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A diagnostic and pathological study of *Cryptosporidium* spp. in some bird species in Diwaniyah Governorate, Iraq

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ABSTRACT

Background: Cryptosporidiosis is a zoonotic parasitic disease caused by *Cryptosporidium* sp. that affects multiple hosts, including birds. It infects birds through water and food contaminated with the infective stage, causing gastrointestinal, respiratory, and kidney diseases in birds. It also causes conjunctival infection in some bird species if the oocysts are deposited directly on the conjunctival sac. **Aim of the study:** This study aimed to diagnose *Cryptosporidium* spp. in some species of birds and histopathological changes in intestine tissues. **Methods:** 200 Samples of the intestinal contents of the birds, including 50 birds each of wild pigeons, ducks, domestic chickens, and field chickens, samples examined using a light microscope with Ziehl-Neelsen stain. The positive samples were diagnosis by polymerase chain reaction technique to confirm the infection. sections of the intestines of infected birds were preserved in 10% formalin for microscopic examination. **Results:** The study was conducted with a total infection rate of 22%, wild pigeons, ducks, local chickens, and field chickens recorded infection rates of 28%, 14%, 24%, and 22%, respectively. Diagnosis using the PCR technique showed the total infection was 77.27%, which is higher than in microscopic diagnosis; microscopic examination of histological sections from the intestines of infected birds revealed atrophy of the villi and infiltration of inflammatory cells as a result of infection with *Cryptosporidium* spp. **Conclusion:** *Cryptosporidium* infection is highly in birds of the province and molecular diagnosis by PCR technique better than microscopic diagnosis.

Introduction

Cryptosporidiosis is a zoonotic parasitic disease caused by *Cryptosporidium* spp. that affects multiple hosts, including birds [1]. It infects birds through water and food contaminated with the infective stage [2]. It causes gastrointestinal and respiratory diseases [3] and kidney diseases in birds [4]. It also causes conjunctival infection in some bird species if the oocysts are deposited directly on the conjunctival sac [5]. Clinical symptoms of *Cryptosporidium* spp.

Range from asymptomatic to severe symptoms leading to death [6]. When wild birds are infected with the parasite, they pose a threat to public health due to the nature of their life in the wild, which makes it difficult to control their infection with *Cryptosporidium* spp. which increases the possibility of this disease being transmitted to humans and pets. It is also necessary to monitor the infection in wild birds, its methods of transmission, and its prevention [7-9]. It was also found that the infection rate of the parasite is higher among children who are in contact with poultry

compared to the infection rate among children who are in contact with livestock and sheep. The infection rate was lower among children not in contact with animals [10]. There are no effective vaccines or medications to treat or prevent infection in animals and humans [11]. Identifying chokes in birds contributes to limiting the spread of the disease, paying attention to strict disinfection and biosecurity, and sterilizing hands to prevent the spread of parasite cysts [12]. Therefore, it is necessary to identify the extent of the spread and rates of infection with this parasite to limit its spread and prevent infection. This study aimed to diagnose *Cryptosporidium* spp. in some species of birds and histopathological changes resulting from infection in intestine tissues.

Materials and Methods

Samples collection and study design

Two hundred birds were collected from September 2024 - March 2025; they included 50 birds each of local chicken, field chicken, duck, and wild pigeon). They were collected from different areas in Diwaniyah province. The birds were dissected in the laboratory, and samples were taken from the intestinal contents. Smears were made and stained with Ziehl-Neelsen Stain according to the method [13]. They were then examined using an oil-lens optical microscope, where the parasite cysts appeared red, while the other organisms appeared green and blue.

Histopathological study

The sections of the intestines of infected birds were preserved in 10% formalin until they were prepared as tissue sections for microscopic examination. The sections were prepared according to the method of [14,15].

