August 2025 Accepted: 27 December 2025 Published: 31 January 2026

*Yu Feng and Feiyan Wang share first authorship. College of Veterinary Medicine & Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses & Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University (YZU), Yangzhou, China. CORRESPONDENCE: D. Liu [ddliu@yzu.edu.cn]. College of Veterinary Medicine - YZU. 12 East Wenhui Road. ZC 225009 Yangzhou, Jiangsu, China.

## INTRODUCTION

Domestic ducks (*Anas platyrhynchos domesticus*) are crucial to rural economies, serving as a key source of meat and eggs [32,38]. However, parasitic infections pose a major challenge to duck production, causing substantial economic losses through widespread infestations [1,34]. The primary parasites affecting ducks include helminths and protozoa, which exhibit high species diversity and broad geographic distribution [1,11,19,43]. Helminth infections often lead to stunted growth, decreased egg production, and poor feed efficiency [8,18], while protozoan infections, particularly coccidiosis, threaten ducklings with 30-60% infection rates and high mortality; survivors frequently suffer severe growth deficits [19,24]. *Cryptosporidium* infections further compound losses by causing both gastrointestinal and respiratory symptoms [15].

The industry's shift toward intensive farming has altered rearing conditions, potentially influencing parasite epidemiology [22,25]. Current diagnostic methods rely heavily on morphological identification, which struggles to differentiate synonymic or cryptic species [5,10,12]. Molecular techniques, especially ribosomal gene sequencing, now enable more precise parasite classification and phylogenetic analysis, overcoming these limitations [4,26,27,29,37].

This study investigated intestinal parasites in commercial ducks across 6 districts in central Jiangsu Province, China. Using integrated morphological and molecular approaches, we analyzed parasites in ducks of varying ages, regions, and farming systems. Our findings provide critical epidemiological data to support targeted control measures, fostering sustainable duck production.

## MATERIALS AND METHODS

### *Study site*

This study was conducted from June to November 2023 in Jiangsu Province, located in eastern China along the lower reaches of the Yangtze River (116°21′~121°57′ E and 30°45′~35°08′ N). We surveyed 36 duck farms across 3 representative prefecture-level cities (Figure 1): Yangzhou (central-northern Jiangsu, including Gaoyou, Guangling, Jiangdu, and Yizheng), Taizhou (central Jiangsu, including Taixing), and Nantong (eastern Jiangsu, including Rugao).

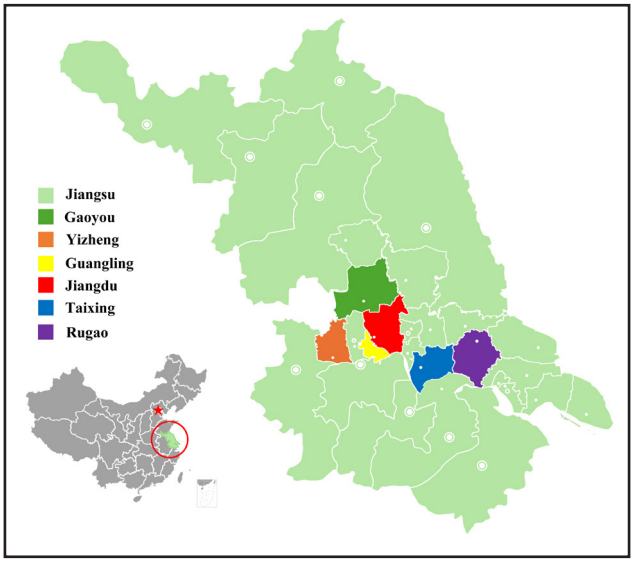



**Figure 1.** Sampling areas of duck farms in Jiangsu province, China.

### *Specimen collection*

Fresh fecal samples were collected from 36 duck farms (Table 1) implementing different rearing systems (Figure 2). A standardized composite sampling method was applied. Within each duck house, feces were collected from 5 distinct points, the 4 corners and the center, to ensure representative coverage of the enclosure [16]. Collected samples were judged as fresh based on moist appearance. Fecal samples that were dry or appeared contaminated in any way (e.g., trampled, covered in dirt, or in contact with other feces) were not collected. The number of samples collected ranged from a minimum of 5 for flocks of 10 birds, to a maximum of 30 for flocks of 300 birds or greater [7]. All samples were placed in sterile, sealed polyethylene bags at 4°C, and each bag was clearly labeled with the sampling location, rearing mode, and the age of the domestic ducks at the time of collection.

### *Morphological observation*

Nematode eggs, cestode eggs and coccidian oocysts were detected by the saturated saline floatation method as previously described [40]. In brief, approximately 10 g of fecal material from domestic ducks was mixed thoroughly with 15 mL of saturated saline solution. The suspension was passed through a 250 μm mesh sieve, and the resulting filtrate was centrifuged at 800 *g* for 5 min. A coverslip was gently placed on the surface of the supernatant and, after standing for 3 min, examined under a light microscope.

Eggs of trematodes were examined by nylon sieve washing method [40]. Fecal samples (10 g) were diluted with water and filtered successively through 60-mesh (250 μm aperture) and 260-mesh (57 μm aperture) sieves. The retained material on the finer mesh was rinsed repeatedly with water until the eluate became clear. The final sediment collected from the 260-mesh sieve was then transferred to a microscope slide for examination. The eggs of nematode, tape worm and trematode were observed and identified under 40x magnification.

*DNA extraction, amplification, and sequencing*

Fecal sample DNA was extracted using fecal DNA extraction kit[1] according to the manufacturer's instructions. The concentration and purity of the extracted genomic DNA were quantified using a NanoDrop 2000/2000c spectrophotometer[2]. All DNA samples were stored at -20°C until further use.

Primers used for PCR amplification were designed based on previously published studies [3,21,35,39,42]. All primers were synthesized by Qingke Biotechnology Co., Ltd.[3], and their sequences are provided in Table 6. PCR reactions were performed in a total volume of 25.0 μL, consisting of 12.5 μL of 2× Taq Plus Master Mix II (Dye Plus)[4], 1.0 μL each of forward and reverse primers (10 mmol/L), 1.0 μL of DNA template, and nuclease-free water to adjust the final volume. The thermal cycling conditions for each target gene are detailed in Table 7, and the amplification products were visualized and preserved by electrophoresis on 1% agarose gels.

The positive PCR products were purified by the EasyPure® Quick Gel Extraction Kit[5], and the purified products were sequenced by Sanger sequencing[6]. The sequencing data were assembled and edited using Clustal X 1.8 program[7], and aligned against GenBank entries via BLAST. Sequence homology was assessed with DNA Star software[8], and a phylogenetic tree was constructed in MEGA 11.0[9] using the Neighbor-Joining method with 1000 bootstrap replicates.

*Statistical analysis*

Statistical analysis of intestinal parasitic infections was conducted using the SPSSAU data science analysis platform (https://spssau.com/indexs.html). The 95% confidence interval (95% CI), odds ratio (OR), and *P*-values were calculated to evaluate potential risk factors associated with intestinal parasitic infections in domestic ducks.

**Table 1.** Duck farm information.

| City | City/District | Duck form | Farming methods |
|---|---|---|---|
| Yangzhou | Guangling | 1 | Intensive floor rearing system |
| | Jiangdu | 3 | Water-based free-range feeding |
| | Gaoyou | 3 | Intensive floor rearing system |
| | | 2 | Water-based free-range feeding |
| | | 1 | Multi-layer cage system |
| | Yizheng | 2 | Water-based free-range feeding |
| Nantong | Rugao | 15 | Intensive floor rearing system |
| Taizhou | Taixing | 10 | Free-range courtyard feeding |

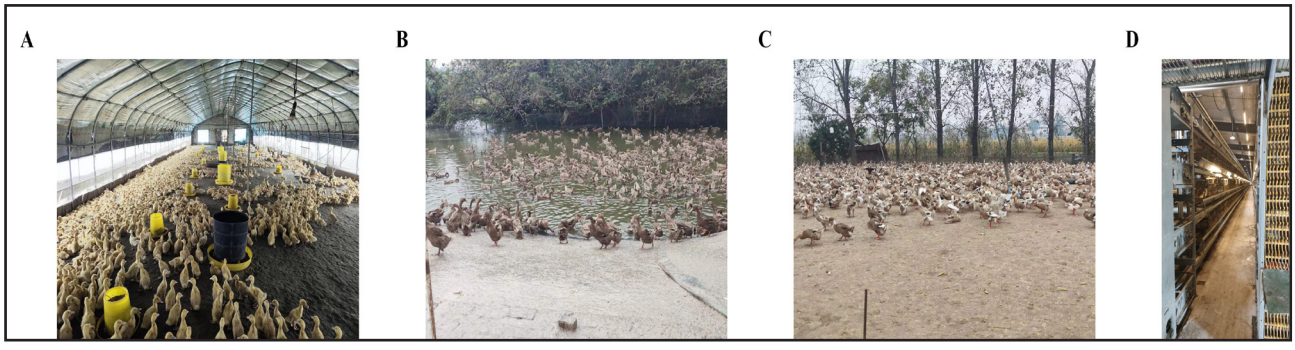



**Figure 2.** Duck farms with different feeding modes. A- Intensive floor rearing system. B- Free-range courtyard feeding. C- Water-based free-range feeding. D- Multi-layer cage system.

