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Detection and Prevalence of *Cryptosporidium* Species in Broiler Chickens and Turkeys in Tikrit Province, Iraq

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Abstract

Cryptosporidium is a parasitic organism that invades the cells of various animals, such as mammals, birds, and humans. Sample collection: 175 fecal samples were collected randomly from poultry, consisting of 127 broilers and 48 turkeys. The Ziehl-Neelsen staining method was used to directly examine all faecal samples for the existence of primary oocysts. Furthermore, the genetic material DNA was extracted from the fecal samples as were found to indicate a positive result. This study showed 2 (1.14%) were positive for Cryptosporidium spp. Through microscopic examination, infection of poultry with the fungus Cryptosporidium spp was diagnosed. Composed of 127 broilers (0.78%) and 48 turkeys (2.08%), 18s rRNA gene sequence analysis indicated the presence of two Cryptosporidium spp: C. meleagridis and C baileyi in turkeys and broiler, respectively. According to sequence analysis, it has been deposited in GenBnk with accession numbers OP868728.1 from Cryptosporidium baileyi and OP868729.1 from Cryptosporidium meleagridis.

Key words: Cryptosporidium spp., broiler chicken, turkey, sequence, Iraq.

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Introduction

Cryptosporidium is a parasitic protozoan that can infect several animal species, such as mammals, reptiles, birds, and fish (Seixas et al., 2019: Lin et al., 2022). Transmission of infection can occur by the consumption of food and water contaminated with infectious agent oocysts. The parasite can be transmitted from animals to human (zoonotic transmission) as well as from humans to humans (Gerace *et al*., 2019; Ryan *et al*., 2018).

Cryptosporidium is a highly prevalent and significant parasite in poultry. More than 30 bird species can be infected by the parasite, and the most frequent Cryptosporidium species in birds are *C. baileyi*, *C. meleagridis*, and *C. galli* (Lin *et al*., 2022; Dong *et al*., 2021; Jian *et al*., 2021), however,

Cryptosporidium spp. detected in wide range of birds species, most prevalent in chickens, turkeys, ducks, quail, pheasants, peacocks, geese, captive birds, and wild birds (Kabir *et al*., 2020). Among *Cryptosporidium* species isolated from birds, *C. meleagridis* can infect humans and cause gastrointestinal obstruction in Immunosuppressed children and adults (Wang *et al*., 2014), a species of the genus *Cryptosporidium* common in poultry, causes respiratory and intestinal infections (Ramirez *et al*., 2004). Nevertheless, additional animals, such as rabbits and poultry, serve as a significant reservoir for newly appearing zoonotic species, such as C. cuniculus and C. meleagridis, respectively. (Robertson *et al*., 2020).

The main clinical signs of cryptosporidiosis are foul-smelling yellow or green diarrhea containing mucus, while respiratory cryptosporidiosis includes sneezing, mucous respiratory secretions, and difficulty breathing. In general, the diagnosis of the parasite in birds depends on microscopic, immunological, histological and molecular methods (ole, 2008; Al-Ghezey and AL-Zubaidi, 2020). Identification of *Cryptosporidium* species is important to treat and prevent the spread of *Cryptosporidium*. disease. But because they have similar morphological traits, it is challenging to distinguish between species based solely on shape (Ryan *et al*., 2014; Lu *et al*., 2022). parasite causing infection in poultry Techniques development Molecular analysis is important for diagnosing species that are morphologically indistinguishable and also plays an important role in determining the relationship between parasite species, hosts and transmission partner.

Materials and methods

Sample collection

overall 175 samples of fresh poultry feces were randomly obtained, including 127 broilers and 48 turkeys. The samples were collected from different regions, throughout the period of June to October 2022. The samples were gathered in a direct manner and promptly transferred into the laboratory at Tikrit University. The Ziehl-Neelsen staining method was used to directly check all stool samples for the presence of primary oocysts. Positive samples were preserved at a temperature of -20°C for future molecular analysis..

