



Detection of *Cryptosporidium* spp in Bobwhite Quails (*Coturnix coturnix*) by Traditional Methods in Baghdad City, Iraq

Khitam J Yahya ^{ID}, Mohammed Thabit Al-Zubaidi* ^{ID}

¹Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad Iraq

A B S T R A C T

Cryptosporidiosis is one of the most important zoonosis diseases distributed worldwide causes severe diarrhea in animals and is lethal in quails. This study detected *Cryptosporidium* spp. in quails in Baghdad province Iraq by flotation and staining methods and compared these results with PCR results from the previous study. This research was carried out from January 2022 to September 2022. Out of 180 quails (*Coturnix coturnix*) in total were investigated. The overall infection rate of *Cryptosporidium* spp. in quails was 23.9% (43/180). Young quail had a greater infection rate of 44.2% (19/43) while adult quails had a lower infection rate of 17.2% (24/137). Finding from the research revealed that there were no significant differences between males and females and highly significant differences among the months of study with the highest infection rate being 45% (9/20) in April, and the lowest infection rate being 10% in June and July. This disease was presented in birds and birds act as a mechanical carrier of *Cryptosporidium* spp. between a conventional acid-fast staining procedure and PCR test, PCR can not only detect *Cryptosporidium* but is also able to differentiate between what appear to be host-adapted genotypes of the parasite with high sensitivity and specificity.

Keywords: *Cryptosporidium*, quail, mZN stain, morphologically, Iraq

*Correspondence:

m.th_s@covm.uobaghdad.edu.iq

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INTRODUCTION

Cryptosporidiosis is a parasitic disease caused by *Cryptosporidium* spp., an obligate intracellular (extracytoplasmic) Apicomplexan parasite, affecting the epithelial cells of the digestive tract in both humans and animals (1). Tyzzer (1929) was the first to report *Cryptosporidium* from chicken caeca, marking a significant milestone in the understanding of this pathogen's impact on a range of hosts. Further discoveries included *C. parvum* in mouse intestines and *C. muris* in mouse stomach glands (2). The species of *Cryptosporidium* that affect birds, including poultry, are *C. meleagridis* and *C. baileyi*, which demonstrate the parasite's diversity with distinct oocyst sizes and host-specific impacts (3).

Transmission of *Cryptosporidium* spp. occurs through inhalation or ingestion of sporulated oocysts from contaminated sources, leading to the infection cycle where sporozoites excyst in the intestine and invade the epithelial cells (4, 5). This cycle can also manifest in respiratory diseases when the parasite colonizes the trachea, bronchi, air sacs, and lungs through inhalation of oocysts (6). The role of domestic and wild birds in the spread of *Cryptosporidium* spp. underscores the importance of understanding the epidemiology of this parasite. Birds act as mechanical vectors, facilitating the environmental dissemination of pathogens, which poses risks to both humans and other animals (7).

Several bird species have been discovered to be infected with *Cryptosporidium* spp., with prevalence rates varying

from 0.41 to 100% depending on the host and methods of detection (1). The low number of oocysts excreted by the infection host, the low dosage needed for infection, the lack of or reduced host specificity, the small size of oocysts, their resistance to environmental pressures and water treatments, their slow sedimentation, and the lack of an effective treatment for cryptosporidiosis are some oocyst-related factors that are thought to be significant in the epidemiology of *Cryptosporidium* spp. Wild birds, particularly vultures living in close proximity to water sources, can become infected with *Cryptosporidium* through contaminated water (8). Water contamination can lead to *Cryptosporidium* infection and transmission in wild birds, especially volant birds, living in habitats close to water resources.

Some animals may play an important role in the epidemiology of Cryptosporidiosis such as wild birds, mice, voles, rats, and darkling beetles, while rodents seem to be vulnerable to *C. meleagridis* (9). Examination of fecal smears with acid-fast stains such as Ziehl-Neelsen which are commonly used by diagnostic facilities. Conventional microscopy, however, is time-consuming and tedious and requires experienced microscopists to accurately identify the oocysts (5, 8). In addition, the detection limits of conventional diagnostic techniques have been reported to be as low as 50,000 to 500,000 oocysts per gram of feces (10). The quail offers more benefits than other birds and it has started to occupy an important place in the poultry industry. Factors, such as high production rates, reduce feed consumption, short generation interval, and more resistance than other poultry to diseases. Infection with cryptosporidiosis cause reduced weight gain, reduces egg production, and may be fatal in quail (11). The Cloaca examination is used to determine gender based on the physical traits of quail by using vent sexing and feather sexing. Quail sexual maturity lasts about 4-6 weeks. The growth in live weight in female birds lasts up until the sixth week of age, when they typically begin to lay eggs. Male birds are distinguished by having a dorsal cloaca. Male birds can be identified by their distinctive behavior because of the hypertrophy of this gland, which is found in the dorsal wall of the cloaca. Male quails can be seen producing cloacal froth during this time (12). Quail less than 6-7 weeks of age sex determined by the feather on the wing the females have those 2 rows of wing tip feathers (and they are longer) and the males have just one, and they are shorter (1).