## RESULTS

*Species of intestinal parasites*

In this study, pathogen identification based on morphological characteristics revealed *Eimeria anatis*, *Isospora mandarin*, *Drepanidotaenia lanceolata*, *Avian Capillaria*, and trematode eggs (Figure 3).

Molecular identification of *Capillaria* eggs based on 18S ribosomal DNA (18S rDNA) sequencing yielded an 1800 bp fragment [GenBank: PV788917.1], exhibiting 99.9% sequence identity with *Capillaria anatis* [GenBank: LC052334.1] isolated from *Gallus gallus domesticus* (Figure 9). Phylogenetic analysis placed the isolate within the *Capillaria* clade, where it closely clustering with *C. anatis*, thereby confirming its taxonomic identity (Figure 4). Sequencing of the internal transcribed spacer (ITS) region from trematode eggs produced a 1170 bp fragment [GenBank: PV785219.1], showing 100% identity to *Echinostoma miyagawai* from *A. platyrhynchos* [GenBank: OR509027.1] (Figure 10). Phylogenetic analysis further supported this identification by grouping the isolate with *E. miyagawai* (Figure 5). The 18S rDNA sequence of *E. anatis* obtained in this study was 1300 bp in length [GenBank: PV785212.1], demonstrating 99.9% identity to a reference sequence from *Anas superciliosa [*GenBank: OL604501.1] (Figure 11). Phylogenetic inference placed the isolate within the waterfowl *Eimeria* clade, closely related to *E. anatis* (Figure 6). The 18S rDNA sequence of the *I. mandarin* isolate was 1384 bp [GenBank: PV785220.1], sharing 98.1% identity with *E. anatis* [GenBank: PV785212.1] (Figure 11). It is classified as *Isospora* sp. and is positioned within the *Eimeria* spp. in Waterfowl branch. Phylogenetic analysis reveals the closest relationship with Eimeria species, particularly *Eimeria fulva* [GenBank: KP789172.1] (Figure 7). Nested PCR and sequencing of the small subunit ribosomal RNA (SSU rRNA) gene yielded a 796 bp fragment [GenBank: PV785213.1], with 100% identity to *Cryptosporidium baileyi* isolated from a Chinese grosbeak (*Eophona migratoria*) [GenBank: MN410723.1] (Figure 12). Phylogenetic analysis clustered the isolate with *C. baileyi*, supporting its molecular identification (Figure 8).

*The infection rate and risk analysis of intestinal parasites in domestic ducks*

Morphological and molecular biological testing of fecal samples from 36 duck farms in central Jiangsu, revealed the highest infection rate of *Cryptosporidium* (58.33%; 21/36), followed by coccidia (30.56%; 11/36), and intestinal helminth (16.66%; 6/36). The risk of infection with *Cryptosporidium* was significantly higher than that of intestinal helminths (OR = 7.00; 95% CI: 2.33-21.00; $P = 0.001$). Although the risk of coccidial infection was also higher than that of intestinal helminths (OR = 2.20; $P = 0.170$), the difference was not statistically significant. Mixed infections were detected in 22.22% (8/36) of the surveyed farms, with the most common co-infections being helminths and *Cryptosporidium* (13.88%; 5/36), followed by coccidia and *Cryptosporidium* co-infections (8.33%; 3/36). Although the infection risk in farms with helminths and *Cryptosporidium* co-infection was slightly higher than in those with coccidia and *Cryptosporidium* co-infection (OR = 1.77; $P = 0.458$), the difference was not statistically significant (Table 2).

*Geographic distribution of positive cases and associated risk*

Significant regional variation in infection rates was observed. The highest overall infection rate was recorded in Nantong City (93.33%; 14/15), followed by Taizhou City (80%; 8/10), with the lowest in Yangzhou City (72.73%; 8/11). Compared to Yangzhou, the risk of infection in Nantong was higher (OR = 5.25; 95% CI: 0.47-59.29; $P = 0.180$); however, the difference was not statistically significant. Similarly, the infection risk in Taizhou was 1.5 times higher than in Yangzhou (OR = 1.50; 95% CI: 0.20-11.55; $P = 0.697$), but the difference was also not statistically significant (Table 3).

*Age-related infection rates for different parasite types*

This study demonstrated that the prevalence of intestinal parasites in domestic ducks was closely related to age. The prevalence of helminth infections was higher in the 21-50-day age group (28.57%; OR = 1.60; $P = 1.00$) compared to ducks over 50 days of age; however, the difference was not statistically significant. No significant difference was observed between ducks over 50 days and those under 20 days ($P > 0.05$). *Coccidia* infections were not detected in the 21-50-day group but were prevalent in ducks over 50 days (52.63%; OR = 4.44; $P = 0.217$) compared to the under 20-day group. *Cryptosporidium* was detected across all age groups, with the highest prevalence in ducks aged 21-50 days (71.43%; OR = 3.75; $P = 0.224$), although none of the observed differences reached statistical significance (Table 4).

*The influence of feeding modes on infection prevalence*

The rearing system had a notable impact on the prevalence of intestinal parasite infections in domestic ducks. Infection rates reached 100% in both the water-based free-range feeding and multi-layer cage system, followed by 89.47% in the intensive floor rearing system and 70% in the free-range courtyard feeding - the lowest among all. Compared to the courtyard pasture system, the infection risk was 6.0 times higher in the water-based free-range feeding (OR = 6.0; *P* = 0.228; 95% CI=0.25-143.12) and 3.64 times higher in the intensive floor rearing system (OR = 3.64; *P* = 0.204; 95% CI=0.50-26.76); however, these differences were not statistically significant. Coccidia was the predominant parasite species, with an infection rate of 60% in the free-range courtyard feeding system. In the water-based free-range system, *Cryptosporidium* spp. was the dominant parasite (71.42%), followed by coccidia (57.14%). *Cryptosporidium* spp. was also the predominant species under the intensive floor-rearing system, with an infection rate of 73.68%. Additionally, *Cryptosporidium* infection was detected in a duck farm practicing a multi-layer cage rearing system (Table 5).

**Table 2.** The infection rate and risk analysis of intestinal parasites in domestic ducks.

| Parasite Species | Type | Positive Rate% | OR | *P* value | 95%CI |
|---|---|---|---|---|---|
| Helminths | Single | 16.66 (6/36) | 1.00 (reference) | - | |
| Coccidia | Single | 30.56 (11/36) | 2.20 | 0.170 | 0.71-6.79 |
| *Cryptosporidium* spp. | Single | 58.33 (21/36) | 7.00 | 0.001 | 2.33-21.00 |
| Helminths, Coccidia | Mixed | 0.00 (0/36) | 0.00 | 0.000 | 0.00 |
| Coccidia, *Cryptosporidium* spp. | Mixed | 8.33 (3/36) | 1.00 (reference) | - | - |
| Helminths, *Cryptosporidium* spp. | Mixed | 13.88 (5/36) | 1.77 | 0.458 | 0.39-8.055 |

**Table 3.** Geographic distribution of positive cases and associated risk.

| City | Positive Rate% | OR | *P* value | 95%CI |
|---|---|---|---|---|
| Yangzhou | 72.73 (8/11) | 1.00 (reference) | - | - |
| Nantong | 93.33 (14/15) | 5.25 | 0.180 | 0.47-59.29 |
| Taizhou | 80.00 (8/10) | 1.50 | 0.697 | 0.20-11.55 |

**Table 4.** Age-related infection rates for different parasite types.

| Parasite Type | Age Group (days) | Positive Rate% | OR | *P* value | 95%CI |
|---|---|---|---|---|---|
| Helminths | <20 | 20.00 (1/5) | 1.00 (reference) | - | - |
| | 21-50 | 28.57 (4/14) | 1.60 | 1.000 | 0.13-19.09 |
| | >50 | 10.53 (2/19) | 0.47 | 0.521 | 0.03-6.57 |
| Coccidia | <20 | 20.00 (1/5) | 1.00 (reference) | - | - |
| | 21-50 | 0.00 (0/14) | 0.00 | 0.000 | 0.00 |
| | >50 | 52.63 (10/19) | 4.44 | 0.217 | 0.42-47.50 |
| *Cryptosporidium* spp. | <20 | 40.00 (2/5) | 1.00 (reference) | - | - |
| | 21-50 | 71.43 (10/14) | 3.75 | 0.224 | 0.45-31.62 |
| | >50 | 42.11 (8/19) | 1.09 | 0.932 | 0.15-8.12 |

**Table 5.** The influence of feeding modes on infection prevalence.

| Rearing System | Positive Rate% | OR | *P* value | 95%CI | Helminths | Coccidia | *Cryptosporidium* spp. |
|---|---|---|---|---|---|---|---|
| Free-range courtyard feeding | 70.00 (7/10) | 1.00 (reference) | - | - | / | 60.00% (6/10) | 10.00% (1/10) |
| Water-based free-range feeding | 100.00 (7/7) | 6.00 | 0.228 | 0.25-143.12 | 28.57% (2/7) | 57.14% (4/7) | 71.42% (5/7) |
| Intensive floor rearing system | 89.47 (17/19) | 3.64 | 0.204 | 0.50-26.76 | 26.31% (5/19) | 5.26% (1/19) | 73.68% (14/19) |
| Multi-layer cage system | 100.00 (1/1) | 0.86 | 1.000 | 0.022-33.12 | / | / | 100.00% (1/1) |

