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Detection of Natural Infection with Cryptosporidiosis in Domestic Poultry from Sulaymaniyah Province/ Iraq

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CRYPTOSPORIDIOSIS is Cryptosporidiosis is a zoonotic protozoan infection caused by Cryptosporidium spp., with a public health concern. It has been reported widely across several countries in both wild and domestic birds. The study was designed to find out the Cryptosporidium parasite in different species of domestic poultry from Sulaymaniyah province northern region of Iraq. The study was carried during the period between June 2022 to February 2023, a total of 215 fresh dropping samples were collected from chicken, turkey, duck, geese and pigeons. All samples were screened for the presence of Cryptosporidium parasite by microscopy using fecal flotation and modified Zeihl- Neelsen staining method for recognition the oocyst stage of the parasite. Intestinal tissue samples were also fixed in 10% buffered formalin and stained with (H&E) for histopathology, to find out the lesions. The overall infection rate of Cryptosporidiosis was 29.30% among the examined poultry species, with the higher prevalence of 38.00% in chicken, followed by pigeon 35.56% then in geese 24.44%, and turkey 22.86%, and a lower prevalence of 22.50% was reported in duck. Histopathology from intestinal tissue revealed sever epithelial damage, ulceration with sloughing of villi, accompanied by infiltration of inflammatory cells with extensive edema. The results of the present study provide the first data on the occurrence of Cryptosporidium infection from domestic poultry in the study area.

Keywords: *Cryptosporidium,* Poultry, Zoonotic, Histopathology, Sulaymaniyah.

Introduction

Cryptosporidiosis is a widely distributed zoonotic infection, caused by protozoan parasites belong to the genus *Cryptosporidium* [1]. Based on molecular investigates and morphological data, nearly 46 species and more than 100 genotypes of Cryptosporidium parasite have been identified from various hosts [2]. Species which belong to the genus Cryptosporidium display slight host specificity, different species have been reported to infect several hosts like mammals, birds, reptiles, and fish [3].

The severity of cryptosporidiosis varies depending on the associated species, some of them only have minor or no illnesses. while others are pathogenic and cause severe symptoms that could be fatal. Besides that, the sternness of condition also depends on host factors, especially immunity [4].

Cryptosporidium parasite has been reported worldwide in nearly thirty different avian species including chicken, turkey, duck, and geese [5]. Until now, birds become infected by five different species of *Cryptosporidium*, including: *C. baileyi, C. meleagridis, C. galli, C. avium*, and *C. proventriculi* [6].

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Cryptosporidium life cycle goes through a single host, the oocysts can withstand a variety of environmental factors and can endure for months in water and soil [7,8]. Transmission of *Cryptosporidium* occurs via fecal-oral route, once the resisting oocysts released in the feces of infected hosts, providing several pathways into the food chain as they contaminate soil and water [9]. Various hosts including domestic and wild animals, livestock, and human are possible reservoirs, and contributing in contamination of environment, food and water by resistance oocysts of different species [10].

The disease in mammals appears as gastrointestinal disorders, while in birds manifest as respiratory and/ or gastrointestinal infections [11]. Since the parasite is an opportunistic pathogen, smaller aged individuals are highly susceptible to infection with probable complications [12].

Cryptosporidium meleagridis is essentially pathogenic species for birds infecting chickens, pigeons, turkeys, parrots and cockatiels [13]. In turkey result in clinical infection leading to decrease in weight gain, diarrhea, intestinal distention, while in chicken infection appear as subclinical intestinal cryptosporidiosis. The species *C. baileyi* can infect domestic fowl, including chickens, turkeys, ducks, and geese in upper respiratory tract, middle ear and ocular conjunctiva. In chickens, cryptosporidiosis is the cause of high mortality as a main reason or in combination with other respiratory tract causative agents such as *Escherichia coli* or infectious bronchitis virus. While oral infection appears as subclinical condition and may lead to reduce in weight gain [14]. *Cryptosporidium galli* causes gastric infections in several species of birds, and results in a subclinical or clinical condition with clinical signs of lethargy, diarrhea, weight loss, and sporadic mortality, as well as chronic proventriculitis can predispose to infection by other pathogens [15].