In Iraq, there is a significant body of research focused on the detection of *Cryptosporidium* spp. in various types of animals including birds (3, 28, 44, 45, 49, 56). However, information specifically related to cryptosporidiosis in quails is scarce. A study conducted by Jasim and Marhoon (7) to study the prevalence using molecular analysis of *Cryptosporidium* spp. in Al-Qadisiya province, Iraq revealed that quails had the highest infection percentage (46/60,

76.7%) among the studied birds. These quails were found to be infected with three *Cryptosporidium* species: *C. parvum*, *C. meleagridis*, and *C. baileyi*. This finding underscores the importance of understanding cryptosporidiosis in quails, as they may serve as potential biological transporters of *Cryptosporidium* spp. that can infect both humans and other animals. Therefore, this study aimed to detect *Cryptosporidium* spp. in quails in Baghdad province, Iraq using traditional method and compared the result with those obtained using molecular method from our previous study.

MATERIALS AND METHODS

Ethical Approval

The procedures of the study were reviewed and approved by the Local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad. Formal approval was granted under reference number 2569/P.G., dated 12 December 2022.

Sample Collection

Dropping samples (2-3 g) from 180 quails were collected from four local markets in different places in Baghdad city from January 2022 to the end of September 2022. Direct samples taken from dropping by birds were gathered with an emphasis on preventative measures such as the use of disposable gloves. The samples were transported in a cool box to the Parasitology Laboratory, College of Veterinary Medicine, University of Baghdad where they were processed and examined. The age of birds was determined according to (14, 15), quails less than 45 days do not reach maturity were considered young quail, while quails with more than 45 days that reach maturity were considered adults. Sex was determined according to (16, 17) by cloacal examination which is present in males and absent in females.

Sample Preparation

According to the sample size, the appropriate quantity of distilled water was added to the mixture, mixed thoroughly, and filtered through four layers of gauze. The suspension was then gathered in test tubes, Direct Wet Smear was done by putting a drop into a glass slide, which was then covered with a coverslip and viewed while submerged in a 100× lens (18). The flotation method was done according to (19) by putting 10 mL of suspension and centrifuging for five minutes at 1500 rpm. Following the removal of the supernatant, 9 mL of the sheathers sugar solution (Jor Vet product support) was added, which was then spun for five minutes at 1500 rpm. The tube bottoms were used to store the pellets. After that, 9 mL of Sheathers sugar solution was added to the test tube, and it was stirred with a wooden stick. Oocysts were detected on the top of the tube after 5 min of spinning at 1500 rpm. For staining smear was made and stained using Modified Ziehl-Neelsen

stains (Sigma Aldrich) to study *Cryptosporidium* parasite (20). The diameter of the parasite was measured using an ocular micrometer (21).

Statistical Analysis

The impact of diverse factors on the study's parameters was assessed using the Statistical Analysis System (SAS) software, version 2018. To evaluate differences in proportions, the Chi-square (χ^2) test was applied, with significance levels set at P-values of 0.05 and 0.01 for determining statistical relevance (22). Any PCR positive result for *Cryptosporidium* spp. considered a true positive; no PCR result consider a false positive; the false negative was defined as *Cryptosporidium* spp. being negative in one test which was positive in the other (23).

RESULTS AND DISCUSSION

Morphological Characterization

The morphological characteristics of *Cryptosporidium* spp. oocysts were observed using a flotation method in Sheather's sugar solution, revealing spherical to oval shapes encased within thick membranes (Figure 1A). Upon examination under the microscope, oocysts that were stained with the Modified Ziehl-Neelsen technique exhibited a distinct red color against a blue background, measuring $5.5 \times 4.6 \mu\text{m}$ in diameter (Figure 1B). These findings align with the morphological descriptions reported in previous research (24, 25). Furthermore, the application of the PCR technique, as documented in a preceding study (26), confirmed the identity of the detected parasite as *C. meleagridis*.

