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Molecular Identification of *Cystoisospora* Protozoan Infecting Dogs

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Abstract | *Cystoisospora* species are protozoan parasites belonging to the phylum Apicomplexa that inhabit the small intestine of their hosts. To date, no previous studies were found regarding *Cystoisospora* infection in dogs in Babylon province, Iraq. Therefore, this study was designed to detect the protozoan *Cystoisospora* in dogs' faeces and identify its species in stray and domestic dogs based on a molecular technique. Fecal samples of 150 stray and domestic dogs were examined from October 2024 to February 2025. All samples were analyzed by PCR targeting the 18S rRNA gene to detect *Cystoisospora* spp. Thirteen PCR-positive samples were selected for partial 18S rRNA gene sequencing to identify the species using the DNA sequencing method and phylogenetic tree analysis. The conventional PCR detected *Cystoisospora* DNA in 15 out of 150 samples (10%). According to sex, infection rates were slightly higher in male dogs (10.46%; 9/86) compared to females (9.37%; 6/64). Age-wise, the maximum infection rate was observed in dogs under 6 months of age (24.44%; 11/45), while the lowest rate was in dogs over one year old (2.22%; 1/45), with **a** significant difference ($p \leq 0.01$). The DNA sequencing identified *Cystoisospora canis* in 10 samples (76.92%) and *Cystoisospora ohioensis* in 3 samples (23.07%). The phylogenetic tree analysis found that *C. canis* and *C. ohioensis* isolates showed high genetic similarity to isolates of GenBank sequences from Canada and China, USA, Australia, and South Korea. This study is suggested to be the first in Babylon province to detect *Cystoisospora* species at the molecular level, identifying both *C. canis* and *C. ohioensis*. The findings highlighted a higher prevalence in younger dogs, demonstrating that molecular methods are crucial for accurate species identification. Especially in Babil city, this effort also offered essential knowledge on the existence and spread of this parasite in Iraq.

Keywords | *Cystoisospora* species, Dog, PCR, DNA sequence, Phylogenetic analysis, Babil

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INTRODUCTION

The protozoa of the genus *Cystoisospora* cause an intestinal parasitic illness known as cystoisosporiasis (previously referred to as isosporiasis). This parasite infects both mammals and humans and is found worldwide, although it is most prevalent in the tropics and subtropics (Dubey, 2018). Coccidia are a kind of protozoan parasite that infects the intestines and is common in animals, people, and pets (Imran and Alsayeqh, 2022). Differences in oocyst shape, host specialization, and developmental phases make the genus *Cystoisospora* difficult to understand (Barta *et al.*, 2005; Raza *et al.*, 2018). Puppies younger than six months and dogs with reduced resistance systems are more likely to get diarrhoea caused by *Cystoisospora* species (Buehl *et al.*, 2006). Contaminated intermediate hosts, such as rats and other animals, can spread the disease through the faecal-oral pathway or by consuming contaminated tissues.

In the first, subclinical stage, no symptoms are present; in the second, clinical stage, symptoms such as oocyst shedding, anorexia, fever, watery diarrhoea, vomiting, dehydration, gastrointestinal haemorrhage, and abdominal pain are noticed (Papazahariadou *et al*., 2007; Lappin, 2010; Mohsen *et al*., 2024). In severe cases, respiratory distress, neurological symptoms, and even mortality can occur. Recovery may lead to the development of varying levels of immunity. In dogs, the survival of oocysts and their environmental persistence may contribute to ongoing transmission (Liberato *et al*., 2018). Dogs serve as hosts for four recognized *Cystoisospora* species. Among them, *C. canis* produces large oocysts (>33 µm), which can be distinguished from the smaller and structurally similar *C. ohioensis, C. neorivolta,* and *C. burrowsi* (<30 µm) (Lindsay *et al*., 1997a). These three are commonly grouped under the term *C. ohioensis*-like due to the challenges of morphological differentiation. Recent molecular tools, such as PCR, sequencing, and RFLP analysis, have enabled the more accurate distinction of these species (Samarasinghe *et al*., 2008; Matsubayashi *et al*., 2011; He *et al*., 2012).

It was initially believed that *Cystoisospora* spp. belonged to the Eimeriidae family. However, phylogenetic studies have shown that they are closer to the Sarcocystidae, which include coccidia that produce tissue cysts, such as Toxoplasma gondii and Neospora caninum (Barta *et al*., 2005; Carreno *et al*., 1998; Franzen *et al*., 2000).