In pigeons, *Cryptosporidium* has been reported as an etiology of enteritis with the signs of diarrhea and intestinal distention. Other species of *C. proventriculi* can also infect the proventriculus of birds, revealing the associated symptoms of anorexia, weight loss and chronic vomiting [14], infection with *C. avium* and *C. baileyi* can also affect the immune system of birds involving the bursa of fabricius responsible organ for the humoral immune response, which become inflamed with exudate formation [16, 17]. Additionally, cryptosporidiosis considered as a health problems condition among human, species naturally affecting birds responsible for human infection [18].

Processing of poultry under unsanitary circumstances, and poultry droppings when not cleaned up regularly, creating a significant risk for *Cryptosporidium* transmission and human infection from poultry [19]. The oocysts of *Cryptosporidium* species are resistant to common sterilizers and they cannot be destroyed by chlorination of water, besides that relatively small numbers of about ten oocysts can cause clinical infections in healthy individual [20].

Cryptosporidium parvum was detected in bird fecal samples [21], so birds act as a mechanical transporter for *C. parvum* oocysts [22]. As well as the marine birds transport the oocysts of zoonotic species mechanically, that affect mammals such as *C. parvum* and *C. hominis*, and might contribute to the epidemiological chain of human cryptosporidiosis by means of environmental contamination [23]. The associated clinical symptoms of human infections include diarrhea, dehydration, vomiting, and weight loss [11].

Cryptosporidiosis can be diagnosed by applying of various diagnostic methods including microscopic examination of wet mount preparation or stained smears with modified Zeihl- Neelsen stain, immune-logically by detecting of antigen and antibody, histopathological examination of tissue biopsy and detection of parasitic DNA through applying of various molecular methods [13, 24].

Microscopy is the least expensive diagnostic method involved for screening *Cryptosporidium* oocysts [14], Nonetheless, species description is not possible through microscopic investigation. due minor variations between the oocysts of different species [5].

Numerous investigations conducted to clarify the connection between human infection and *Cryptosporidium*, although the documented studies related to cryptosporidiosis in animals are limited, and no study have been done for detection of a *Cryptosporidium* species in domestic poultry in Sulaymaniyah province, the study aimed to detect cryptosporidiosis in different species of domestic poultry as preliminary data.

Material and Methods

Study areas and sampling

The study was conducted from March 2021

to August 2022 to investigate the occurrence of cryptosporidiosis in domestic poultry. The study population comprised 215 dropping samples from poultry including chicken (n = 50), turkey (n = 35), ducks (n = 40), geese (n = 45) and pigeons (n = 45), of various age; poultry were obtained from different districts and from local market of Sulaymaniyah city. Fresh fecal samples, or intestinal content of slaughtered bird were collected in an individual clean container labeled, and transferred to the laboratory at Veterinary Medicine College, University of Sulaimani. All samples were subjected to microscopic examination by flotation technique and modified Ziehl-Neelsen stain for investigation of *Cryptosporidum* species oocysts.

For flotation technique, 1 g of the sample was suspended in 9 ml of saturated sucrose solution, following filtration, the mixed solution was added to the test tube, Then, the tube was filled to create a convex surface by adding drops of solution and putting a coverslip over the tube following 5–10 min, the coverslip was put on a glass slide and examined under 40 X for observing the oocysts [25].

After microscopic examination Modified Ziehl-Neelsen stains were also used for inspection of all collected samples. Fine fecal smears were prepared from each sample, allowed to be dried at room temperature fixed with methanol for 5 min then followed by staining procedure with Carbol-fuchsin and methylene green for detection of Cryptosporidium oocysts after examination under 100 X [26].

Tissue samples were collected from positively slaughtered birds including different parts of intestine and bursa of fabricious, fixed in 10% buffered formalin, followed by histological preparation and stained with hematoxylin and eosin (H&E), to be observed microscopically at 40X and 100X by light microscopy [16].

Results

The study results revealed the presence of *Cryptosporidium* oocysts in 29.30% (63 of the 215) poultry samples higher infection rate was reported in chicken 38%, and a lower prevalence of 20.50% was found in duck with no significant differences (Table 1).

The photomicrograph in (Figure 1) represented *Cryptosporidium* oocysts, which were colorless, spheroid to oval in shape, and stained bright red by modified acid-fast staining procedure.

Histopathological lesions from intestinal tissue of infected chicken revealed sever epithelial damage, ulceration with sloughing of villi, accompanied by infiltration of inflammatory cells with extensive edema (Figure 2), and the intestinal section in (Figure 3) represented the parasitic stages at border of intestinal mucosa.