Molecular method: Genetic materials (DNA) was extracted from positive stool samples for Cryptosporidium spp.by using the company (Bioneer Korea), examined with a Nano Drop spectrophotometer, stored at -20 °C, and PCR amplified approximately 435 bp of the 18s rRNA gene using specific primers forward 5'-AAGCTCGTAGTTGGATTTCTG-3' and reverse 5'-TAAGGTGCTGAAGGAGTAAGG-3' to detect. A 20 µl volume was used for the PCR reaction. Each reaction contains 5 DNA templates and 0.5 µl of each primer. The double-stranded DNA was denatured by a single session at 95 degrees Celsius for four minutes, which was then followed by:

- Denaturation (thirty-five cycles of 30 seconds at 95°C).
- Annealing The temperature is 54 degrees Celsius for a duration of 30 seconds.
- Extension (The temperature should be set to 72°C and maintained for a duration of 30 seconds).
- Finally, extension at 72 °C for five minutes. In order to separate and visualise the PCR products, 1.5% agarose gel electrophoresis was utilised.

Sequencing: BLAST was used to match SSu-rRNA sequences to NCBI nucleotide database sequences.

Result

Out of the 175 fecal samples, 2 (1.14%) tested positive for Cryptosporidium species. Using the microscopic examination method (Figure 1), poultry were infected with *Cryptosporidium* spp. It was made up of 127 broiler chickens (0.78%) and 48 turkeys (2.08%) as shown in Table 1.

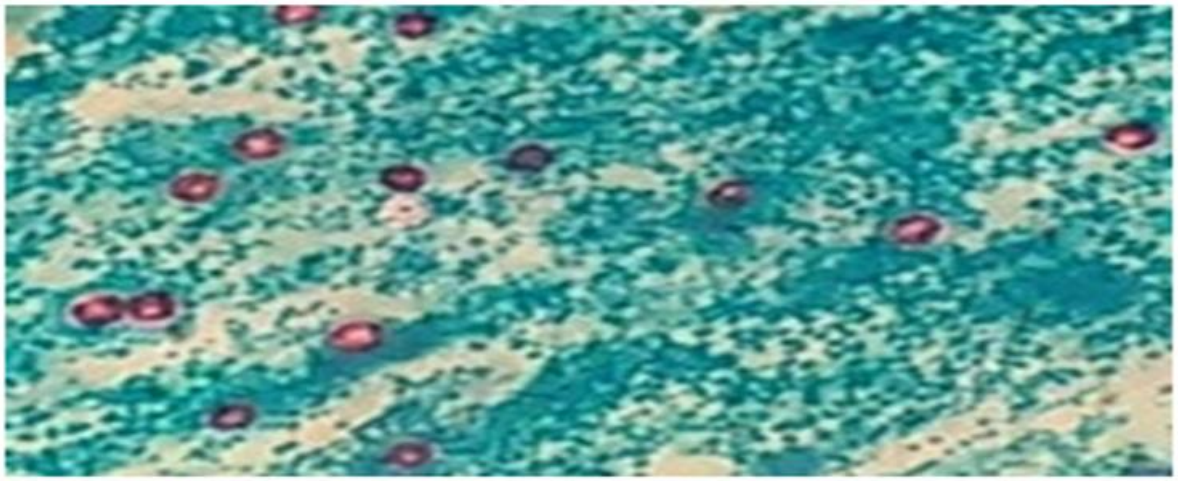

Fig 1 oocysts of Cryptosporidium in feces diagnosed using modified Ziehl-Neilson (MZN)staining method

Table 1: *Cryptosporidium* prevalence in turkeys and broiler chickens

Host	Collected	Positive samples %	Species
Broiler chickens	127	1(0.78%)	*C.baileyi*
Turkey	48	1(2.08%)	*C.meleagridis*
Total	175	2(1.14%)	-

Positive stool samples were analyzed by Amplify PCR with approximately 435 bp of the 18s rRNA gene. (Figure 2), 18s rRNA gene sequence analysis indicated the existence of two *Cryptosporidium* spp: *C. baileyi* and *C. meleagridis* which found in broiler chickens and turkeys, respectively. According to sequence analysis, it has been deposited in GenBnk with accession numbers OP868728.1 from *Cryptosporidium* baileyi and OP868729.1 from *Cryptosporidium* meleagridis (Figure 3).

