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ORIGINAL ARTICLE

Cryptosporidium Infection in Ducks and Pigeons Collected From Live Bird Markets in Two Nile Delta Governorates Dakahlia and Gharbia, Egypt

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Abstract

BACKGROUND: Cryptosporidiosis is a zoonotic parasitic disease that infects a wide range of hosts, including birds. *Cryptosporidium* infections vary across avian species and cause considerable economic losses. Data on cryptosporidiosis in ducks and pigeons are scarce.
OBJECTIVES: The present study aimed to investigate *Cryptosporidium* infections in household ducks and pigeons collected from live bird markets in two Nile Delta governorates (Dakahlia and Gharbia) in Egypt.
METHODS: The intestinal contents of household ducks ($n = 109$) and pigeons ($n = 57$) collected from live bird markets in two Nile Delta governorates (Dakahlia and Gharbia) were tested using a sugar flotation test coupled with modified Ziehl Neelsen acid fast-fast (MZN) staining. Tracheal and bursal smears from some birds were also modified Ziehl Neelsen tested. Thin (4–5 μm) histological sections of tissues containing *Cryptosporidium* oocysts were examined. *Cryptosporidium* oocysts were harvested from 10 intestinal content samples and PCR-tested for the *Cryptosporidium* oocyst wall protein (COWP) gene.
RESULTS: *Cryptosporidium* oocysts were detected in the intestinal contents of 23 (21.1%) ducks and 15 (26.3%) pigeons. Oocysts were also detected in one out of 84 tracheal smears from ducks and one out of 15 bursal smears from pigeons. However, all infections were subclinical, most likely because all birds examined were healthy, and a few *Cryptosporidium* oocysts were detected in the tested samples. Variations in *Cryptosporidium* infections among districts, age groups, and seasons were not statistically significant. Morphometric analysis of the detected oocysts revealed infections with two *Cryptosporidium* species, small-sized *Cryptosporidium meleagridis* (4–5.5 × 4–5 μm), and large-sized *Cryptosporidium baileyi* (6–7 × 5–6 μm). The identity of these oocysts was not confirmed because PCR testing of the harvested oocysts failed, probably because the number of oocysts was lower than the PCR detection limits. A few histopathological changes were detected in *Cryptosporidium* oocyst-infected tissues. However, these changes were not specific and were not accompanied by exclusion tests for other pathogens.
CONCLUSIONS: Although the survey was small, high *Cryptosporidium* prevalence of Cryptosporidium was high among household ducks and pigeons in Egypt. Oocysts were preliminarily identified as *C. meleagridis* (the third most zoonotic *Cryptosporidium* species) and *C. baileyi*. However, accurate species genotype identification requires successful PCR sequencing assays. This underlines the importance of conducting large-scale molecular surveys of both ducks and pigeons. Samples from household and farmed birds, as well as healthy and clinically ill birds, should be included in these future surveys.

Keywords: Cryptosporidium, Duck, Egypt, Pigeon

1. Introduction

Cryptosporidiosis is an important parasitic foodborne zoonotic disease that can virtually infect almost all mammals, humans, birds, and fishes [1,2]. This disease is a habitual cause of fatalities due to diarrhea in toddlers. In 2015, cryptosporidiosis caused over 600,000 human deaths [3]. Cryptosporidiosis can also cause chronic and life-threatening diseases in immunocompromised

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individuals, especially those seriously affected by the HIV [4].

Tyzzer [5] published the first description of *Cryptosporidium* infection in birds, which involved the cecal epithelium of chicken. *Cryptosporidium* can cause infections in several avian species with different morbidity and mortality rates, resulting in considerable economic losses [6,7]. *Cryptosporidium* spp. can inhabit various tissues in birds, including the respiratory and gastrointestinal epithelium as well as the bursa of Fabricius [8,9].