**Table 6.** List of primers for PCR.

| Species | Gene name | Primer name | Primer sequence 5′→3′ |
|---|---|---|---|
| *Eimeria* spp. | 18S rDNA | Outer Primer | Forward: 5′- GCTTGTCTCAAAGATTAAGCC -3′<br>Reverse: 5′- ATGCATACTCAAAAGATTACC -3′ |
| | | Inner Primer | Forward: 5′- CTATGGCTAATACATGCGCAATC -3′<br>Reverse: 5′- ATGCATACTCAA AAGATTACC -3′ |
| *Cryptosporidium* spp. | SSUrRNA | Outer Primer | Forward: 5′- CCCATTTCCTTCGAAACAGGA -3′<br>Reverse: 5′- TTCTAGAGCTAATACATGCG -3′ |
| | | Inner Primer | Forward: 5′- GGAAGGGTTGTATTTATTAGATAAAG -3′<br>Reverse: 5′- AAGGAGTAAGGAACAACCTCCA -3′ |
| Avian *Capillaria* | 18S rDNA | NSF4/18<br>18S-1192R/20 | Forward: 5′- CTGGTTGATCCTGCCAGT -3′<br>Reverse: 5′- CAGGTGAGTTTTCCCGTGTT -3′ |
| | | NSF4/18<br>NSR1438/20 | Forward: 5′- CTGGTTGATCCTGCCAGT -3′<br>Reverse: 5′- GGGCATCACAGACCTGTTAT -3′ |
| | | NSF573/19<br>NSR1787/18 | Forward: 5′- CGCGGTAATTCCAGCTCCA -3′<br>Reverse: 5′- CGACGGGCGGTGTGTACA -3′ |
| | | NSF573/19<br>SSU18R | Forward: 5′- CGCGGTAATTCCAGCTCCA -3’<br>Reverse: 5′- TGATCCTTCYGCAGGTTCAC -3’ |
| Trematodes | ITS | | Forward: 5′- GTAGGTGAACCTGCGGAACGATCATT -3′<br>Reverse: 5′- TTAGTTTCTTTTCCTCCGCT -3′ |

**Table 7.** Standard PCR procedure.

| Species | Target Gene | Cycles | Initial Denaturation | Denaturation | Annealing | Extension | Final Extension |
|---|---|---|---|---|---|---|---|
| *Eimeria* spp. | 18S rDNA (1st round) | 30 | 95°C for 3 min | 95°C for 15 s | 52.5°C for 20 s | 72°C for 1 min 30 s | 72°C for 10 min |
| | 18S rDNA (2nd round) | 30 | 95°C for 3 min | 95°C for 15 s | 52.5°C for 20 s | 72°C for 1 min 30 s | 72°C for 10 min |
| *Cryptosporidium* spp. | SSU rRNA (1st round) | 30 | 95°C for 3 min | 95°C for 15 s | 52.5°C for 20 s | 72°C for 1 min 30 s | 72°C for 10 min |
| | SSU rRNA (2nd round) | 30 | 95°C for 3 min | 95°C for 15 s | 52.5°C for 20 s | 72°C for 1 min 30 s | 72°C for 10 min |
| Avian *Capillaria* | 18S rDNA | 30 | 95°C for 3 min | 95°C for 15 s | 64.0°C for 20 s | 72°C for 1 min 30 s | 72°C for 10 min |
| Trematodes | ITS | 30 | 95°C for 3 min | 95°C for 15 s | 52.5°C for 20 s | 72°C for 1 min 30 s | 72°C for 10 min |

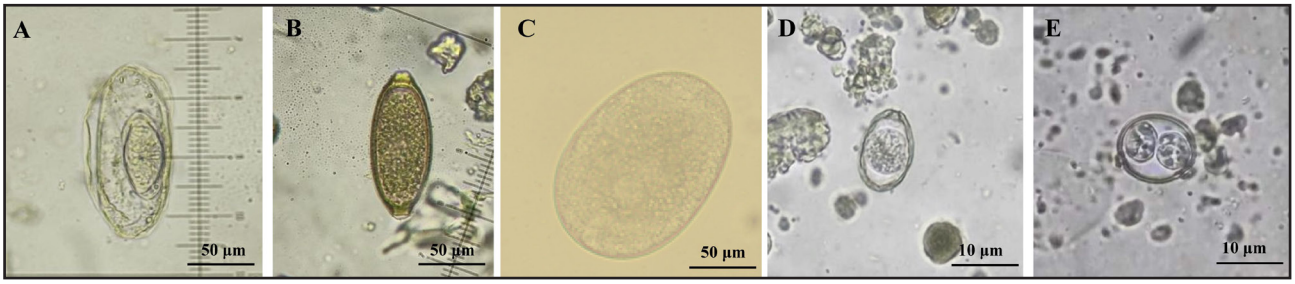



**Figure 3.** Microphotographs of various parasitic eggs taken at 40 × magnification. A- *Drepanidotaenia lanceolata*. B- *Avian Capillaria*. C- Trematode egg. D- *Eimeria anatis* (40 ×). E- *Isospora mandarin* (40 ×). [Scale bars: 50 µm (A, B, C) and 10 µm (D, E)].

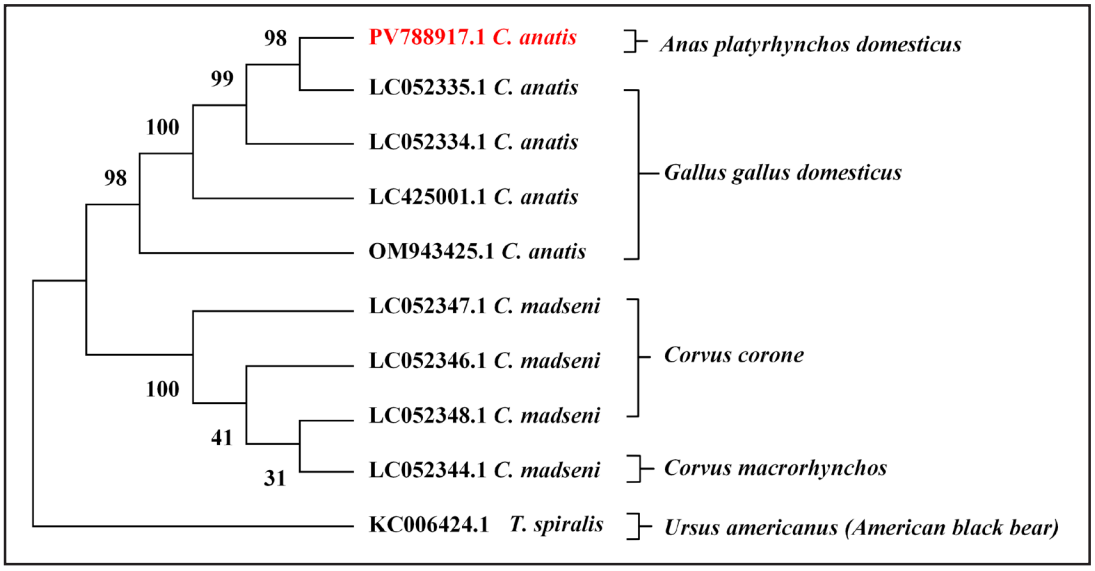



**Figure 4.** Phylogenetic tree of *Capillaria* based on 18S rDNA (Bootstrap = 1,000). The sequence PV788917.1 of *C. anatis* is highlighted in red, and it is associated with the domestic duck (*A. platyrhynchos domesticus*). The bootstrap values reflect the support for each node in the tree.