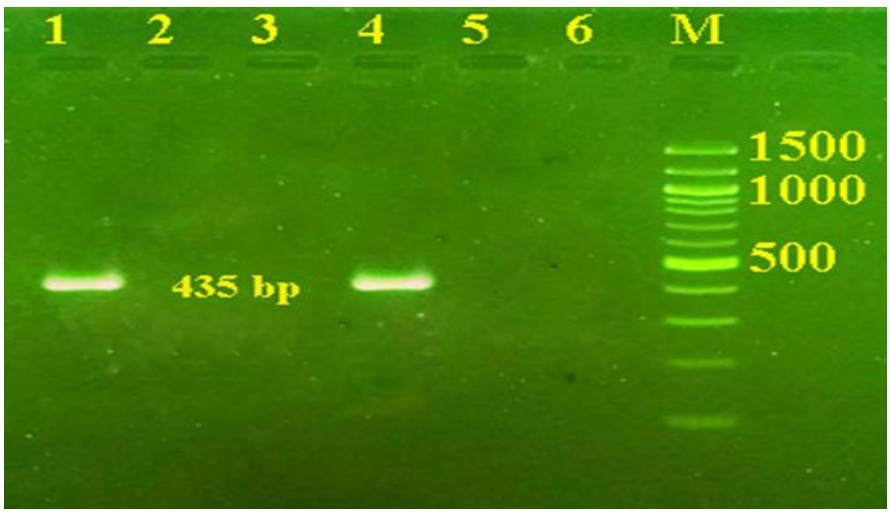

Figure 2. Agarose gel electrophoresis of *18s rRNA* gene, the bands in the tracks 1,4 with size of 435 bp is a typical band of the *18s rRNA* gene of *Cryptosporidium spp.* M: DNA ladder (100-1500bp)