Discussion

Cryptosporidiosis is a zoonotic protozoan infection among wide range of hosts including birds which is considered as a problematic state to poultry industry worldwide. Due to the lack of the study regarding the importance of cryptosporidiosis in poultry as reservoir, the current study detects *Cryptosporidium* parasite through microscopy as preliminarily effort, with the overall infection rate of 29.30%, such high prevalence was disagreed with the reported lower infection rate of 2.9% by Gong et al. [27].

The current data revealed a higher prevalence of cryptosporidiosis among chickens 38% than in pigeons 35.56% contrary to the current findings [19], was reported higher prevalence of 50% from pigeons than in chicken 40%. The finding of AL-Zubaidi et al.[28] in broiler 35% somewhat harmony with current data 38%. In different to the study data lower prevalence of 1.3% was reported by [27], although 12.6% and 13.2% were reported by some investigators [29] and [30] from free range and broiler chicken respectively. Contrary to the current prevalence of cryptosporidiosis in pigeon 35.56% lower prevalence of 20% and 21% were reported through applying of modified acid fast, and PCR by some authors [31,32].

Higher prevalence of cryptosporidiosis 37% was reported from turkey by applying of Acid-fast microscopy in compare to chicken 30% [31] such findings were disagreed with the study data 22.86% from turkey and 38% from chickens. Similarly low prevalence of 9.3% was also reported from turkey by molecular assay [33].

Regarding other inspected poultry species, current data represent the occurrence of cryptosporidiosis in geese and ducks with a prevalence rate of 24.44% and 22.5% respectively. Contrary to the current data [27] reported a low prevalence of 7.3% and 1.4% in both ducks and geese respectively. Besides that, higher prevalence of 39.9% and 50% was reported in ducks by some researchers [34, 35]. However lower prevalence of 11% and 5.38% was found by others [36, 37] from Spain and Brazil respectively. Furthermore,

in geese, a lower prevalence of cryptosporidiosis than current finding 4.1% was reported by Zhao et al. [38], and a high prevalence of 44.7% was reported by Binkley et al.[39].

It was found that free-living birds could be a possible disseminator for *Cryptosporidium* oocysts making environmental contamination [40]. Moreover, several factors could be attributed to the variation in the prevalence rate of cryptosporidiosis amongst different poultry breeds, environmental influences, management practices as well as the immune status of the birds [41] As well as various poultry breeds have different life spans and genetic variation which may affect dissimilarity in prevalence rates [19].

In areas where different avian species are raised together for production, there is a possibility of recurrent infection of birds via contamination of the environment. Nevertheless, the opportunity of potential contamination by wild birds should be taken into account, especially those of the Columbiforme order [37].

Although the current study did not differentiate between different species due to morphological similarity in oocysts of *Cryptosporidium* spp. It was found by Liao et al.[13] that *Cryptosporidium baileyi* was highly prevalent in chicken and detected in 69%, *C. meleagridis* was the second most prevalence species and reported in 31%, infection with *C. baileyi* accompanying with lower weight gain in broilers and reduced egg production in laying chickens, even in the absence of obvious clinical signs. While in pigeon the most prevalence *Cryptosporidium* spp. was *C. meleagridis* with 61.90%, followed by *C. baileyi* with 33.33%, and *C. hominis* was reported in 4.76% [32].

Similarly, the reported species by Kabir et al.[19] from high prevalence to low were *C. baileyi, C. meleagridis*, and *C. parvum* from chicken and pigeon. Additionally, *C. baileyi, C. galli* and *C. meleagridis* were identified in chickens [27]. In other study, *C. parvum* was the predominant species and was identified in broilers, laying chickens and turkeys [33]. According to the finding of Zhao et al. [38] the main reported species in geese was *C. baileyi*, also Nakamura and Meireles [14] was identified *C. baileyi* from ducks and geese, while other studies have specified the role of geese as possible vectors for *C. parvum* [42].

Concerning the potential zoonotic aspects of the reported *Cryptosporidium* species, genetic analyses showed the similarity between sub types of *C. meleagridis* reported from poultry including chicken, pigeon and duck with that of humans which suggest that chickens may contribute to the transmission of *C. meleagridis* to humans [1]. Furthermore, there was similarity between other subtypes of *C. meleagridis* reported from turkey and human [43].

Cryptosporidium meleagridis is considered the third most common species causing infection in humans after *C. hominis* and *C. parvum* [44], in some areas *C. meleagridis* is the source for approximately 10% of human cases [18].