Molecular study

The positive samples were examined for microscopic diagnosis by polymerase chain reaction technique. The DNA of parasites was extracted from the intestinal contents of the birds using the Presto™ Stool DNA Extraction Kit from Geneaid / Taiwan, which was provided by a Scientific Researcher. Co. Ltd. The extraction was carried out according to the steps in the company's instruction manual. A Nanodrop spectrophotometer was used to measure the concentration and purity of DNA. Primers specific for the Small-subunit 18 Sr RNA gene were

used for the detection of *Cryptosporidium* spp. Using PCR technique. They were designed in this study using NCBI-Genbank and Primer 3 Plus software. These primers were prepared by a Scientific Researcher. Co. Ltd, Iraq. The primers are forward primer 5'-AGACGGTAGGGTATTGGCCT-3', reverse primer 5'-TACGAATGCCCAACTGTC-3', and the product size is 784 bp. The PCR product was analyzed by Agarose gel electrophoresis.

Results

Microscopic study

Ziehl-Neelsen stain was used to detect *Cryptosporidium* spp. Oocysts. The oocysts appeared spherical or oval in shape and red, while the rest of the organisms were blue, as shown in Figure (1). The results of examining 200 birds showed that 44 birds were infected with *Cryptosporidium* spp., representing 22% of the total examination birds. Microscopic examination revealed that 14/50 wild pigeons, 7/50 ducks (14%), 12/50 domestic chickens (24%), and 11/50 field chickens (22%) of the total examined birds were infected; Table (1) shows the percentage of infection in different types of examined birds.

Molecular study

positive samples were examined using polymerase chain reaction (PCR) techniques. The results of electrophoresis on agarose gels of DNA amplification using the Small gene primer Subunit ribosomal RNA showed DNA bands of approximately (784 bp) and 34 samples were positive, at a rate of 77.27% when confirmatory diagnosis was performed on 44 examined samples to detect the *Cryptosporidium* spp. The infection rate of the examined bird samples, including wild pigeons, ducks, domestic chickens, and field chickens, was recorded as follows: 12 (85.71%), 5 (71.42%), 9 (75%), and 8 (72.72%), respectively, as shown in Table (2).

Histopathological changes

The results of examining the tissue sections under the microscope showed atrophy of the villi with infiltration of inflammatory cells (Figure 2) and (Figure 3), showing atrophy of the villi and the death of some of them compared to healthy villi (Figure 4).

Table 1. The infection rates of *Cryptosporidium* spp. in the examined birds.

Bird	No. of Examined birds	No. of Infected Birds	Percent of the infected birds	No. of Uninfected birds
Pigeon	50	14	28	36
Duck	50	7	14	43
Local chicken	50	12	24	38
field chicken	50	11	22	39
Total	200	44	22	156
Chi-square value x2	3.03			
Calculated Likelihood Value	0.387*			

* There are no significant differences at 0.05.

Table 2. The number of examined samples for detection of *Cryptosporidium* spp. and percentage of infection by PCR technique.

Bird type	No.Examined	No.Infected	Percentage	Non-infected
Pigeon	14	12	85.71	2
Duck	7	5	71.42	2
Domestic chickens	12	9	75	3
field chickens	11	8	72.72	3
Total	44	34	77.27	10
0.869				X ² Value
0.833*				calculated probability value

No 05).significant deferences (≤ 0.05).

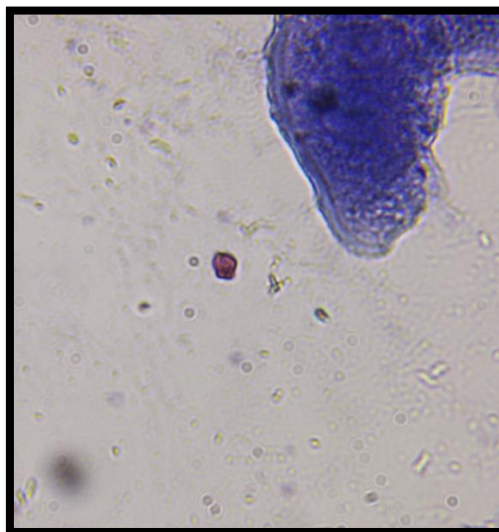
Figure 1. Oocyst of *Cryptosporidium* spp. (4x).