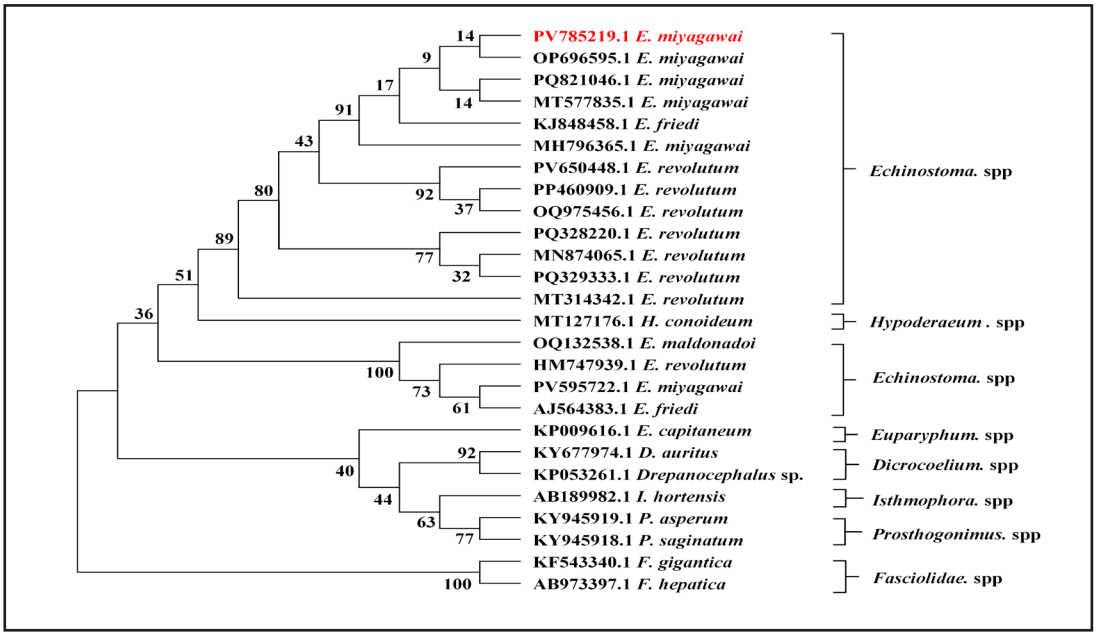



**Figure 5.** Phylogenetic tree of Trematode based on ITS region (Bootstrap = 1,000). The sequence PV785219.1 of *Echinostoma miyagawai* is highlighted in red, and it is classified within the *Echinostoma* genus, closely related to other *E. miyagawai* sequences. This clade is part of the larger Echinostomatidae family. The bootstrap values reflect the support for each node in the tree.

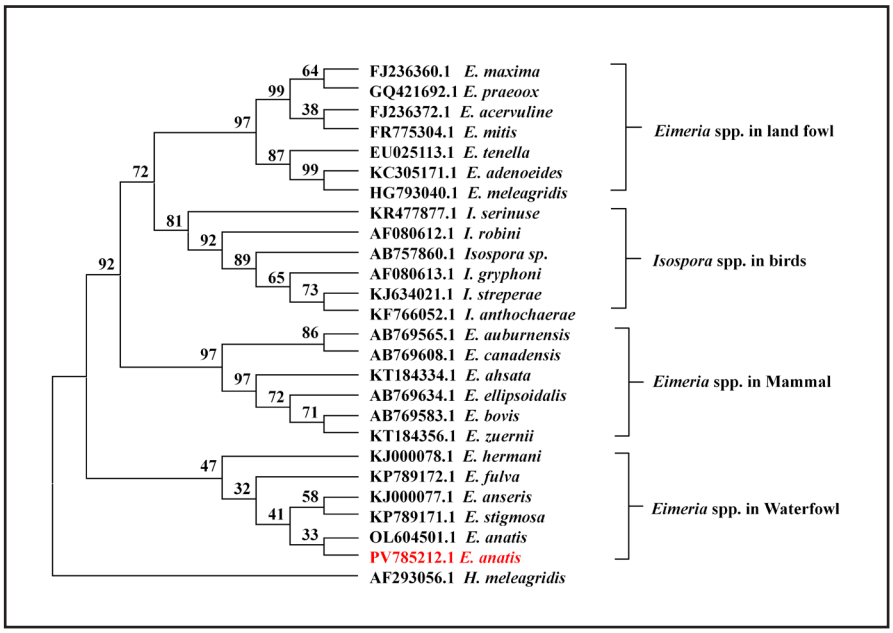



**Figure 6.** Phylogenetic tree of *Eimeria anatis* based on 18S rRNA (Bootstrap = 1,000). The sequence PV785212.1 of *E. anatis* is highlighted in red, and it is belonged to the *Eimeria* genus, clustering with waterfowl species and showing evolutionary links to *Eimeria* in land fowl and mammals. The bootstrap values reflect the support for each node in the tree.

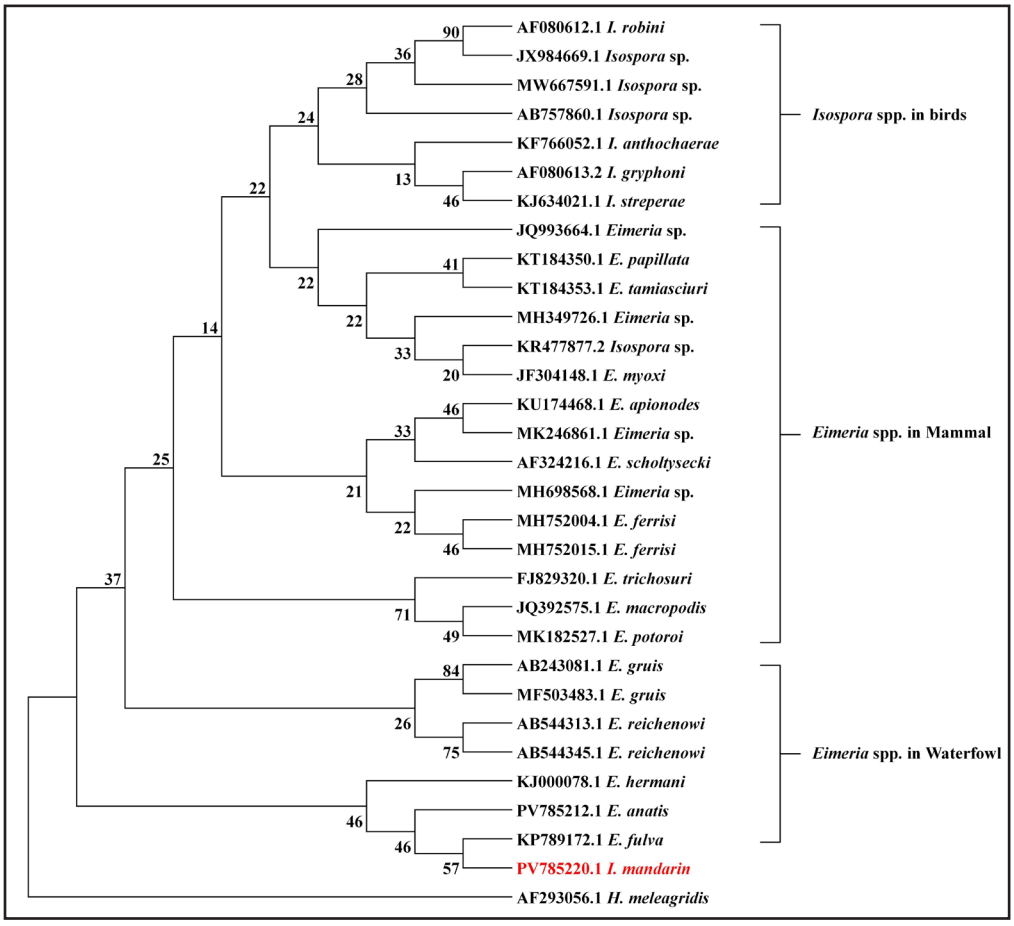



**Figure 7.** Phylogenetic tree of *Isospora mandarin* based on 18S rRNA (Bootstrap = 1,000). The sequence PV785220.1 of *I. mandarin* is highlighted in red, and it is positioned within the *Eimeria* species found in waterfowl, indicating its close genetic relationship with other waterfowl-associated species. The bootstrap values reflect the support for each node in the tree.

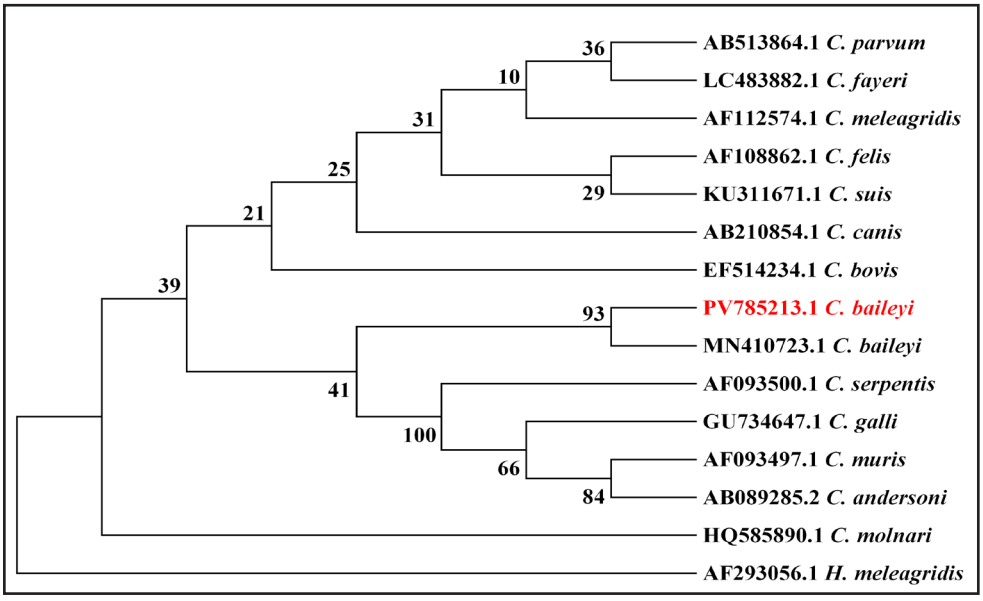
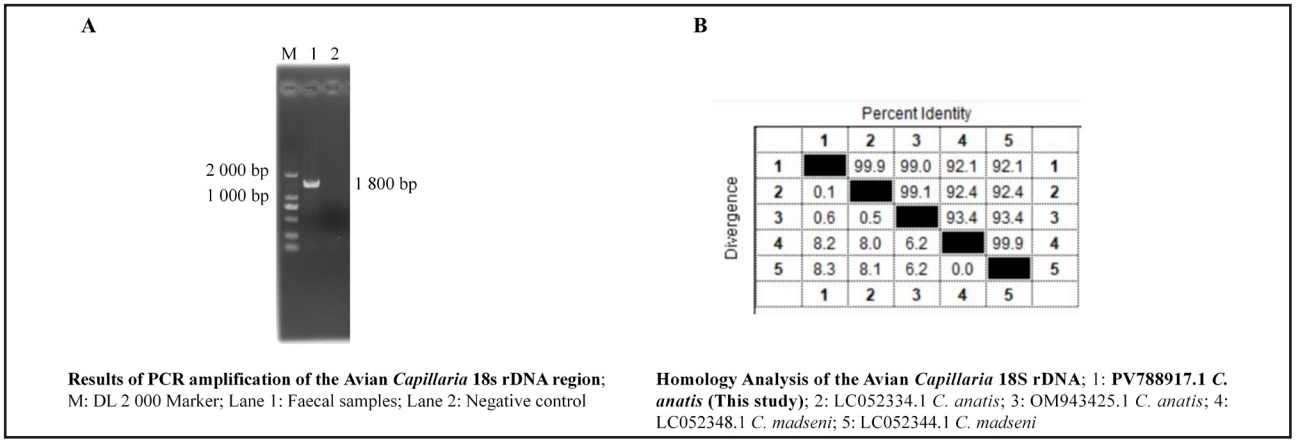