The parasite circulates in a direct life cycle, in which the host is infected after ingesting food or water contaminated with *Cryptosporidium* oocysts. The oocysts excyst in the small intestine, and the sporozoites release and invade the intestinal epithelium. The sporozoites multiply asexually in two generations of schizogony, and the resulting merozoites initiate sexual reproduction by gametogony, giving rise to the oocysts, which undergo sporulation in the intestinal lumen and pass as sporulated oocysts in the feces of infected hosts. Oocysts are highly infectious and can resist adverse environmental conditions and various disinfectants [4,10,11].

Four *Cryptosporidium* species have been frequently detected in birds including: *Cryptosporidium baileyi*, *Cryptosporidium meleagridis*, *Cryptosporidium galli*, and *Cryptosporidium avium.* Several *Cryptosporidium* genotypes have been detected, including avian genotypes I, II, III, IV, and V, duck genotype, and goose genotype. A few mammalian-specific *Cryptosporidium* species have rarely been detected, such as *C. parvum*, *C. hominis, C. andersoni,* and the Eurasian woodcock genotype [12]. *C. meleagridis* is the third most significant zoonotic species known to infect humans [13]. The parasite has been isolated from immunocompromised patients and children [14]. A few human cases have been found to be infected with *C. baileyi* [15,16].

A few reports have been published on *Cryptosporidium* infections in birds in Egypt. However, two recent reports on pigeons and ducks are of interest. Abou Elez et al. [17] found *C. parvum* (the most pathogenic *Cryptosporidium* species worldwide) in seven out of 150 molecularly tested pigeons from Sharqia governorate (located in the Nile Delta). On the other hand, Kalifa et al. [18] found high *Cryptosporidium* prevalence in ducks from various Egyptian governorates (39.9%), and *C. meleagridis* oocysts were molecularly detected in the tested ducks. This highlights the importance of pigeons and ducks in maintaining the life cycle of these two *Cryptosporidium* species and may indicate their potential role, particularly pigeons, in *Cryptosporidium* zoonosis in Egypt. Given that, household-reared birds have a higher prevalence of *Cryptosporidium* infections and have a greater role in the circulation of zoonotic *Cryptosporidium* species (e.g., *C. parvum*) than intensively-farmed birds [19]. The majority of the household birds in Egypt are marketed in live bird markets (LBMs). This study aimed to determine the prevalence of different types of *Cryptosporidium* oocysts in ducks and pigeons collected from LBMs in selected Egyptian governorates (Dakahlia and Gharbia) in the Nile Delta, Egypt.

2. Materials and methods

2.1. Study area, samples collection and microscopy

Between May 2022 and April 2023, a total of 109 ducks and 57 pigeons of different ages and sexes were collected from various LBMs in some districts (Mansoura, Dekernes, and Aga) at Dakahlia governorate, Egypt. A few (11 ducks and 16 pigeons) samples were also collected from a LBM in Elmahalla Elkubra, a city at the border of Dakahlia, but belonged to Gharbia governorate. Both governorates fall inside the Nile Delta (31°50′N, 31°00′E), which is the largest agricultural region in Egypt (Fig. 1).

The collected ducks and pigeons were clinically examined. After slaughter, fresh contents of the whole intestinal tract were collected in a plastic cup and tested for *Cryptosporidium* oocysts using the modified Wisconsin sucrose flotation test [20]. The floats were harvested and used to prepare air-dried smears. The smears were stained using the modified Ziehl Nelssen acid-fast (MZN) stain [21]. Bursal smears from seven pigeons and tracheal smears from 15 pigeons and 84 ducks were also MZN-stained. Thin sections from the intestines, bursae of Fabricius, and tracheae were prepared and stained with hematoxylin and eosin, according to Slaoui and Fiette [22]. Smears/tissue sections were microscopically examined using a light microscope (Carl Zeiss, Oberkochen, Germany), and micrographs were captured using a 12 megapixels AmScope camera (United Scope, Irvine, CA, USA).