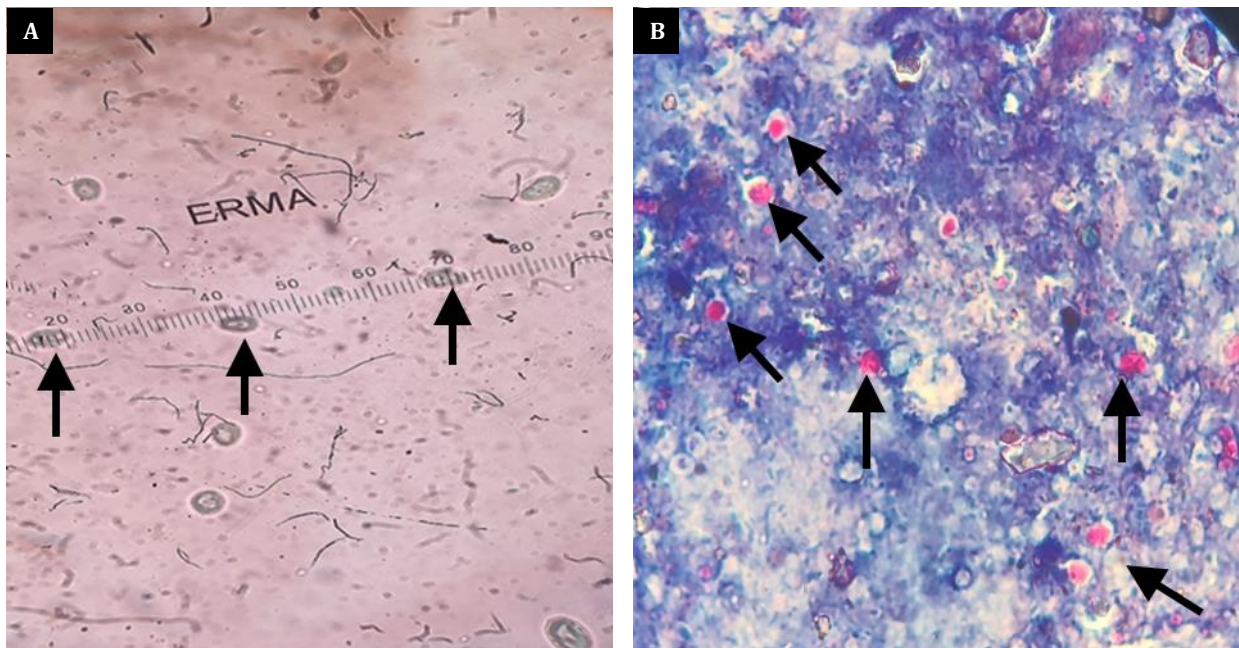


Figure 1. *Cryptosporidium* spp. oocysts under 100× lens by using (A) flotation method and (B) Modified Ziehl-Neelson Stain (black arrows) pink spherical body against purple background

Total Infection Rate

A total of 180 dropping samples were examined by flotation and modified Ziehl-Neelsen staining, giving an infection rate of 23.9% (43/180) in Baghdad city. This result is higher than previous studies conducted in Iraq by (27), who recorded an infection rate of 15.2% (19/125) in domestic pigeons and 8.8% (11/125) in wild pigeons, in Babylon (28) who found an infection rate of 14% (14/100) of *Cryptosporidium* spp. in pigeons, and in Diyala (29) who found an infection rate of 11% (22/200) in ostriches. The infection rate in other countries, such as Egypt (11), Iran (30), Nigeria (31), and Bangladesh (32), was also lower than this result, with infection rates of 19%, 10.5%, 11.9%,

and 19.8%, respectively. However, the infection rate was similar to the result obtained by (33) in Azerbaijan, which found an infection rate of 27.16% in birds infected with *C. meleagridis* and *C. baileyi*. This result disagrees with (34), who found an infection rate of 40% in Baghdad pigeons, and with (35), who found an infection rate of 63% among three different species of birds in pet shops in Baghdad. Additionally, (7) and (36) found infection rates of 58.1% and 51.45%, respectively. (37) found an infection rate of 55% in chickens and 41% in turkeys and concluded that the climate of the central region of Algeria is favorable for the survival of the parasite (heat and humidity).

The application of various detection techniques (histology, serology, and microscopy) management

practices, farm control measures seasonal variations in this study locations and so on, high immune system resistance of quails to the disease, the timing of sample collection, stress factor, feeding style, subpar hygiene, farm management practices and may be related to increased stocking densities and intensive husbandry management techniques this may all contribute to the differences in prevalence rates (31). The amount of resistance and the start of most diseases are unquestionably influenced by breeding variables, such as an appropriate location, a healthy overall environment, and balanced food. Farm bird breeding programs seek to increase output by hastening growth and minimizing feed use. Fast-growing (heavy strain) boilers are more prone to illnesses because of their weakened immune systems; this results in skeletal difficulties as well as the advent of metabolic disease (38).