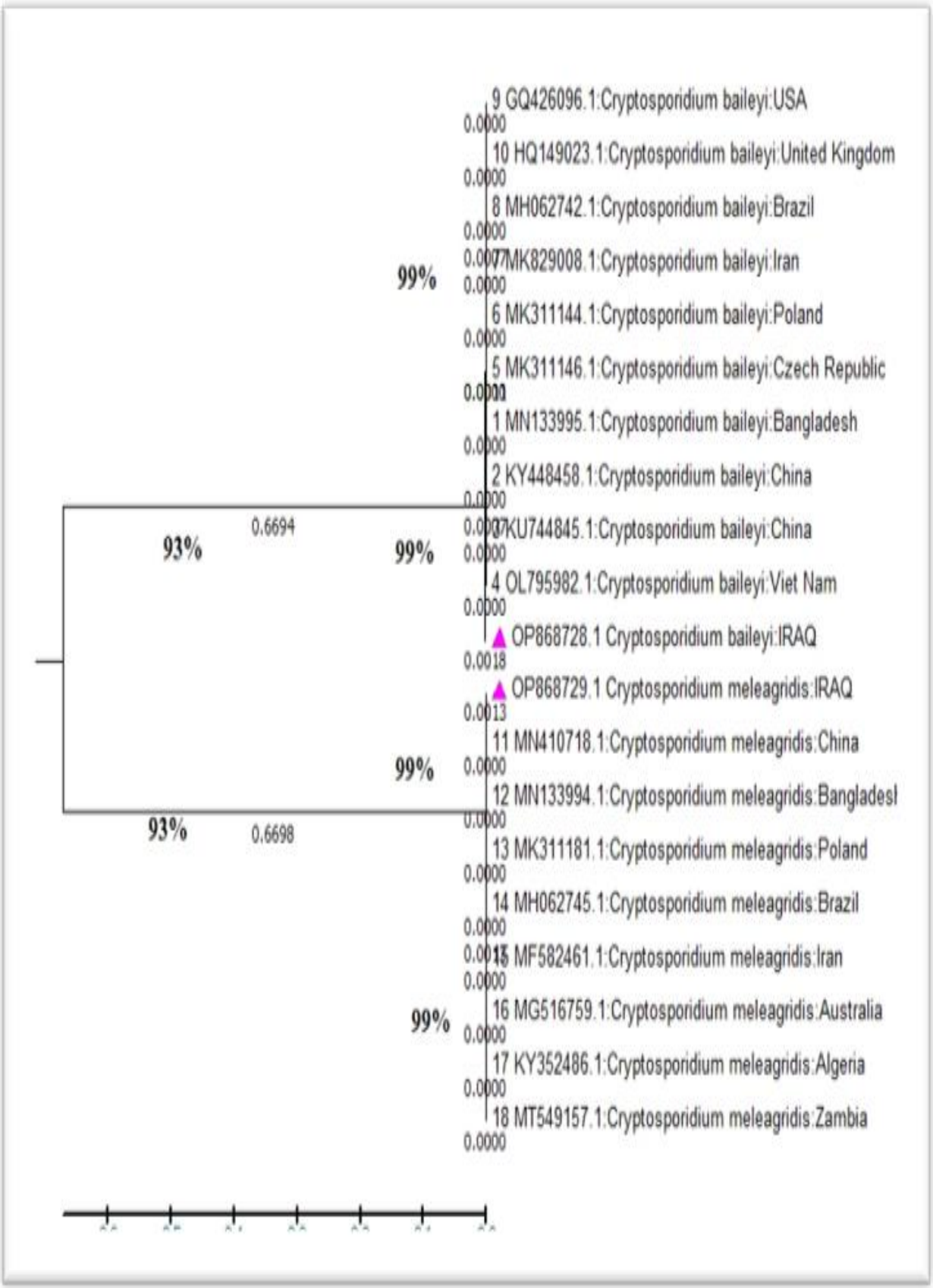

Figure 3. Phylogenetic relationships between reference sequences from Gen Bank and the 18s rRNA of Cryptosporidium species found in the current work.

Table 2. Genetic rapprochement of 18s rRNA for the isolate of *Cryptosporidium* spp

	Accession	Country	Source	Isolation source	Compatibility
1.	**ID: MN133995.1**	**Bangladesh**	***Cryptosporidium baileyi***	**Chicken**	**99%**
2.	**ID: KY448458.1**	**China**	***Cryptosporidium baileyi***	**Chicken**	**99%**
3.	**ID: KU744845.1**	**China**	***Cryptosporidium baileyi***	**crested myna**	**99%**
4.	**ID: OL795982.1**	**Viet Nam**	***Cryptosporidium baileyi***		**99%**
5.	**ID: MK311146.1**	**Czech Republic**	***Cryptosporidium baileyi***	**Taeniopygia guttata**	**99%**
6.	**ID: MK311144.1**	**Poland**	***Cryptosporidium baileyi***	**Erythrura gouldiae**	**99%**
7.	**ID: MK829008.1**	**Iran**	***Cryptosporidium baileyi***	**Broilers**	**99%**
8.	**ID: MH062742.1**	**Brazil**	***Cryptosporidium baileyi***	**Quail**	**99%**
9.	**ID: GQ426096.1**	**USA**	***Cryptosporidium baileyi***	**Falco cherrug**	**99%**
10.	**ID: HQ149023.1**	**United Kingdom**	***Cryptosporidium baileyi***	**Chicken**	**99%**
11.	**ID: MN410718.1**	**China**	***Cryptosporidium meleagridis***	**Parrot**	**99%**
12.	**ID: MN133994.1**	**Bangladesh**	***Cryptosporidium meleagridis***	**Chicken**	**99%**
13.	**ID: MK311181.1**	**Poland**	***Cryptosporidium meleagridis***	**Homo sapiens**	**99%**
14.	**ID: MH062745.1**	**Brazil**	***Cryptosporidium meleagridis***	**Quail**	**99%**
15.	**ID: MF582461.1**	**Iran**	***Cryptosporidium meleagridis***	**Exotic birds**	**99%**
16.	**ID: MG516759.1**	**Australia**	***Cryptosporidium meleagridis***	**Birds**	**99%**
17.	**ID: KY352486.1**	**Algeria**	***Cryptosporidium meleagridis***	**Anser**	**99%**
18.	**ID: MT549157.1**	**Zambia**	***Cryptosporidium meleagridis***	**Homo sapiens**	**99%**