2.2. Molecular examination of *Cryptosporidium*-positive samples

After microscopic examination, the modified Wisconsin sucrose flotation test was used to harvest oocysts from *Cryptosporidium* positive intestinal contents of five pigeons and five ducks. The

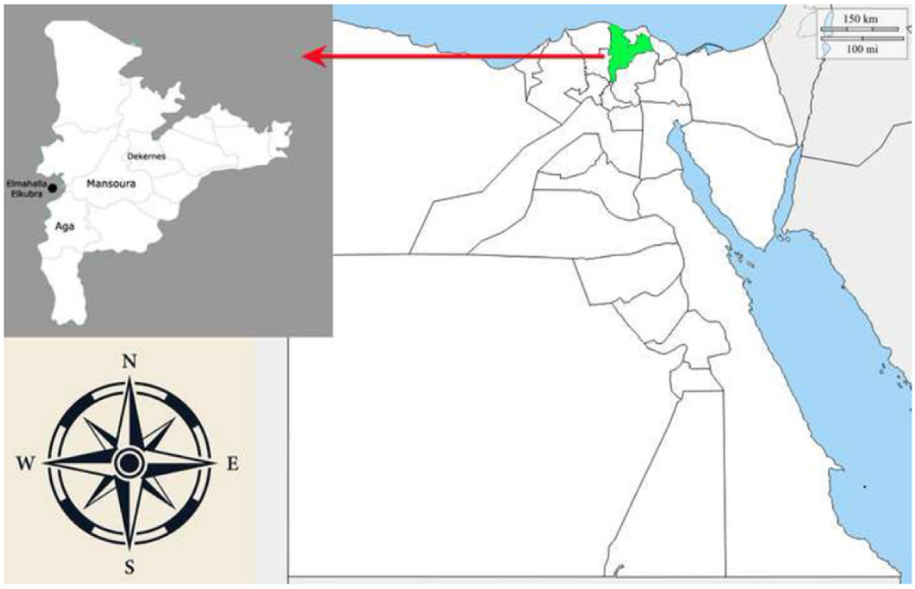

Fig. 1. Map of Egypt including Dakahlia governorate (insert), and showing districts from which samples were collected. These districts are located on a latitude 31°50′N and a longitude 31°00′E.

harvested floats were subjected to DNA extraction using the QIAamp DNA stool Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The extracted DNA was tested using PCR, and the primer pair Cry9 (5′-GGACTGAAA-TACAGGCATTATCTTG-3′) and Cry15 (5′-GTA-GATAATGGAAGAGATTGTG-3′) was used to amplify the 553 bp section of the *Cryptosporidium* oocyst wall protein (COWP) gene. The reactions were conducted in a total volume of 25 μl containing 5 μl of the extracted DNA, 12.5 μl of Emerald Amp GT PCR master mix (Takara, Japan), 1 μl of each primer, and 5.5 μl of PCR-grade water. The following cycling conditions were used: a single cycle of primary denaturation at 94 °C for 5 min, 35 cycles of each of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 40 s, and extension at 72 °C for 45 s. A final extension step at 72 °C for 10 min was also included [23]. The amplified PCR products were electrophoresed on agarose gels stained with ethidium bromide (10 mg/ml) [24].

2.3. Data analysis

Data were tested for statistical significance using GraphPad Prism software (www.graphpad.com). A variety of models were used, including a one-way analysis of variance coupled to the Kruskal–Wallis test and an unpaired *t*-test coupled to the Mann–Whitney *U* test. Differences were considered statistically significant when *P* value was less than 0.05.

3. Result

3.1. Prevalence of Cryptosporidium oocysts in ducks and pigeons

All examined birds were healthy, i.e., no disease symptoms were observed. Twenty-three (21.1%) out of 109 and 15 (26.3%) out of 57 intestinal content samples from ducks and pigeons, respectively, contained *Cryptosporidium* oocysts, with no significant difference (*P* value = 0.56). On the other hand, a single tracheal smear out of 84 tested from ducks had *Cryptosporidium* oocysts. However, no oocysts were detected in any tracheal smear from pigeons ($n = 15$). In contrast, oocysts were detected in a single bursal smear of pigeons ($n = 7$).