Cystoisospora spp. is common in dogs worldwide, according to many epidemiological studies; 4.9% of dogs in South Korea tested positive (Liyanagama *et al*., 2024), 7.3% in Germany (Murnik *et al*., 2023), 24.6% in Spain (Ortuño *et al*., 2014), and 10.4% in Canada (Villeneuve *et al*., 2015). Microscopic and molecular studies revealed a canine infection incidence of 9.5% in Basrah, Iraq (Jassim *et al*., 2024), in contrast to previous research from Diwaniyah, Iraq, which indicated a prevalence rate of 17.3% by PCR and 9% by microscopic inspection (Jarad *et al*., 2025). The microscopic study revealed a 17.33% infection rate of *Cystoisospora* spp. in Babylon, Iraq (Abd Alhamza and Al-Hasnawy, 2025).

As they scavenge, dogs in urban areas of Iraq frequently encounter polluted surfaces since they are allowed to wander freely in neighbourhoods and households. This environmental exposure may increase the risk of parasite transmission through faeces, soil, and water. Although zoonotic parasites have received some attention, infections with *Cystoisospora* remain underreported. Although previous studies in Babylon province investigated some coccidian protozoa, such as *Cryptosporidium* spp, which revealed the infection rate was 22% in dogs (Alasady and Al-Hasnawy, 2024), *Sarcocystis* spp. (88.33%) in cattle (Kadhim and Ameer, 2025).

Molecular techniques were not successful in identifying canine illnesses caused by *Cystoisospora* species in this province. Therefore, this study set out to determine the molecular characteristics and prevalence of *Cystoisospora*.

MATERIALS AND METHODS

Samples collection

From October 2024 to February 2025, researchers in Babylon province gathered 150 faecal samples from dogs, both stray and domestic, spanning a range of ages and sexes. Each sample was placed in a clean, labelled plastic container with a tightly sealed lid, and metadata such as age, sex, and faecal consistency were recorded at the time of collection. The samples were promptly placed in a cool box and sent to the Parasitology Laboratory, where they were stored at 4°C until the DNA extraction process could commence.

Molecular diagnosis

DNA extraction

After flotation purification, genomic DNA was extracted from 150 faecal samples following the directions of the Easy Pure® Stool Genomic DNA Kit (TransGen Biotech, China) (Abed *et al*., 2024).

Primer preparation and PCR amplification

For the molecular identification of *Cystoisospora* spp., an internal transcribed spacer-targeting primer pair (ITS-1) in the 18S rRNA gene region (450 bp) was used, as defined by Samarasinghe *et al*. (2008). The lyophilized primers were initially dissolved in nuclease-free ddH_2O to create a 100 pmol/µL standard solution. A working explanation of 10 pmol/µL was prepared by diluting 10 µL of the stock in 90 µL of ddH_2O.

PCR reactions were performed using a 25 µL final volume that included 12.5 µL of GoTaq® Green Master Mix (Promega, USA), one microlitre each of forward and reverse primers, 2 microlitres of template DNA at a concentration of 10 pmol/µL, and enough nuclease-free water to make up

Table 1: The sequence of primers used in this study.

Primer	Sequence	Primer sequence 5'- - 3'	Tm (°C)	GC%	Size of product (bp)	Source
ITS	F	GATCATTCACACGTGGC CCTTG	63. 7	55		(Samara *et al*., 2008)
	R	GACGACGTCCAAATCCA CAGAGC	65. 1	57	450 bp	

the whole volume. In a heat cycler, the PCR amplification was carried out using Applied Biosystems™ ProFlex™ PCR System (Fisher Scientific/ USA), subjected to the following cycling conditions: A three-minute denaturation at 94°C, forty cycles of two-minute denaturation, one-minute annealing, two-minute extension, and seven-minute extension, as indicated in Table 2.

Electrophoresis on 1.5% agarose gels was used to resolve PCR products, which were stained with RedSafe™ dye (Mebep Bio Science, China), and then viewed under UV light using a microscope. To measure the molecular size, a 100 bp DNA ladder (lane M) was used. The presence of a ~450 bp band was considered indicative of a positive result for *Cystoisospora* spp.

Table 2: The optimum condition of detection.