Identification and description of *Cryptosporidium* species, genotypes and subtypes by applying of molecular approaches is a significant step for outlining the contamination sources and evaluation its public health status [30].

The morphology of intestinal tissue was changed due to parasitic infection including *Cryptosporidium* spp. Histopathology of intestinal tissue from infected chicken revealed sever epithelial damage, ulceration with sloughing of villi, also infiltration of inflammatory cells with extensive edema were observed, similar findings were also reported by Al-Zubaidi et al. [28].

Conclusion

Although the study could not detect the *Cryptosporidium* species. The current findings signify the establishment of natural infections with *Cryptosporidium* parasites in different types of domestic poultry, which may potentially serve as its potential reservoir for the parasite due to the zoonotic potential of *Cryptosporidium* in humans and other domestic animals. The impact of natural infection by *Cryptosporidium* spp. should be determined by applying advanced and accurate diagnostic techniques, as well as human precautions should be directed toward contacts with different species of domestic poultries especially in local markets of live poultry.

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Confect of interest

The author declares that there was no conflict of interest.

TABLE 1. Infection rates of cryptosporidiosis according to poultry spp.

Poultry spp.	Number of examined poultry	Number of infected poultry	Percentage of infection	X2 (p-value)
Chicken	50	19	38**%**	
Turkey	35	8	22.86%	4.783
Geese	45	11	24.44**%**	[0.31]
Duck	40	9	22.5**%**	
Pigeon	45	16	35.56**%**	
Total	215	63	29.3%	

*No significant differences $p < 0.05$.

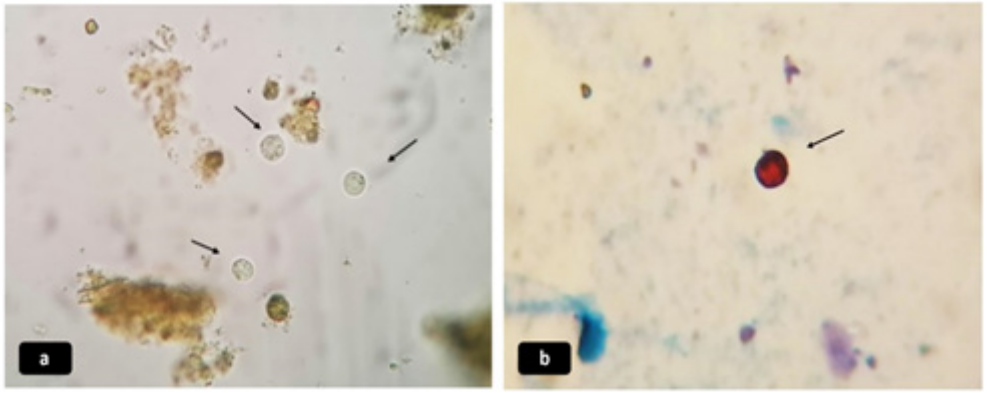

Fig. 1. *Cryptosporidium* spp. oocysts recovered from intestinal contents of infected chicken

fecal floatation methods, 40 X, b- Modified Ziehl-Neelsen stain, 100 X.

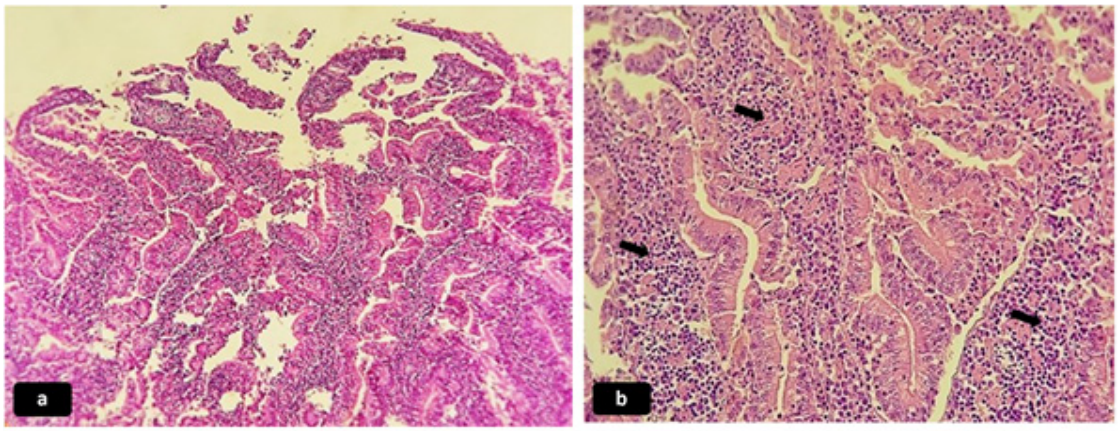

Fig. 2. Histopathological lesions from intestinal tissue of infected chicken with cryptosporidiosis (H&E stain), a. Ulceration with sloughing of villi, 100 X, b. Infiltration of inflammatory cells with extensive edema, 100 X.