Infection Percentage According to Age

This result showed a significant difference between two different ages of birds that were infected with *Cryptosporidium* spp. young birds reported the highest infection rate 44.2% (19/43), and adult birds 17.2% (24/137) with a significant difference ($P < 0.01$).

According to earlier research conducted in Iraq by (34), the infection rate was higher in young birds (31.25%) and lower in mature birds (10%). Additionally, this study agreed with (11), who discovered that young quails had 10% infection rate and it was more than the adult. Infection rates were 12.26% in 9-month-old chicks and 9.57% in adult chicks according to (29). According to research from Algeria (37), the prevalence rate of infection chicken increases significantly up to the age of 40 days before declining till the age of 64 days. Young pigeon infection rates in Egypt were 40% and adult pigeon infection rates were 20% (39).

Young animals appear to have a higher prevalence of *Cryptosporidium* because their immune systems are still developing at this stage, making them more vulnerable to illness than older animals, who most likely have antibody levels sufficient to fight the infection at this stage. Additionally, a higher percentage of the birds in the hatchery had omphalitis, which may indicate unsanitary circumstances.

Infection Percentage According to Sex

This result showed no significant differences between both sexes, the infection rate was recorded highest in females at 26.43 (23/87), while the lowest percentage of 20.5% (20/93) was in males.

The result of this study is consistent with those of (28, 29, 36, 40, 41), who asserted that both sexes are vulnerable to infection with *Cryptosporidium* oocysts. In comparison to male birds, female birds had a higher prevalence of

Cryptosporidium oocysts, which may be explained by female birds' heightened vulnerability to infection due to lower immunity during a particular stage of the reproductive cycle (31). This was also in agreement with (42) findings in a study conducted in North China, though. According to the prevalence, there is an identical probability of infection during feeding and epidemic (43).

Infection Percentage According to Month

The infection rate of Cryptosporidiosis increased during a certain month of the year and infection was reported at a higher rate in February March and April (34.6%, 40%, and 45%), respectively. However, the infection rate dropped in the other months.

These findings concur with those of (35), who discovered that the infection rate peaked in April (15.6%) and peaked at 2.6% in June. Findings of (7) revealed a substantial variation between the study months in terms of infection ratios, with the highest ratio occurring in spring, followed by autumn, winter, and summer, with infection ratios of 88.4%, 64.1%, 56.1%, and 32.97%, respectively. Similarly in terms of months evaluation as reported by (44), who concluded that the rate of infection was highest in April 26% (23/50) and lowest in June 20% (10/50). In agreement with (36), who discovered that there were significant differences in infection rates over the study months and that March had the highest prevalence of infection at 11.9%, while June and July had the lowest infection rates at 5.23% and 5.23%, respectively. (45) summarized that the higher infection rate was 30% in March and the lowest infection rate was 11.42% in May. The infection rate in March 2020 by (29) was 50%, and the lowest in Summer (June, July, and August) was 0%. In Egypt, (11) discovered that the rate of total parasite infection in the quails he tested was higher in the cold season (57%) than in the warm season (53%). This high percentage was observed when both mixed and naturally infected quails were tested. In Algeria (37), the infection rate was higher in winter (54.17%), whereas it was lower in Autumn, Spring, and Summer (48.39%, 48.35%, and 47.08%). And demonstrated that there are no discernible seasonal fluctuations.

Oocysts of *Cryptosporidium* spp. stay viable in 28-30 °C for six months; higher temperatures cause a rapid loss of viability; freezing kills oocysts; desiccation kills oocysts. After 2 h of desiccation, just 3% of oocysts were discovered to be alive (4, 46).

The finding of the current investigation points to birds as potential mechanical carriers of *Cryptosporidium* spp. oocysts and the diseases were a wild spread among quails which led to economic loss, and it is a danger of infection to people of all ages and genders. Hence it has an effect on public health.