Phase	Tm (°C)	Time	No. of cycle
Initial Denaturation	94°C	3 min	1 cycle
Denaturation -2	94°C	2 min	40 cycles
Annealing	68°C	1 min	
Extension-1	72°C	2 min	
Extension -2	72°C	7 min.	1 cycle

DNA sequencing technique

The *Cystoisospora* species was determined using DNA sequencing, and the genotypes of thirteen local isolate products that tested positive for PCR were transferred to the MacroGene Company in Korea for further analysis. The entire sequence of the 18S rRNA gene was cast-off to confirm that all four isolates were *C. canis* (Jarad *et al*., 2025). We used BioEdit, version 7.2, in conjunction with the Basic Local Alignment Search Tool (BLAST) offered by the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) to perform a homology search (Yun *et al*., 2023). To validate the findings, they were cross-referenced with information from the NCBI database and the ExPASy software (SIB Swiss Institute of Bioinformatics, Switzerland).

Phylogenetic tree analysis

To calculate evolutionary distances, a phylogenetic tree was constructed using the UPGMA technique (Sneath and Sokal, 1973). This is the ideal tree of *C. canis*, with a total branch length of 0.04032703. Here we can see the ideal *C. ohioensis* tree, where the total branch length is 7.22043733 (Tamura *et al*., 2004). The MEGA6 system was used to carry out evolutionary analyses (Tamura *et al*., 2013).

Statistical analysis

We expressed the results as a percentage. For the statistical analysis, we relied on the chi-square (χ^2) test. Findings were considered statistically significant when the p-value was less than or equal to 0.05, and highly significant when the p-value was less than 0.001.

RESULTS

Conventional PCR

Ten per cent, or fifteen out of one hundred fifty cases, had positive results for *18S rRNA* gene PCR amplification, according to the molecular diagnostic data (Figure 1). A higher number of oocysts was found in the faecal samples of males (10.46%) compared to females (9.37%), with no significant difference (Table 3, Figure 2).

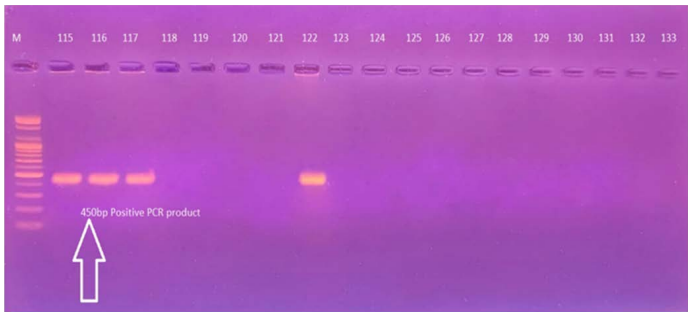

Figure 1: Agarose gel electrophoresis image shows PCR product of *Cystoisospora* spp. based on *18S rRNA* gene. Bands were fractionated by electrophoresis on a 1.5% agarose gel, and visualized under U.V. light after staining with red stain. Lane: M (M: 100bp ladder)

Table 3: Infection rate of *Cystoisospora* spp. in dogs according to the sex of dogs.

Sex	Examined sample	Infected samples	Infection rate
Male	86	9	10.46 %
Female	64	6	9.37%
Total	150	15	10 %
Chi-square (x^2)	4.200		
p-value	0.122		

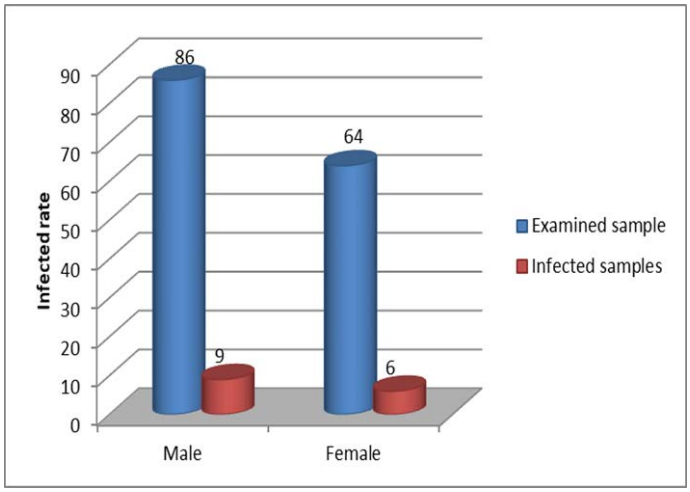

Figure 2: Infection rate of *Cystoisospora* spp. in dogs according to the sex of dogs.

Puppies less than six months of age had the highest infection rate in this study's PCR analysis, at 24.44% (11 out of 45). In contrast, the lowest infection degree was observed in dogs older than one year, with only 1 out of 45 animals testing positive (2.22%). These differences were statistically significant ($p \leq 0.01$), as shown in Table 4 and Figure 3.