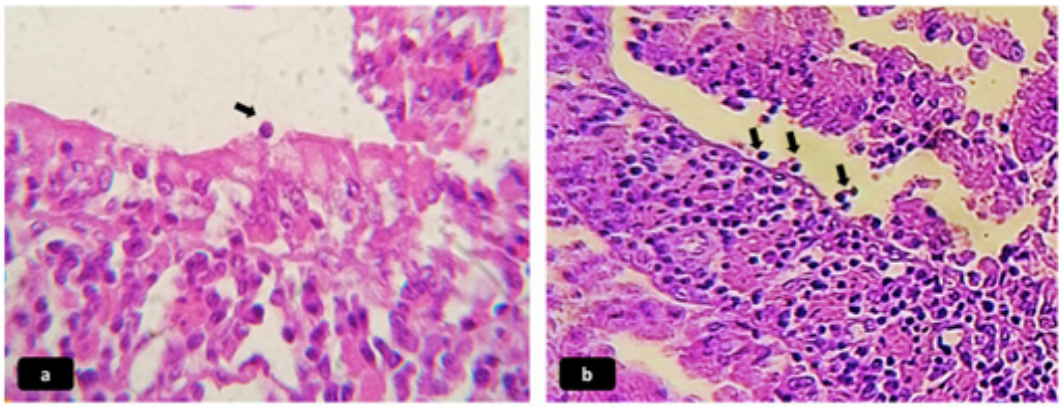

Fig. 3. Intestinal tissue from infected chicken with cryptosporidiosis (H&E stain), the arrows in a and b refer to *Cryptosporidium* stages 100X.

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الكشف للاصابة الطبيعية بداء الابواغ الخبيئة في الدواجن الداجنة في محافظة السليمانية/ العراق

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داء الأبواغ الخبيئة هومرض حيواني المنشأ مسببه طفيلي الأبواغ الخبيئة ، ذو تاثيربالصحة العامة. تم الكشف عنه على نطاق واسع في العديد من البلدان في كل من الطيور البرية والداجنة. صممت الدراسة للكشف عن طفيلي الأبواغ الخبيئة في أنواع مختلفة من الدواجن الداجنة من محافظة السليمانية شمال العراق.اجريت الدراسة في الفترة ما بين حزيران 2022 وشباط 2023 ، تم جمع215 عينة براز من الدجاج والديك الرومي والبط والإوز والحمام. تم فحص جميع العينات للكشف عن طفيلي الأبواغ الخبيئة بالفحص المجهري ، باستخدام التطويف وطريقة التصبيغ بصبغة الزيل-نلسن المحورة للتعرف على مرحلة اكياس بيض الطفيلي، كما وتم تثبيت عينات النسيج المعوي في 10٪ من الفورمالين والمصبوغة بـ (H&E) للدراسة النسيجية المرضية. بلغت نسبة الاصابة الكلية بالطفيلي 29.30٪ لكل أنواع الدواجن المفحوصة ، وبلغت أعلى نسبة اصابة 38٪ في الدجاج ، يليها الحمام 35.56٪ ثم الأوز 24.44٪ ،و الديك الرومى 22.86٪ ، و بلغت أقل نسبة اصابة 22.50 ٪ في البط. أظهرت نتائج الدراسة النسجية للنسيج المعوي حدوث الضرر ظهاري شديد ، تقرح مع انسلاخ الزغابات ، مصحوبًا بارتشاح الخلايا الالتهابية مع وذمة شديدة. تعد هذه الدراسة الاولى للكشف عن داء الابواغ الخبيئة في الطيور الداجنة في محافظة السليمانية.

الكلمات الدالة: الأبواغ الخبيئة ، دواجن ، حيواني المنشأ ، أمراض الأنسجة، السليمانية.