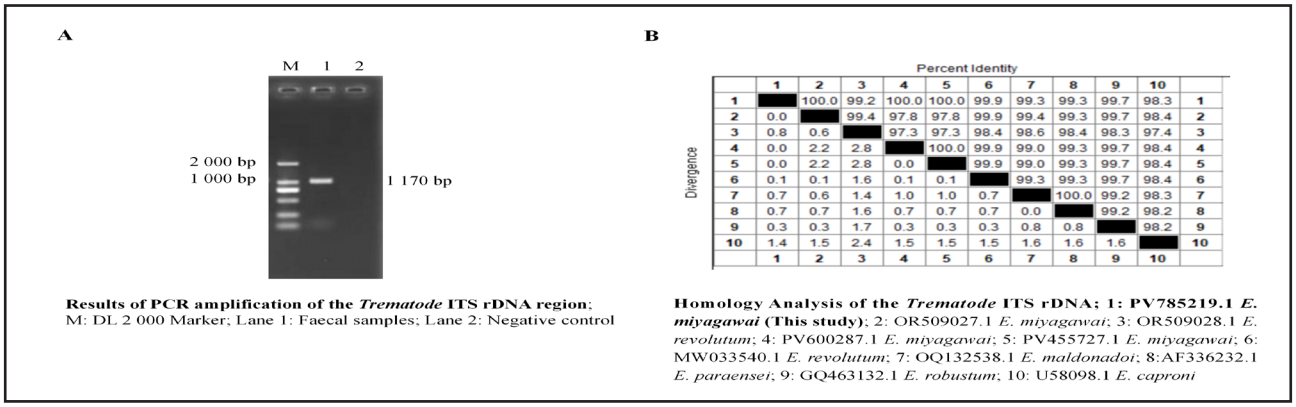

**Figure 8.** Phylogenetic tree of *Cryptosporidium* spp. based on SSU rRNA. The sequence PV785213.1 of *C. baileyi* is highlighted in red, and it is located within the same clade as other closely related *Cryptosporidium* species. The bootstrap values reflect the support for each node in the tree.




**Results of PCR amplification of the Avian *Capillaria* 18s rDNA region**; M: DL 2 000 Marker; Lane 1: Faecal samples; Lane 2: Negative control

**Homology Analysis of the Avian *Capillaria* 18S rDNA**; 1: **PV788917.1 *C. anatis* (This study)**; 2: LC052334.1 *C. anatis*; 3: OM943425.1 *C. anatis*; 4: LC052348.1 *C. madseni*; 5: LC052344.1 *C. madseni*

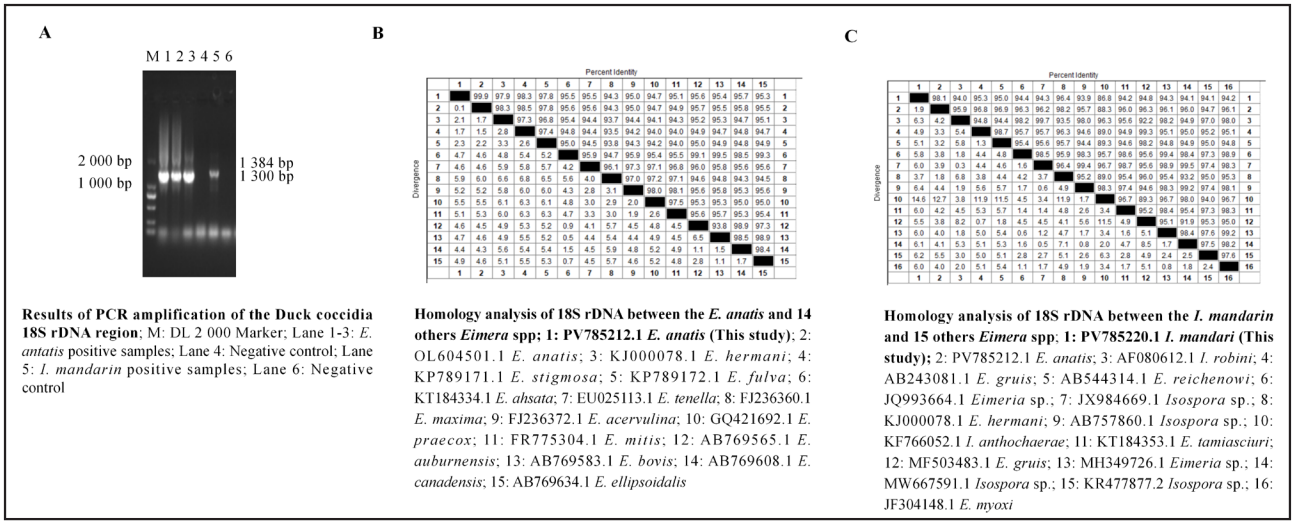
**Figure 9.** PCR Amplification and Sequence Homology Analysis of the Avian *Capillaria* 18s rDNA. A- Results of PCR amplification of the Avian *Capillaria* 18S rDNA region. [Lane M: DL 2 000 Maker; lane 1: Fecal samples; Lane 2: Negative control]. B- Homology Analysis of the Avian *Capillaria* 18S rDNA. The sequence PV788917.1 of the Avian *Capillaria* exhibits 99.9% sequence identity with *Capillaria anatis* (GenBank: LC052334.1), which was isolated from *Gallus gallus domesticus*.




**Results of PCR amplification of the *Trematode* ITS rDNA region**; M: DL 2 000 Marker; Lane 1: Faecal samples; Lane 2: Negative control

**Homology Analysis of the *Trematode* ITS rDNA; 1: PV785219.1 *E. miyagawai* (This study)**; 2: OR509027.1 *E. miyagawai*; 3: OR509028.1 *E. revolutum*; 4: PV600287.1 *E. miyagawai*; 5: PV455727.1 *E. miyagawai*; 6: MW033540.1 *E. revolutum*; 7: OQ132538.1 *E. maldonadoi*; 8:AF336232.1 *E. paraensei*; 9: GQ463132.1 *E. robustum*; 10: U58098.1 *E. caproni*

**Figure 10.** PCR Amplification and Sequence Homology Analysis of the *Trematode* ITS region. A- Results of PCR amplification of the *Trematode* ITS region. [Lane M: DL 2 000 Maker; lane 1: Fecal samples; Lane 2: Negative control]. B- Homology Analysis of the *Trematode* ITS region]. The sequence PV785219.1 of *E. miyagawai* exhibits 100% identity with *E. miyagawai* from *A. platyrhynchos* (GenBank: OR509027.1).