Discussion

A whole collection of 175 poultry feces samples were collected at random, 127 sample from chicken and 48 from turkey. were examined by ziehl-Neelsen staining and PCR methods, the overall prevalence of *Cryptosporidium* spp. were (1.14%) which is lower than result of Saeed and Meteab (2021) they determined that Cryptosporidium spp. were 19.4% (wild duck with 26.6%, domestic chicken 20% and turkey 17%). Another study showed the prevalence of *Cryptosporidium* spp. in layers chickens and broiler, it was 64%, 36% respectively (AL-Ghezey and AL-Zubaidi, 2020)

However, other study reported the occurrence of Cryptosporidiosis such as in Jordan 4.8% (Hijjawi *et al*., 2016), China 2.43% (Cao *et al*., 2020), Tunisia 4.5% (Soltane *et al*., 2007). but, higher the overall prevalence in chicken was 55% and in turkeys 41% (Guechtouli *et al*., 2022). The variations in incidence may arise from a number of variables, including strains of birds, environmental factors, management and the immune status of birds (Bouzid *et al*., 2013).

Identification and identification of Cryptosporidium spp. Genotypes, when analysed using molecular techniques, have a significant impact on identifying the origin of an infection and assessing its overall health condition (Lin *et al*., 2022). Two isolates of *Cryptosporidium* spp were isolated. It has been registered with NCBI based on the sequence of the ribosomal RNA gene subunit (18s rRNA) and has been transferred to Iraq, the Middle East and the world under accession numbers OP868728.1 of *Cryptosporidium* baileyi and OP868729.1 of *Cryptosporidium* meleagridis, strains registered in the National Registry. The Center for Biotechnology Information (NCBI) has 93-99% concordance with Bangladesh (MN133995.1), China (KY448458.1), China (KU744845.1), Vietnam (OL795982.1), Czech Republic (MK311146.1), Poland (MK311181.1), Brazil (MH062745.1), Iran (MF582461.1), Australia (MG516759.1) and Algeria (KY352486.1), as shown in Table 2 and Figure 3 of the *Cryptosporidium* spp. phylogenetic tree.

The sequence analysis revealed the existence of two species of Cryptosporidium: *C. baileyi* which is the predominant type found in chickens and has the ability to infect more than 20 different avian species and *C. meleagridis* in turkeys (Ryan *et al*., 2021) in current study, the prevalence of *C. baileyi* was (0.78%) in previous studies the found of *C.baileyi* in domestic and broiler chicken (Jasim and marhoon, 2015). Another investigation revealed that only C.baileyi was found in chickens, with an incidence rate of 2.43%(Cao *et al*., 2020)

The species *C. meleagridis* is the third most common cause of infection in humans, and it is also capable of infecting birds and mammals (Xiao, 2010). In the current study, the occurence of *C.meleagridis* was (2.08%). in contrast, other author found at a high prevalence in turkeys aged over 4 weeks was 29% Baroudi *et al*. (2013). humans are infected with the *Cryptosporidium* parasite through transmission from domestic and wild bird, which are considered a reservoir for human infection (Bomfim *et al*., 2013).

Conclusion

In this study, two species of *Cryptosporidium* were identified, *C. baileyi* and *C. meleagridis* which exist in broiler chickens and turkeys, respectively. *C. meleagridis* is more widespread than *C. baileyi* and the turkey may serve as a reservoir host for cross-transmission of disease.

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