Ducks and pigeons from the Gharbia governorate had higher *Cryptosporidium* prevalence (36.4 and 31.3%, respectively) than those from Dakahlia (19.4 and 24.4%, respectively), however, these differences were not statistically significant; the estimated *P* values were 0.24 for ducks and 0.74 for pigeons. Similarly, no statistical differences were detected when comparing the prevalence rate among various districts of Dakahlia governorate, *P* values were 0.48 for ducks and 0.99 for pigeons (Table 1).

In general, the prevalence was higher during the hot season than during the cold season. Ducks in Autumn and Summer had almost equal prevalence rates, which were higher than those in Winter and Spring. However, these variations were not statistically significant (*P* value = 0.80). Similarly, in pigeons, the seasonal variations were statistically

Table 1. Prevalence of Cryptosporidium oocysts in different bird species from various districts included in the present study.

Bird/district	Duck		Pigeon	
	Number tested	Number positive (%)	Number tested	Number positive (%)
Gharbia (Elmahalla Elkubra)	**11**	**4 (36.4)**	**16**	**5 (31.3)**
Dakahlia	**98**	**19 (19.4)**	**41**	**10 (24.2)**
Mansoura	10	3 (21.4)	31	8 (25.8)
Dekernes	53	8 (15.1)	–	–
Aga	31	8 (25.8)	10	2 (20.0)
Total	**109**	**23 (21.1)**	**57**	**15 (26.3)**

insignificant (P value = 0.60); nonetheless, spring also had a higher prevalence along with Autumn and Summer, when comparing to winter (Table 2). On the other hand, *Cryptosporidium* prevalence in the two tested bird species was higher in young aged birds than in old aged birds. Ducks aged less than 6 months and pigeons aged less than 3 months had higher prevalence rates than ducks aged greater than 6 months and pigeons aged greater than 3 months, respectively, however, these differences were not statistically significant (Table 3).

3.2. Morphology of Cryptosporidium oocysts

The MZN-stained *Cryptosporidium* oocysts detected in intestinal smears were oval to spherical, double-walled, pink-colored, and belonged to two types of different sizes. The smaller oocysts measured 4–5.5 × 4–5 μm and were the predominant (21/23 ducks and 13/15 pigeons) (Fig. 2a). Larger oocysts measured 6–7 × 5–6 μm and were found in two ducks and two pigeons (Fig. 2b). Oocysts detected in tracheal smears from ducks belonged to the larger type only, whereas oocysts detected in bursal smears from pigeons belonged to the smaller type. The mean oocyst count detected in the MZN-stained slides from positive samples was three oocysts/slide for ducks and two oocysts/slide for pigeons.

3.3. Histopathological changes associated with Cryptosporidium infections

Cryptosporidium-positives intestinal sections showed mild to moderate destruction and loss of

Table 2. Prevalence of Cryptosporidium oocysts in different bird species in different seasons.

Bird/season	Autumn		Winter		Spring		Summer		*P* value
	Number tested	Number positive (%)	Number tested	Number positive (%)	Number tested	Number positive (%)	Number tested	Number positive (%)	
Duck	35	9 (25.7)	32	6 (18.7)	30	5 (16.7)	12	3 (25.0)	0.80
Pigeon	22	7 (31.8)	15	2 (13.3)	11	3 (27.3)	9	3 (33.3)	0.60

Table 3. Prevalence of Cryptosporidium oocysts in different bird species in relation to age.