Table 4: Infection rate of *Cystoisospora* spp. in dogs according to age groups.

Age (months)	Examined sample	Infected samples	Infection rate
Less than 6 months	45	11	24.44%
6-12 months	60	3	5%
More than 1 year	45	1	2.22%
Total	150	15	10%
Chi-square (χ^2)	17.467		
p-value	0.001**		

*Highly significant difference at (P≤0.05). ** refer to significant difference at (p≤0.01).

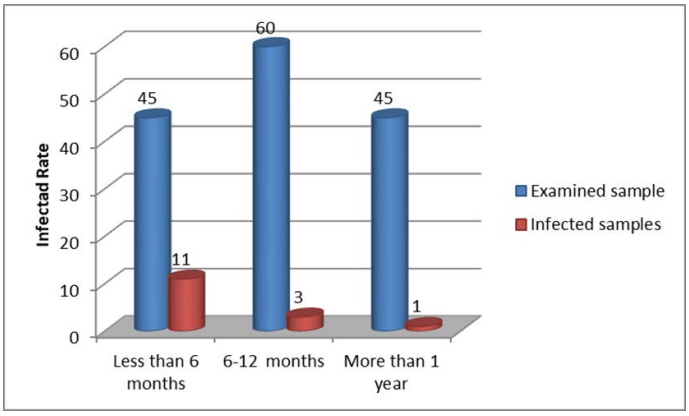

Figure 3: Infection rate of *Cystoisospora* spp. in dogs, according to age groups.

DNA SEQUENCE ANALYSIS OF *CYSTOISOSPORA* SPP.

Thirteen canine faecal sample isolates from Babylon province were genetically sequenced and characterized using 18S ribosomal RNA gene partial sequencing in order to study and identify the species of genus *Cystoisospora* that

Table 5: DNA sequence analysis of *Cystoisospora* spp. according to 18S ribosomal RNA gene.

Identities	Sequence ID with comparison	Source	Nucleotide	Location	Type of substitution	No. of sample
99%	ID: OP837796.1	*C. ohioensis*	G\A	187	Transition	1
			C\G	246	Transvertion	
99%	ID: OP837796.1	*C. ohioensis*	A\T	282	Transvertion	2
			A\C	289	Transvertion	
			T\C	300	Transition	
99%	ID: OP837796.1	*C. ohioensis*	A\T	282	Transvertion	3
99%	ID: MH790139.1	*Cystoisospora canis*	C\A	968	Transvertion	4
			A\C	1217	Transvertion	
99%	ID: MH790139.1	*Cystoisospora canis*	C\A	968	Transvertion	5
			C\G	1086	Transvertion	
			A\G	1087	Transition	
99%	ID: MH790139.1	*Cystoisospora canis*	C\A	968	Transvertion	6
			C\A	1204	Transvertion	
99%	ID: MH790139.1	*Cystoisospora canis*	C\A	968	Transvertion	7
			A\T	1078	Transvertion	
			C\T	1245	Transition	
99%	ID: MH790139.1	*C. canis*	C\A	968	Transvertion	8
99%	ID: MH790139.1	*C. canis*	C\A	968	Transvertion	9
99%	ID: MH790139.1	*Cystoisospora canis*	C\A	968	Transvertion	10
			A\G	1144	Transition	
99%	ID: MH790139.1	*Cystoisospora canis*	C\A	968	Transvertion	11
			G\T	1146	Transvertion	
99%	ID: MH790139.1	*C. canis*	T\G	920	Transvertion	12
			C\A	968	Transvertion	
99%	ID: MH790139.1	*Cystoisospora canis*	T\G	920	Transvertion	13
			C\A	968	Transvertion	
			A\T	1078	Transvertion	
			T\G	1210	Transvertion	

had been identified by traditional PCR. The results showed that certain *C. canis* (10/13, 76.92%) and *C. ohioensis* (3/13, 23.07%) were present. Isolates of *Cystoisospora* spp. were entered into the National Centre for Biotechnology Information's GenBank database as accession numbers (ID: PV606919.1; ID: PV606920.1; ID: PV606921.1) and identified and registered for the specific species *C. ohioensis*. The sequences (ID: PV606922.1; ID: PV606923.1; ID: PV606924.1; ID: PV606925.1; ID: PV606926.1; ID: PV606927.1; ID: PV606928.1; ID: PV606929.1; ID: PV606930.1; ID: PV606931.1) were identified and registered for the specific species *C. canis*, expressed in Table 5.