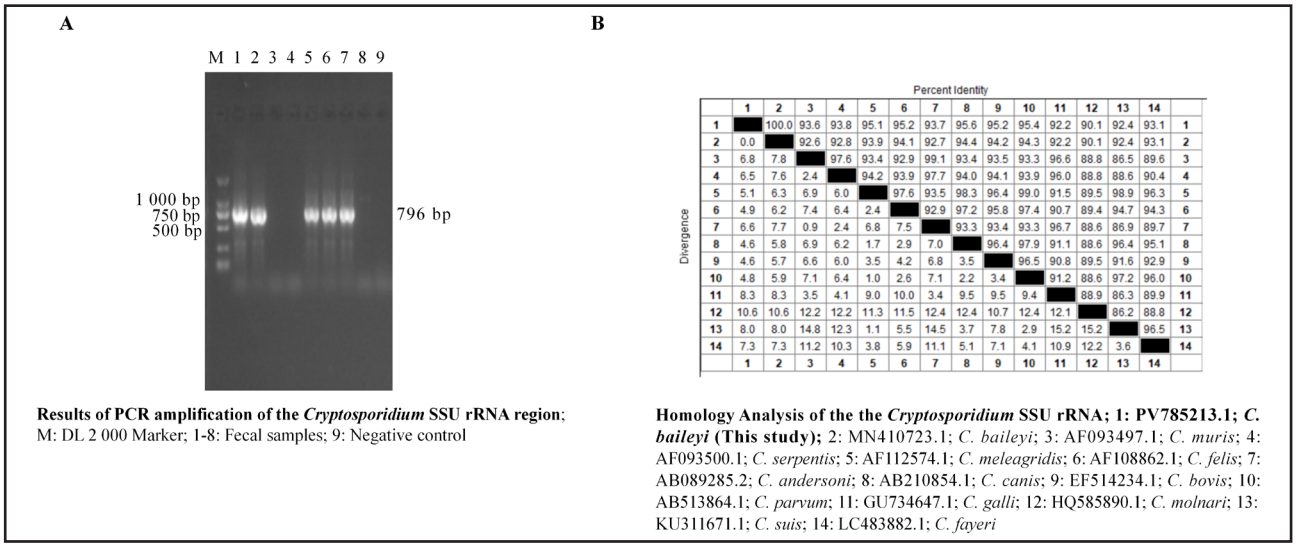



**Results of PCR amplification of the Duck coccidia 18S rDNA region**; M: DL 2 000 Marker; Lane 1-3: *E. antatis* positive samples; Lane 4: Negative control; Lane 5: *I. mandarin* positive samples; Lane 6: Negative control

**Homology analysis of 18S rDNA between the *E. anatis* and 14 others *Eimera* spp; 1: PV785212.1 *E. anatis* (This study)**; 2: OL604501.1 *E. anatis*; 3: KJ000078.1 *E. hermani*; 4: KP789171.1 *E. stigmosa*; 5: KP789172.1 *E. fulva*; 6: KT184334.1 *E. ahsata*; 7: EU025113.1 *E. tenella*; 8: FJ236360.1 *E. maxima*; 9: FJ236372.1 *E. acervulina*; 10: GQ421692.1 *E. praecox*; 11: FR775304.1 *E. mitis*; 12: AB769565.1 *E. auburnensis*; 13: AB769583.1 *E. bovis*; 14: AB769608.1 *E. canadensis*; 15: AB769634.1 *E. ellipsoidalis*

**Homology analysis of 18S rDNA between the *I. mandarin* and 15 others *Eimera* spp**; **1: PV785220.1 *I. mandari* (This study);** 2: PV785212.1 *E. anatis*; 3: AF080612.1 *I. robini*; 4: AB243081.1 *E. gruis*; 5: AB544314.1 *E. reichenowi*; 6: JQ993664.1 *Eimeria* sp.; 7: JX984669.1 *Isospora* sp.; 8: KJ000078.1 *E. hermani*; 9: AB757860.1 *Isospora* sp.; 10: KF766052.1 *I. anthochaerae*; 11: KT184353.1 *E. tamiasciuri*; 12: MF503483.1 *E. gruis*; 13: MH349726.1 *Eimeria* sp.; 14: MW667591.1 *Isospora* sp.; 15: KR477877.2 *Isospora* sp.; 16: JF304148.1 *E. myoxi*

**Figure 11.** PCR Amplification and Sequence Homology analysis of the coccidia 18S rDNA. A- Results of PCR amplification of the Duck coccidia 18S rDNA region [M: DL 2 000 Marker; Lane 1-3: *Eimeria anatis* positive samples; Lane 4: Negative control; Lane 5: *Isospora mandarin* positive samples; Lane 6: Negative control]. B- Homology analysis of 18S rDNA between the *E. anatis* and 14 others *Eimeria* spp. The sequence of PV785212.1 *E. anatis* exhibits 99.9% identity with *E. anatis* from *Anas superciliosa* (GenBank: OL604501.1). C- Homology analysis of 18S rDNA between the *I. mandarin* and 15 others *Eimeria* spp. The sequence of PV785220.1 *I. mandarin* exhibits 98.1% identity with *E. anatis* from *A. platyrhynchos domesticus* (GenBank: PV785212.1).




**Results of PCR amplification of the *Cryptosporidium* SSU rRNA region**; M: DL 2 000 Marker; 1-8: Fecal samples; 9: Negative control

**Homology Analysis of the the *Cryptosporidium* SSU rRNA; 1: PV785213.1; *C. baileyi* (This study);** 2: MN410723.1; *C. baileyi*; 3: AF093497.1; *C. muris*; 4: AF093500.1; *C. serpentis*; 5: AF112574.1; *C. meleagridis*; 6: AF108862.1; *C. felis*; 7: AB089285.2; *C. andersoni*; 8: AB210854.1; *C. canis*; 9: EF514234.1; *C. bovis*; 10: AB513864.1; *C. parvum*; 11: GU734647.1; *C. galli*; 12: HQ585890.1; *C. molnari*; 13: KU311671.1; *C. suis*; 14: LC483882.1; *C. fayeri*

**Figure 12.** PCR Amplification and Sequence Homology Analysis of the *Cryptosporidium* SSU rRNA. A- Results of PCR amplification of the *Cryptosporidium* SSU rRNA region [M: DL 2 000 Marker; 1-8: Fecal samples; 9: Negative control]. B- Homology Analysis of the *Cryptosporidium* SSU rRNA. The sequence *C. baileyi* of PV785213.1 exhibits 100% identity with *C. baileyi* from a Chinese grosbeak (*Eophona migratoria*) [GenBank: MN410723.1].

## DISCUSSION

This study conducted an epidemiological survey on intestinal parasites in duck farms from 6 districts in the central Jiangsu region of China, and employed both pathogenic and molecular biological methods to identify the parasite species. The results showed that the overall infection rate of intestinal parasites in the duck farms was 83.33%. Among these, *Cryptosporidium* spp. had the highest infection rate at 58.33%, followed by coccidia at 30.56%, and helminths at 16.66%. Higher parasite infection rates were observed in farms employing water-based free-range and intensive floor-rearing systems, likely due to increased exposure to contaminated water sources and poor management practices such as infrequent bedding replacement and inadequate manure removal [9,36]. In contrast to the absence of helminth and coccidia infections, a 100% infection rate of *Cryptosporidium*

spp. was observed in the multi-layer cage system. This unexpected finding may be attributed to factors such as automated equipment and routine cleaning, which are generally believed to interrupt parasite transmission routes [33]. Nevertheless, due to the extremely limited sample size, this result should be interpreted with caution and warrants further investigation.

Using the nylon sieve washing method in combination with the saturated salt flotation technique, eggs of *Drepanidotaenia lanceolata*, *Capillaria* spp., and trematodes were successfully detected in fecal samples. The eggs of *D. lanceolata* were morphologically distinguishable from those of *Fimbriaria fasciolaris*, which are characterized by prominent polar filaments - a feature absented in *D. lanceolata* [6]. Microscopic examination further confirmed the presence of *D. lanceolata*. Given the morphological similarity among various *Capillaria* spp. and trematode species, molecular identification was subsequently performed using PCR amplification of the ITS and 18S rDNA genes [21,39]. Phylogenetic analysis placed the trematode sequences within the same clade as *Echinostoma miyagawai* previously identified in *Anas platyrhynchos*, and grouped the *Capillaria* sequences with those derived from *Gallus gallus domesticus*.

In terms of duck coccidiosis, preliminary identification based on pathogen morphology indicated the presence of *Isospora mandarin* and *Eimeria anatis*. The oocysts of *Eimeria anatis* are ovoid in shape, with distinct oocyst wall and micropyle structures that serve as key morphological criteria for species identification. However, due to the elevated ambient temperature during sampling, the oocysts failed to undergo sporulation, making it difficult to accurately distinguish *Tyzzeria perniciosa* or *Wenyonella philiplevinei* based solely on the morphology of unsporulated oocysts. Phylogenetic analysis revealed that the obtained sequence clustered within the same evolutionary clade as *Eimeria anatis*. The oocysts of *I. mandarin* exhibited typical morphological features, including 2 sporocysts each containing 4 sporozoites, with clearly visible refractile bodies and dark granular residues within the sporocysts. Phylogenetic analysis revealed that the sequence PV785220.1, although taxonomically classified under the genus *Isospora*, exhibited high genetic similarity to *Eimeria* spp. isolated from waterfowl. While *Isospora* and *Eimeria* differ in certain morphological and molecular characteristics, both genera are members of the family Eimeriidae and share key biological features, particularly in sporozoite development and host cell invasion mechanisms [2,23,41]. These similarities may account for the close phylogenetic clustering observed in our analysis[31]. Notably, waterfowl represent a unique host group with specific ecological and immunological traits that could support overlapping niches for both *Isospora* and *Eimeria* [20]. Such ecological convergence may have driven parallel or intertwined evolutionary trajectories [20,30], resulting in shared phylogenetic signals between the 2 genera. This finding provides valuable insights into the evolutionary dynamics and complexity of parasite community structure in avian hosts[17]. In addition, this study employed nested PCR targeting SSU rRNA gene, which confirmed that the *Cryptosporidium* species infecting the duck farms was *C. baileyi*. The isolate showed 100% sequence identity with *C. baileyi* isolated from *Passer montanus*, and phylogenetic analysis placed them within the same lineage. *C. baileyi* is a common parasite in both domestic and wild birds, primarily colonizing the trachea, esophagus, and cloaca [28]. In China, studies on avian *Cryptosporidium* have largely focused on mammals and a limited number of bird species, with molecular data on ducks being relatively scarce[13-15].