Figure 2. Shows atrophy of the villi with infiltration of inflammatory cells(10x).

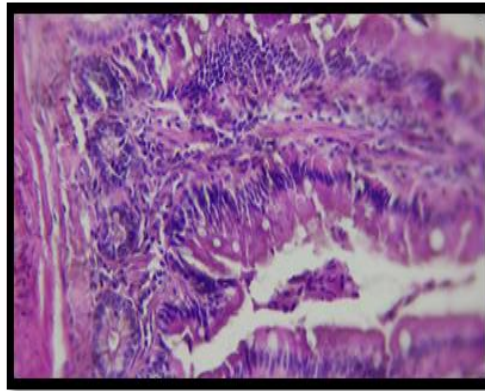


Figure 3. Shows atrophy and death of some villi(40x).

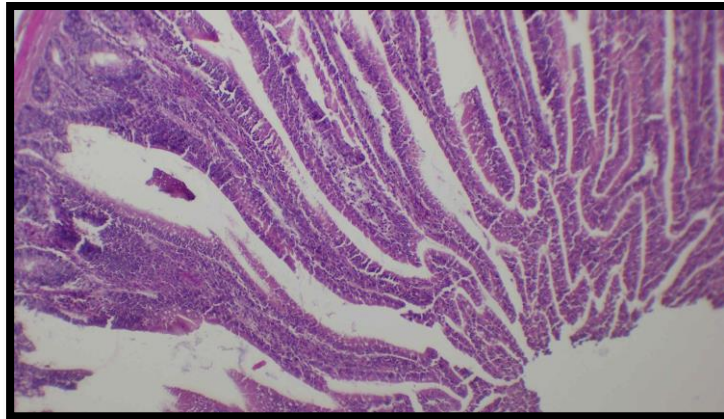
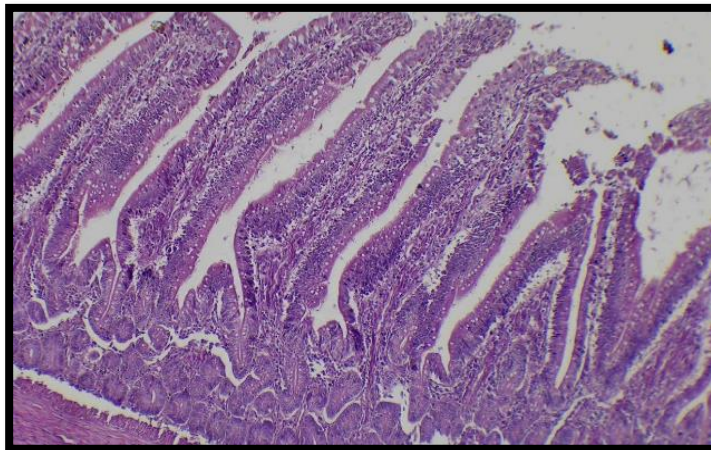


Figure 4, Showed the intact villi of the intestine (400x).



Discussion

Upon microscopic examination, infection with the parasite *Cryptosporidium* spp. was recorded at a total rate of 22% in the birds under study. This was close to what was reported by [16] in their study of domesticated birds in Al-Diwaniyah province, where they recorded a total infection rate

of 19.4% in birds, and close to the results of the study conducted by [15], where a total infection rate of 29.30% in poultry species in Sulaymaniyah Governorate in Iraq was recorded. The results of the study showed that the wild pigeons were infected with the parasite at a rate of 28%, which was consistent with what [17] in Mosul had reached, where he recorded a rate of wild pigeons being

infected with the parasite of 30%. It was also consistent with what [1] in Egypt had recorded, where they stated that the rate of pigeons being infected with the parasite was 26.3%.