Table 1. Prevalence and statistical analysis of *Cryptosporidium* spp. infection by age, sex, and monthly distribution in quails

Category	Subcategory	Sample Examined		%	95% Confidence Interval	χ^2	P-value
		Number	Number Positive				
Age	Young	43	19	44.2	29.3 - 59.0	11.307	0.004
	Adult	137	24	17.2	10.8 - 23.5		
Sex	Male	93	20	20.5	12.3 - 28.7	0.209	0.647
	Female	87	23	26.4	17.2 - 35.7		
Month	January	14	1	7.14	1.27 - 31.5	16.855	0.032
	February	26	9	34.6	19.4 - 53.8		
	March	20	8	40.0	21.9 - 61.3		
	April	20	9	45.0	25.8 - 65.8		
	May	20	5	25.0	11.2 - 46.9		
	June	20	2	10.0	2.79 - 30.1		
	July	20	2	10.0	2.79 - 30.1		
	August	20	4	20.0	8.07 - 41.6		
	September	20	3	15.0	5.24 - 36.0		
	Total	180	43	23.9	17.7 - 30.1		

Table 2. *Cryptosporidium* spp. infection percentage by microscopic examination and PCR in quails

Method	Number of samples examined	Number of positives detected	Sensitivity (%)	Specificity (%)
PCR	100	37	75.67%	96.82
Microscopy	100	30	93.33%	87.14

Sensitivity and Specificity

Microscopic examination of quail feces indicated an infection rate of 30% for *Cryptosporidium* spp. while the infection rate in the previous study was 37% with molecular identification (Table 2). No statistically significant difference was between the two infection rates. This comparison demonstrated that PCR improves the detection of *Cryptosporidium* spp. in quails diagnosis. This result was in agreement with (4V) who found the infection rate was 9.4% by microscopic examination and 11.6% by PCR, also (48) obtained a lower infection rate recorded by microscopic examination (3.86%) as compared to molecular technique (3.96%). The same result was obtained by (49) with 6% infection rate by microscopic examination and 11% by PCR, in disagreement with (50) who found the infection rate was 15.83 % by microscopic examination and 28% by PCR.

These results indicate that the PCR method has a higher specificity (96.82%) than recorded in the microscopic examination (87.14%). However, the PCR method had lower sensitivity (75.67%) compared to the microscopic examination (93.33%), similar study was obtained by (51) who recommended that PCR was very sensitive and specific techniques for the detection of *Cryptosporidium* spp. genes. This result was different from the other studies on the comparison of cryptosporidiosis diagnostic tests conducted in the United Kingdom (52) and Ethiopia (53) which showed the lower sensitivity of microscopy procedures used to diagnose *Cryptosporidium* spp.

The laboratory diagnosis of *Cryptosporidium* typically relies on microscopic analysis by mZN stain, which is considered a suitable diagnosis technique, especially in low-income countries (54).

This study demonstrated a wide range of oocyst power detection using common parasitological techniques, demonstrating that mZN staining can be utilized as a useful diagnostic tool in clinical laboratories for *Cryptosporidium* identification. The simultaneous detection of the parasite will cut down on costs and time.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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التحري عن طفيلي الابواغ الخبيثة في طائر السمان باستخدام الطرق التقليدية

ختام جاسم يحيى، محمد ثابت صالح الزبيدي

فرع الطفيليات، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

داء الابواغ الخبيثة هو واحد من أهم الامراض الحيوانية المصدر الواسعة الانتشار في جميع أنحاء العالم والتي تسبب الاسهال الشديد للحيوانات ويُعدّ مميتاً لطائر السمان. خلال الدراسة الحالية تم التحري عن طفيلي الابواغ الخبيثة في طائر السمان بمحافظة بغداد العراق باستخدام طريقة التعويم و باستخدام طريقة التصبيغ، أجريت الدراسة الحالية للفترة من كانون الثاني ٢٠٢٢ لغاية ايلول ٢٠٢٢. تم فحص ١٨٠ كشفت طرق الفحص التقليدية عن معدل الإصابة الكلية ٢٣,٨٨٪ (٤٣٨٨٠) وكانت نسبة الإصابة في طيور السمان الاصغر عمرا أعلى من نسبة الإصابة في طيور السمان البالغة حيث بلغت ٤٤,١٨٪ (١٩٤٣) و ١٧,٥١٪ (٢٤٣٧) على التوالي. نتيجة البحث بينت لا توجد فروق ذات دلالة احصائية بين الذكور والاناث مع وجود فروق ذات دلالة احصائية عالية بين شهر الدراسة حيث بلغت اعلى نسبة اصابة ٤٥٪ (٢٠/٩) في شهر نيسان واقل نسبة اصابة كان ١٠٪ (٢٠/٢) كان في شهري حزيران وتموز ي بعد المرض موجودا في الطيور والطيور تُعدّ ناقلاً ميكانيكياً للمرض.

الكلمات المفاحية: كربتوسبورديم، السمان، الطرق التقليدية، شكليا، العراق