Bird species	Age group	Number tested	Number Positives (%)	*P* value
Duck	<6 months	43	10 (23.2)	0.81
	>6 months	66	13 (19.7)	
Pigeon	Squabs (<3 months)	28	9 (32.1)	0.38
	Adults (>3 months)	29	6 (20.7)	

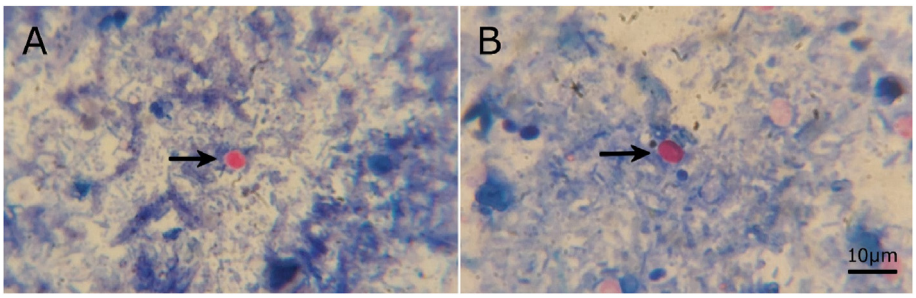

Fig. 2. Cryptosporidium oocysts detected in the modified Ziehl Neelsen-stained smears and were captured at ×100. A. Small size oocysts (arrow). B. Large size oocysts (arrow).

intestinal villi, as well as heavy leukocytic cell infiltration (Fig. 3a). *Cryptosporidium* oocysts detected in these sections appeared as round or oval structures among desquamated cells in the intestinal lumen (Fig. 3b). *Cryptosporidium*-positive tracheal sections showed deciliation and desquamation of the tracheal epithelium, thickened lamina propria, and heavy leukocytic cell infiltration (Fig. 3c). *Cryptosporidium* oocysts detected in the tracheal sections appeared as round or oval structures among desquamated cells in the tracheal lumen or on the upper surface of the tracheal epithelium (Fig. 3d).

3.4. Molecular detection of Cryptosporidium oocysts from positive intestinal contents

Unfortunately, *Cryptosporidium* DNA was not amplified in the PCR tested samples.

4. Discussion

Cryptosporidiosis can cause serious disease in humans and animals. *Cryptosporidium* is a leading cause of diarrhea in children and an important contributor to calf scour disease that occurs during the first few days of life. In birds, the role of *Cryptosporidium* in the clinical disease remains largely unknown. However, a few reports have isolated *Cryptosporidium* oocysts from chicken/turkeys with enteric and/or respiratory lesions [25]. On the other hand, little is known about the role of birds in *Cryptosporidium* zoonosis. Household and free-range birds are assumed to be a significant source of *Cryptosporidium* oocysts for human infections since these birds can mechanically spread the oocysts over great distances in the environment [26–28]. In the present study, various samples were collected from household ducks and pigeons marketed in LBMs in Mansoura, Dekernes, and Aga districts (Dakahlia governorate) and Elmahalla Elkubra district (Gharbia governorate) in the Nile Delta, to evaluate their *Cryptosporidium* infections. The prevalence of *Cryptosporidium* oocysts is relatively high in both ducks and pigeons tested in the present study. However, the prevalence of 21.1% detected in ducks was lower than the previously detected 39.9% prevalence in 915 ducks tested in various Egyptian governorates [18]. In contrast, the 26.3% prevalence detected in pigeons in the present study was considerably higher than the previously detected 20.0% prevalence in 50 pigeons from Assiut