The results of the study showed that the infection rate of ducks with *Cryptosporidium* spp. Parasite reached 14%, which is higher than what Al-Taie and Karwan [18] reached, where they mentioned that the infection rate of ducks was 8% in Babil Governorate. The reason may be due to the difference in the sample collection areas, as their study showed differences in the infection rates between the different sample collection areas in their study. The highest infection rate was recorded in the Hillah area at 20%, followed by the Al-Kifl area at 10%. At the same time, the infection rate in the study results agreed with the infection rate of ducks with the *Cryptosporidium* spp. Parasites recorded by [19] where recorded the infection of ducks with the parasite at a rate of 15.4% when they studied the spread of intestinal parasites in domestic ducks in Ibadan, southwest Nigeria. The results of the study showed that the local chicken was infected with the parasite at a rate of 24%, while in farm chickens, the infection rate was recorded at 22%, which is close to what was reached by [20], whose study recorded the prevalence of the parasite in broiler chickens in Al-Qadisiyah Governorate at 29.33% upon microscopic examination. [21] also recorded rates close to this study, as it recorded the infection of domestic chickens with the parasite *Cryptosporidium* spp. At a rate of 28.6%, and the infection of farm chickens (broiler) at a rate of 20.7%. The results of the molecular examination using the polymerase chain reaction technique showed the total infection at a rate of 77.27%, which is higher than the infection rates in microscopic diagnosis, as the samples were fewer in number and diagnosed microscopically in advance and not randomly as in microscopic examination samples. It was also shown that a number of samples did not show positivity in the examination using the (PCR) technique, despite being positive upon microscopic examination, which may be due to the small number of oocysts in the sample, and this is supported by what was mentioned by [1].

Microscopic examination of histological sections from the intestines of infected birds revealed atrophy of the villi and infiltration of inflammatory cells as a result of infection with *Cryptosporidium* spp. This is consistent with what [22] reported in their study of cryptosporidiosis in

chickens in Mosul. They found atrophy of the villi, distortion, and infiltration of inflammatory cells.

Although two *Cryptosporidium* species were identified using PCR, they were unable to confirm the identity of the parasite using

morphometric analysis of the oocysts. This was believed to be due to the small number of oocysts below the detection limit; the infection rates of the birds studied using polymerase chain reaction (PCR) were higher than those detected by microscopic examination. This is due to the fact that the samples in our current study were previously diagnosed positive for *Cryptosporidium* spp. by microscopic examination. Therefore, the sample was considered selective, not random, as was the case with microscopic examination.

Conclusion:

From the results of the present study, it was concluded that *Cryptosporidium* spp. DNA is found in the guts of birds. Birds are large sources of human infection by contamination, Specifically in Al- Diwaniyah province. Health and veterinary departments must give health guidelines to lactation breeders and consumers every day in order to interfere with infection of animals and then transmission to human beings.

Cryptosporidium infection is highly in birds of the province and molecular diagnosis by PCR technique Peter than microscopic diagnosis,

Ethical Approval

The study was conducted following prior approval in accordance with ethical guidelines for the use of animals in research. Ethical clearance was granted by the Institutional Animal Care and Use Committee (IACUC) of the Department of Biology, College of Education, University of Al-Qadisiyah, under approval number [546], dated 07/03/2025. All procedures involving animals adhered to ethical standards, ensuring their welfare, and were carried out in line with both national and international guidelines for the humane treatment of animals.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

Data is available upon reasonable request.

Authors' contribution

- Hind Qasim Neamah Al Qasimi: Conception and design of the study, acquisition of data, analysis, and interpretation of data.

- Khaled Thamer Matter Alshaebani: Drafting the article, revising it critically for important intellectual content, final approval of the version to be submitted.

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