Overall, the findings of this study underscore the complexity of parasitic infections in duck populations and the importance of incorporating both morphological and molecular approaches to improve diagnostic accuracy. Further studies with larger sample sizes and broader geographical coverage are needed to better understand the factors influencing the distribution and prevalence of these parasites in different farming systems and to inform control strategies for improving duck health management in China.

## CONCLUSIONS

In summary, this study systematically investigated intestinal parasite infections in duck farms in central Jiangsu, utilizing both morphological and molecular approaches to identify the parasite species present. The high overall infection rate of 83.33% underscores the urgent need for enhanced control measures, particularly in free-range and floor-rearing systems. The detection of multiple parasite species, including *Cryptosporidium baileyi*, *Eimeria anatis*, *Isospora mandarin*, and various helminths, reveals the diversity of intestinal parasites in domestic ducks and highlights potential zoonotic risks. These findings emphasize the importance of improving farming environments, strengthening biosecurity, and implement-

ing effective hygiene management practices to reduce infection risks, promote duck health, and mitigate economic losses associated with parasitic diseases.

MANUFACTURERS

[1]Tiangen Biotech Co. Ltd. Beijing, China.

[2]Thermo Fisher Scientific. Waltham, MA, USA.

[3]Beijing Qingke Biotechnology Co. Ltd. Beijing, China.

[4]Takara Biotechnology Co. Ltd. Dalian, China.

[5]Transgen Biotech Co. Ltd. Beijing, China.

[6]BGI-Huada Genomics Co. Ltd. Shenzhen, Guangdong, China.

[7]Clustal X software, version 1.8. University College Dublin (UCD). Dublin, Ireland.

[8]DNASTAR Inc. Madison, WI, USA.

[9]MEGA (Molecular Evolutionary Genetics Analysis). Arizona State University (ASU). Tempe, AZ, USA.

***Funding*.** This work was supported by the Innovation and Entrepreneurship Training Program for College Students at Yangzhou University (grant numbers XCX20240779 and XCX20250785); the 111 Project D18007, the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

***Acknowledgements*.** The authors are grateful to Htet Aung Naing and Lele Wang for their hard work and dedication in sample collection. Special thanks to ChatGPT for assisting with language polishing.

***Ethical approval*.** The animal study protocol was approved by the ethical standards of the Animal Experiment Ethics Committee of Yangzhou University (SYXK (Su) 2021-0026).

***Declaration of interest*.** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## REFERENCES

1 **Anggrahini S., Widiyono I., Baihaqi Z.A., Sofyan A., Mulianda R., Wulandari W., Ekawasti F., Fauziah I., Sadarman S., Sigit M., Herdian H., Ahmad R.Z. & Rokana E. 2025.** Occurrence of gastrointestinal parasites in local ducks at varying altitudes in Yogyakarta, Indonesia. *Veterinary World*. 18(3): 616-623. DOI: 10.14202/vetworld.2025.616-623.

2 **Augustine P.C. 2001.** Cell: sporozoite interactions and invasion by apicomplexan parasites of the genus *Eimeria*. *International Journal for Parasitology*. 31(1): 1-8. DOI: 10.1016/S0020-7519(00)00150-8.

3 **Berto B.P., Brice B., Thomas G., Elloit A., Zahedi A. & Yang R. 2022.** *Eimeria* spp. and *Tyzzeria perniciosa* Allen, 1936 (Apicomplexa: Eimeriidae) from a Pacific black duck, *Anas superciliosa* Gmelin (Aves: Anseriformes), in western Australia. *Current Research in Parasitology & Vector-Borne Diseases*. 2: 100075. DOI: 10.1016/j.crpvbd.2022.100075.

4 **Chan A.H.E., Chaisiri K., Morand S., Saralamba N. & Thaenkham U. 2020.** Evaluation and utility of mitochondrial ribosomal genes for molecular systematics of parasitic nematodes. *Parasites & Vectors*. 13(1): 364. DOI: 10.1186/s13071-020-04242-8.

5 **Cháves-González L.E., Morales-Calvo F., Mora J., Solano-Barquero A., Verocai G.G. & Rojas A. 2022.** What lies behind the curtain: Cryptic diversity in helminth parasites of human and veterinary importance. *Current Research in Parasitology & Vector-Borne Diseases*. 2: 100094. DOI: 10.1016/j.crpvbd.2022.100094.

6 **Chubb J.C., Pool D.W. & Veltkamp C.J. 2010.** A key to the species of cestodes (tapeworms) parasitic in British and Irish freshwater fishes. *Journal of Fish Biology*. 31(4): 517-543. DOI: 10.1111/j.1095-8649.1987.tb05256.x.

7 **Cornell K.A., Smith O.M., Crespo R., Jones M.S., Crossley M.S., Snyder W.E. & Owen J.P. 2022.** Prevalence Patterns for Enteric Parasites of Chickens Managed in Open Environments of the Western United States. *Avian Diseases*. 66(1): 60-68. DOI: 10.1637/21-00079.

8 **Dalal D., Saha S., Dalal D.D., Roy A., Talapatra S.N. & Ghosh P. 2024.** Comparative study on the outbreak of helminth parasites in domestic duck and chicken. *International Journal of Fauna and Biological Studies*. 11(2): 23-26. DOI: 10.22271/23940522.2024.v11.i2a.1015.

9 **EFSA Panel on Animal Health and Animal Welfare (AHAW), Nielsen S.S., Alvarez J., Bicout D.J., Calistri P., Canali E., Drewe J.A., Garin-Bastuji B., Gonzales Rojas J.L., Gortázar Schmidt C., Herskin M., Miranda Chueca M.Á., Padalino B., Pasquali P., Roberts H.C., Spoolder H., Stahl K., Velarde A., Viltrop A., Winckler C., Estevez I., Guinebretière M., Rodenburg B., Schrader L., Tiemann I., Van Niekerk T., Ardizzone M., Ashe S., Hempen M., Mosbach Schulz O., Rojo Gimeno C., Van der Stede Y., Vitali M. & Michel V. 2023.** Welfare of laying hens on farm. *EFSA Journal*. 21(2): 1-188. DOI: 10.2903/j.efsa.2023.7789.

10 **Escalante G.C., Sweet A.D., Mccracken K.G., Gustafsson D.R., Wilson R.E. & Johnson K.P. 2016.** Patterns of cryptic host specificity in duck lice based on molecular data. *Medical and Veterinary Entomology*. 30(2): 200-208. DOI: 10.1111/mve.12157.

**11 Essam A., Abbas I., Elmishmishy B. & Elwafa S.A. 2024.** *Cryptosporidium* infection in ducks and pigeons collected from live bird markets in two Nile Delta governorates (Dakahlia and Gharbia), Egypt. *Mansoura Veterinary Medical Journal*. 25(4): 1247. DOI: 10.35943/2682-2512.1247.

**12 Gomes-Gonçalves S., Rodrigues D., Santos N., Gantois N., Chabé M., Viscogliosi E. & Mesquita J.R. 2024.** Molecular Screening and Characterization of Enteric Protozoan Parasites and Microsporidia in Wild Ducks from Portugal. *Animals*. 14(20): 2956. DOI: 10.3390/ani14202956.

**13 Gong C., Cao X.-F., Deng L., Li W., Huang X.-M., Lan J.-C., Xiao Q.-C., Zhong Z.-J., Feng F., Zhang Y., Wang W.-B., Guo P., Wu K.-J. & Peng G.-N. 2017.** Epidemiology of *Cryptosporidium* infection in cattle in China: a review. *Parasite (Paris, France)*. 24: 1. DOI: 10.1051/parasite/2017001.

**14 Gong Z., Kan Z., Huang J., Fang Z., Liu X., Gu Y. & Li W.-C. 2021.** Molecular prevalence and characterization of *Cryptosporidium* in domestic free-range poultry in Anhui Province, China. *Parasitology Research*. 120(10): 3519-3527. DOI: 10.1007/s00436-021-07191-y.

**15 Graaf D.C., Vanopdenbosch E., Ortega-Mora L.M., Abbassi H. & Peeters J.E. 1999.** A review of the importance of cryptosporidiosis in farm animals. *International Journal for Parasitology*. 29(8): 1269-1287. DOI: 10.1016/s0020-7519(99)00076-4.

**16 Investigations Operations Manual. 2022.** United States: U.S. Food and Drug Administration (FDA). [Fonte: <https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-references/investigations-operations-manual>].