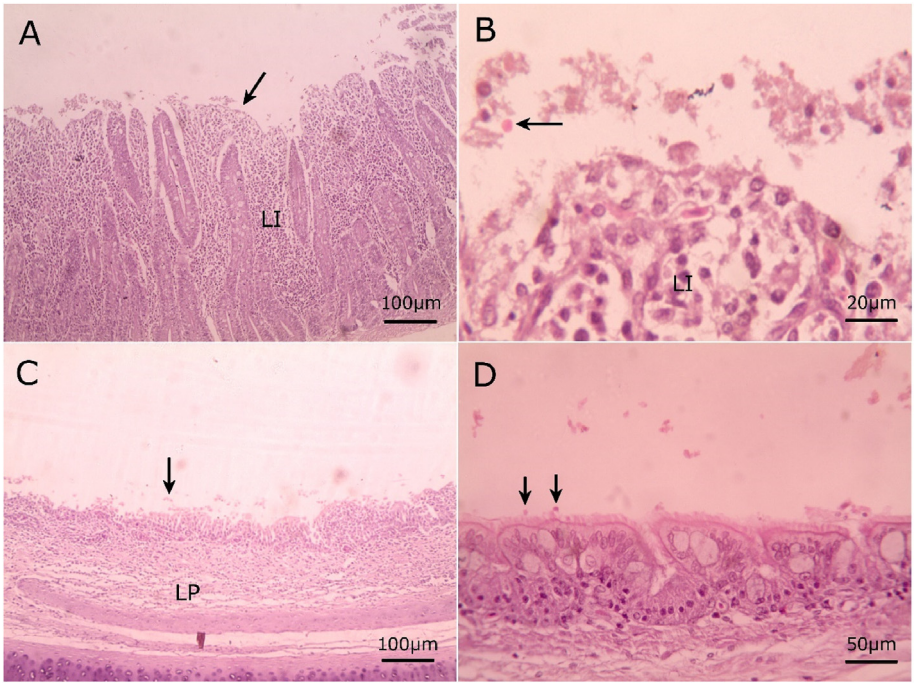

Fig. 3. Histopathological findings in H and E-stained tissue sections from pigeons and ducks infected with Cryptosporidium oocysts. A. Duck intestine, note loss of the intestinal villi (arrow), associated with heavy leukocytic cells infiltration (LI) in the mucosa, captured at X 10. B. Higher magnification of duck intestine, note round to oval Cryptosporidium oocysts (arrows) among desquamated cells in the intestinal lumen, and the leukocytic cells infiltration (LI) in the mucosa, captured at ×100. C. Duck trachea, note deciliation of the mucous epithelium (arrow) and thickened lamina propria (LP), captured at X 10. D. Higher magnification of duck trachea, note Cryptosporidium oocysts (arrows) on the upper surface of the tracheal epithelium, captured at ×100.

Table 4. Earlier reports on Cryptosporidium infections in ducks worldwide.

Country	Sampling year	Number tested	Age range	Source of samples	Tissues or samples examined	Tests used	Number of positives (%)	Genera/species	Reference
Egypt	2014	915	1 w–6 m	NS	Intestinal content, bursal samples	MZN, PCR-sequencing	365 (39.9) in bursae, 333 (36.4) in intestines	[b]*C. meleagridis*, *C. baileyi*	[18]
Egypt/Gharbia	2012–2013	403	Various	Various	Intestinal content	Sugar flotation technique, SMB	56 (13.8)	[a]*C. baileyi*	[30]
Nigeria	NS	11	NS	Various	Dropping	MZN	1 (9.09)	NS	[31]
Egypt/Behera	2001–2003	110	3–12 m	Market	Various tissues	MZN	0	NS	[32]
Australia	1983–1984	6	NS	NS	Colonic, bursal contents	Formol ether sedimentation, MZN	6 (100)	NS	[33]
China	2006–2007	564	Various	Farm	Droppings	Sugar flotation, nested PCR-sequencing	92 (16.3)	[b]*C. baileyi*	[34]
Brazil	NS	60	<1 m	Market	Dropping	Sugar flotation, nested PCR-sequencing	46 (76.6)	[b]*C. baileyi*	[35]
Japan	2018	200	NS	Migratory ducks	Dropping	Nested PCR- sequencing	23 (11.5)	*C. baileyi*, *Cryptosporidium* avian genotype III	[36]
Brazil	NS	315	1 ->4 m	Free range	Dropping	Sugar flotation, nested PCR-sequencing	10 (3.17)	[b]*C. baileyi*	[37]
China	2018–2019	487	NS	Free range	Dropping	Nested PCR-sequencing	36 (7.4)	*C. baileyi*	[38]
Iraq	NS	30	NS	NS	Dropping	ACMV, nested PCR	7 (23.3)	[b]*C. meleagridis*	[39]

Abbreviations: ACMV, aniline-carbol- methyl violet staining; m, month; MZN, modified Ziehl Neelsen staining; NS, not stated; SMB, safranin-methylene blue staining; w, week.
[a] These species were identified based on the oocyst morphometrics.
[b] These species were identified after PCR testing of the oocysts.

Table 5. Earlier reports on Cryptosporidium infections in pigeons worldwide.

Country	Sampling year	Number tested	Age range	Source of samples	Tissues or samples examined	Tests used	Number of positives (%)	Genera/species	Reference
Egypt/Assiut	2018	50	Various	NS	Droppings	KS	10 (20)	NS	[29]
Egypt/Gharbia	2012–2013	322	Various	Various	Intestinal content	Sugar flotation technique, SMB	39 (12.1)	[a]*C.baileyi*	[30]
Iraq	2013	120	NS	Household	Droppings	MZN	48 (40)	[a]*C. baileyi*, *C. meleagridis*, and *C. galli*	[40]
Iraq	2013–2014	30	NS	NS	Droppings	MZN, PCR-Sequencing	8 (26.7)	[b]*C. parvum*, *C. baileyi*	[41]
China	2012–2013	244	NS	Farms	Droppings	Sugar flotation, PCR-sequencing	2 (0.82)	[b]*C. baileyi*, *C. meleagridis*	[42]
Iran	2012–2013	40	NS	NS	Droppings	MZN	1 (2.5)	NS	[43]
Nigeria	NS	8	NS	Various	Droppings	Sugar flotation, MZN	0	NS	[31]
Bangladesh	2016	65	NS	NS	Droppings	Flotation direct wet smear, Giemsa staining	9 (13.84)	[a]*C. baileyi*	[44]
Brazil	2015	100	Various	Farms	Droppings	MGN, nested PCR-sequencing)	4 (4.0) by microscopy and 7 (7.0) by nested PCR	[b]*C. parvum*	[45]
Iraq	2019	100	NS	Wild pigeons	Droppings	MZN, nested PCR-sequencing)	6 (6.0) by microscopy and 11 (11.0) by nested PCR	[b]*C. baileyi*, *C. parvum*	[46]
Nepal	2020	155	NS	Household	Droppings	Direct wet smear, formalin ethyl acetate sedimentation, NACL flotation, MZN	5 (3.9)	NS	[47]

Abbreviations: KS, Kinyoun acid-fast staining; MGN, malachite green negative staining; MZN, modified Ziehl Neelsen staining; NS, not stated; SMB, Safranin-methylene blue staining.
[a] These species were identified based on the oocyst morphometrics.
[b] These species were identified after PCR testing of the oocysts.

governorate [29]. It is worth mentioning that the present study is the first to detect *Cryptosporidium* oocysts in tracheal smears from ducks and bursal smears from pigeons. Reports of cryptosporidiosis in ducks and pigeons are scarce. Tables 4 and 5 summarize the important findings of reports published on duck and pigeon cryptosporidiosis in Egypt and worldwide, respectively. These reports documented diversified prevalences; 0.0–76.6% in ducks and 0.0–40.0% in pigeons. These variations are likely due to differences in the number of tested birds, as well as their ages, breeds, and immune status. The management practices, methods used for detection of *Cryptosporidium* oocysts, and environmental factors might also have a role in these variations.

On the other hand, the low oocyst numbers detected in the MZN-stained slides from ducks and pigeons tested in the present study suggest the dominance of subclinical cryptosporidiosis in ducks and pigeons. This is not confirmatory, since only a few birds were tested, and all were healthy. In addition, the histopathological lesions detected in *Cryptosporidium*-infected tissues tested in the present study do not necessarily indicate that *Cryptosporidium* is responsible for these lesions since no exclusion testing for other pathogens (including bacteria and viruses) was conducted. This highlights the need for large-scale studies recruiting clinically ill (particularly diarrheic) ducks and pigeons from various sources, including farms. Subclinical cryptosporidiosis with mild or no clinical symptoms is common in ducks [48]. Pigeons, in contrast, occasionally suffer from clinical enteric cryptosporidiosis, but mostly in squabs [49,50].