**17 Jirků M., Jirků M., Oborník M., Lukeš J. & Modrý D. 2009.** A Model for Taxonomic Work on Homoxenous Coccidia: Redescription, Host Specificity, and Molecular Phylogeny of *Eimeria ranae* Dobell, 1909, with a Review of Anuran-Host Eimeria (Apicomplexa: Eimeriorina). *Journal of Eukaryotic Microbiology*. 56(1): 39-51. DOI: 10.1111/j.1550-7408.2008.00362.x.

**18 Kerroucha R., Medjoual I., Bourguig L. & Senouci K. 2022.** Identification of The Main Intestinal Helminths of Local Breed Chickens (*Gallus gallus domesticus* Linnaeus, 1758) Reared in Traditional Mode in The Oran Region. *Helminthologia*. 59(2): 170-178. DOI: 10.2478/helm-2022-0015.

**19 Larki S., Alborzi A., Chegini R. & Amiri R. 2018.** A Preliminary Survey on Gastrointestinal Parasites of Domestic Ducks in Ahvaz, Southwest Iran. *Iran Journal of Parasitology*. 13(1): 137-144.

**20 Li Q., Wang C., Gong Z. & Liu G. 2019.** Phylogenetic relationships of 52 *Eimeria* species based on COI sequences. *Mitochondrial DNA*. 4(2): 3956-3958. DOI: 10.1080/23802359.2019.1688709.

**21 Li Y. 2019.** Comparison and phylogenetic analyses of complete mitochondrial genomes of three trematodes in duck. 102f. Daqing, China. Dissertation (Master's Degree in Preventive Veterinary Medicine) - Graduate Program in Preventive Veterinary Medicine, Heilongjiang Bayi Agricultural University.

**22 Liu K., Wang M., Zhang Y., Fang C., Zhang R., Fang L., Sun J., Liu Y. & Liao X. 2024.** Distribution of antibiotic resistance genes and their pathogen hosts in duck farm environments in south-east coastal China. *Applied Microbiology and Biotechnology*. 108(1): 136. DOI: 10.1007/s00253-023-12842-4.

**23 Lopez-Osorio S., Chaparro-Gutiérrez J.J. & Gomez-Osorio L.M. 2020.** Overview of Poultry *Eimeria* Life Cycle and Host-Parasite Interactions. *Frontiers in Veterinary Science*. 7: 384. DOI: 10.3389/fvets.2020.00384.

**24 Aboulaila M., El-Bahy N., Hilali M., Yokoyama N., Igarashi I. 2011.** Prevalence of the enteric parasites of ducks from Behera governorate, Egypt. *Journal of Protozoology Research*. 21: 36-44. DOI: 10.32268/jprotozoolres.21.2_36.

**25 Ma L., Lyu W., Zeng T., Wang W., Chen Q., Zhao J., Zhang G., Lu L., Yang H. & Xiao Y. 2024.** Duck gut metagenome reveals the microbiome signatures linked to intestinal regional, temporal development, and rearing condition. *iMeta*. 3(4): e198. DOI: 10.1002/imt2.198.

**26 Mejías-Alpízar M.J., Porras-Silesky C., Rodríguez E.J., Quesada J., Alfaro-Segura M.P., Robleto-Quesada J., Gutiérrez R. & Rojas A. 2024.** Mitochondrial and ribosomal markers in the identification of nematodes of clinical and veterinary importance. *Parasites & Vectors*. 17(1): 77. DOI: 10.1186/s13071-023-06113-4.

**27 Miller M.L., Rota C. & Welsh A. 2024.** Transforming gastrointestinal helminth parasite identification in vertebrate hosts with metabarcoding: a systematic review. *Parasites & Vectors*. 17(1): 311. DOI: 10.1186/s13071-024-06388-1.

**28 Nakamura A.A. & Meireles M.V. 2015.** *Cryptosporidium* infections in birds - a review. *Revista Brasileira de Parasitologia Veterinária*. 24(3): 253-267. DOI: 10.1590/S1984-29612015063.

**29 Nisa R.U., Tantray A.Y. & Shah A.A. 2022.** Shift from morphological to recent advanced molecular approaches for the identification of nematodes. *Genomics*. 114(2): 110295. DOI: 10.1016/j.ygeno.2022.110295.

**30 Ogedengbe M.E., Brash M. & Barta J.R. 2016.** The complete mitochondrial genome sequence of an *Isospora* sp. (Eimeriidae, Eucoccidiorida, Coccidiasina, Apicomplexa) causing systemic coccidiosis in domestic Canaries (*Serinus canaria* Linn.). *Mitochondrial DNA*. 27(5): 3315-3317. DOI: 10.3109/19401736.2015.1018201.

**31 Ogedengbe M.E., El-Sherry S., Ogedengbe J.D., Chapman H.D. & Barta J.R. 2018.** Phylogenies based on combined mitochondrial and nuclear sequences conflict with morphologically defined genera in the eimeriid coccidia (Apicomplexa). *International Journal for Parasitology*. 48(1): 59-69. DOI: 10.1016/j.ijpara.2017.07.008.

**32 Onyeabor A.I. & Onunkwo D.N. 2023.** Preliminary survey on the intestinal protozoan and helminthes parasites of domestic ducks reared in Umuahia Area of Abia State. *The International Journal of Agriculture, Management and Technology*. 7(1): 637-640.

**33 Ozenturk U., Chen Z., Jamone L. & Versace E. 2024.** Robotics for poultry farming: challenges and opportunities. *Computers and Electronics in Agriculture*. 226: 15-16. DOI: 10.1016/j.compag.2024.109411.

**34 Patil S.S., Shinduja R., Suresh K.P., Phukan S., Kumar S., Sengupta P.P., G. Amachawadi R., Raut A., Roy P., Syed A., Marraiki N., Elgorban A.M., Al-Harthi H.F., Bahkali A.H., Shivamallu C. & Shiva Prasad K. 2021.** A systematic review and meta-analysis on the prevalence of infectious diseases of Duck: A world perspective. *Saudi Journal of Biological Sciences*. 28(9): 5131-5144. DOI: 10.1016/j.sjbs.2021.05.034.

**35 Sato H., Ihara S., Inaba O. & Une Y. 2010.** Identification of *Euryhelmis costaricensis* metacercariae in the skin of *Tohoku hynobiid* salamanders (*Hynobius lichenatus*), northeastern Honshu, Japan. *Journal of Wildlife Diseases*. 46(3): 832-842. DOI: 10.7589/0090-3558-46.3.832.

**36 Sherwin C.M., Nasr M.A.F., Gale E., Petek M., Stafford K., Turp M. & Coles G.C. 2013.** Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. *British Poultry Science*. 54(1): 12-23. DOI: 10.1080/00071668.2012.757577.

**37 Stout L., Daffe G., Chambouvet A., Correia S., Culloty S., Freitas R., Iglesias D., Jensen K.T., Joaquim S., Lynch S., Magalhães L., Mahony K., Malham S.K., Matias D., Rocroy M., Thieltges D.W. & de Montaudouin X. 2024.** Morphological vs. molecular identification of trematode species infecting the edible cockle *Cerastoderma edule* across Europe. *International Journal for Parasitology. Parasites and Wildlife*. 25: 101019. DOI: 10.1016/j.ijppaw.2024.101019.

**38 Subagja H., Nadia Z.A. & Zubaidah S. 2025.** Effect of Hygiene on Hatching Performance of Duck Eggs (*Anas platyrhynchos domesticus*). *Advances in Animal and Veterinary Sciences*. 13(5): 1060-1065. DOI: 10.17582/journal.aavs/2025/13.5.1060.1065.

**39 Tamaru M., Yamaki S., Jimenez L.A. & Sato H. 2015.** Morphological and molecular genetic characterization of three *Capillaria* spp. (*Capillaria anatis*, *Capillaria pudendotecta*, and *Capillaria madseni*) and *Baruscapillaria obsignata* (Nematoda: Trichuridae: Capillariinae) in avians. *Parasitology Research*. 114(11): 4011-4022. DOI: 10.1007/s00436-015-4629-2.

**40 Taylor M.A., Coop R.L. & Wall R.L. 2007.** General parasitology including taxonomy, diagnosis, antiparasitics. In: *Veterinary Parasitology*. 3rd edn. Oxford: Blackwell Publishing, pp.270-290.

**41 University of New Mexico. 2001.** Alphabetical list of the Eimeriidae of the world. Albuquerque, NM: University of New Mexico. [Fonte: http://eimeria.unl.edu/list.html.].

**42 Xiao L., Escalante L., Yang C., Sulaiman I., Escalante A.A., Montali R.J., Fayer R. & Lal A.A. 1999.** Phylogenetic Analysis of *Cryptosporidium* Parasites Based on the Small-Subunit rRNA Gene Locus. *Applied and Environmental Microbiology*. 65(4): 1578-1583. DOI: 10.1128/AEM.65.4.1578-1583.1999.

**43 Yegani M. & Korver D.R. 2008.** Factors Affecting Intestinal Health in Poultry. *Poultry Science*. 87(10): 2052-2063. DOI: 10.3382/ps.2008-00091.