The limited number of published studies makes it difficult to interpret seasonal variations in *Cryptosporidium* infections in ducks and pigeons. *Cryptosporidium* oocysts have thick walls and are highly resistant to harsh environmental conditions. However, temperature extremes (less than −5 °C and over 60 °C) inactivate the oocysts [51]. The oocysts can also survive for months in moist soil or water, but the oocysts can be rapidly inactivated due to desiccation occurring during the hot months [52]. A study by Nagwa et al. [30] on ducks and pigeons from Egypt supports this assumption. The authors found that winter had the highest prevalence. In the present study, the prevalence was higher during hot months than during cold months; nonetheless, this variation was not statistically significant. Likewise, there were no significant variations in *Cryptosporidium* prevalence between different age groups, and there is scarce data in the literature linking the age group of ducks or pigeons to the prevalence of *Cryptosporidium*.

Birds can be infected with various *Cryptosporidium* species and genotypes. Of these species, *C. meleagridis* and *C. baileyi* are the most prevalent [2]. However, a few other species/genotypes can be detected at lower frequencies, including *C. galli*; *C. avium*, and avian genotypes II, III, and IV. Some of these species have specific habitats, for example, *C. galli* develops in the epithelial lining of the proventriculus, but not in the intestine or respiratory tract. In contrast, *C. meleagridis* develops in the intestine and bursa of Fabricius [2]. *C. meleagridis* oocysts are small in size and are closely similar to the smaller oocyst-type (4–5.5 × 4–5 μm) detected in the present study, in the intestinal contents of ducks and pigeons, as well as the bursa of Fabricius of pigeon. On the other hand, the larger oocyst-type (6–7 × 5–6 μm) that was detected in the intestinal contents of ducks and pigeons, as well as in the trachea of ducks, is consistent with *C. baileyi*. This species has a wide range of habitats and has been detected in the intestine, trachea, air sacs, cloaca, bursa of Fabricius, kidneys, and urinary tract of various avian species [2]. It is worth mentioning that accurate identification of various *Cryptosporidium* species cannot rely only on microscopic examination, since oocysts of different species may display identical morphometrics in some cases. In contrast, various PCR sequencing assays can be used for the accurate delimitation of various *Cryptosporidium* species. However, the presence of a few oocysts harvested from subclinical-infected (apparently healthy) hosts can occasionally fail PCR amplification, which could explain the failure to molecularly characterize the oocysts revealed in the present study. Other reasons that can explain this failure include uneven distribution of oocysts in droppings of infected birds, thick oocyst walls, and the presence of PCR inhibitors in the intestinal contents (e.g., bilirubin) [53,54].

5. Conclusion

The present study surveyed *Cryptosporidium* infections in small numbers of ducks and pigeons collected from LBMs in two governorates in the Nile Delta, Egypt. A high prevalence was documented, but was accompanied by subclinical infections. The oocysts detected were roughly described based on their habitat and morphometrics, and two *Cryptosporidium* species were identified: *C. meleagridis* (the most common) and *C. baileyi*. Trials to molecularly confirm the identity of the revealed *Cryptosporidium* oocysts failed, most likely because of the small

number of harvested oocysts. This highlights the need to conduct a large-scale molecular survey on *Cryptosporidium* infection in ducks and pigeons. This survey would be beneficial in illustrating the potential role of these birds in *Cryptosporidium* zoonosis.

Ethics approval

This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (reference number M/73).

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

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Authors' contributions

conceptualization: AE, IA, SA; sample collection: AE; experimental work: AE, BE; data analysis: IA; writing original draft: AE, IA; writing review and editing: AE, IA, BE, SA. All authors have read and approved the final manuscript.

Conflict of interest

There are no conflicts of interest.

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