Phylogenetic analysis of *C. canis* and *C. ohioensis*

The evolutionary history of *C. canis* and *C. ohioensis* was inferred using the UPGMA method (Sneath and Sokal, 1973), based on 18S ribosomal RNA gene sequences. To determine evolutionary distances, we employed the Maximum Composite Likelihood method (Tamura *et al*., 2004), which is based on the total number of nucleotide substitutions (or base changes) that occurred at each location. We used MEGA6 for all our analyses (Tamura *et al*., 2013), which included the first, second, third, and noncoding codon locations, and excluded any gaps or missing data.

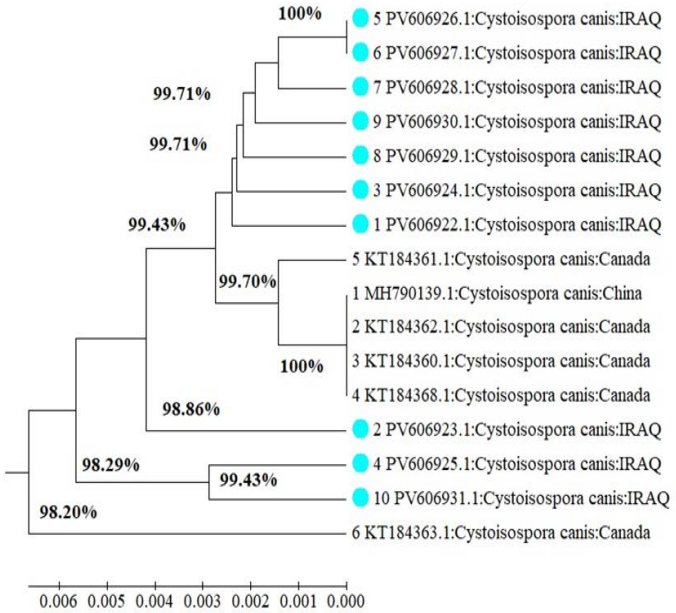

Figure 4: Phylogenetic tree analysis based on the partial sequence of 18S ribosomal RNA gene explains identity between the local isolates of *Cystoisospora canis* in dogs and NCBI-BLAST isolates.

For *C. canis*, the analysis included 16 nucleotide sequences with 350 aligned positions, yielding a best tree with a whole-branch distance of 0.04032703 (Figure 4). Isolates from Iraq shared 99% sequence similarity with strains from Canada and China (Table 6).

In comparison, the *C. ohioensis* phylogenetic tree was based on 10 sequences with 271 aligned positions, and a total branch length of 7.22043733 (Figure 5). Iraqi *C. ohioensis* isolates clustered closely with sequences from China, the USA, Australia, and South Korea, showing 91%–99% sequence similarity (Table 7).

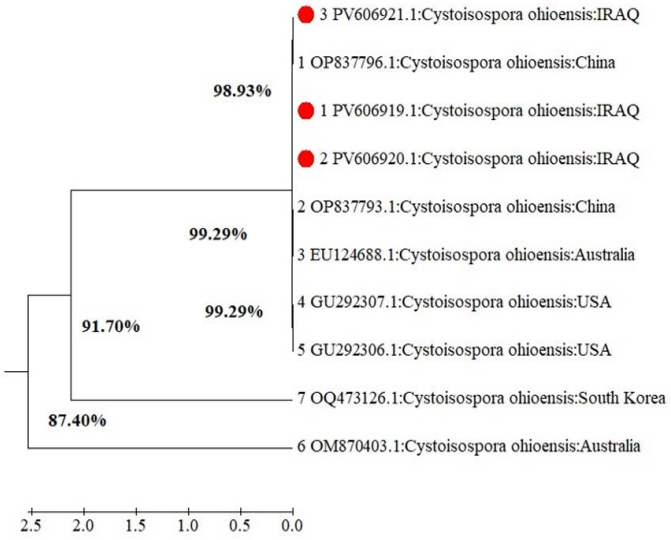

Figure 5: Phylogenetic tree analysis based on the partial sequence of the 18S ribosomal RNA gene explains identity between the local isolates of *C. ohioensis* in dogs and NCBI-BLAST isolates.

Table 6: The NCBI-BLAST Homology Sequence Identity (%) between local *C. canis* in dog stool isolates and NCBI-BLAST deposited strains.

Source: *Cystoisospora canis*; 18S ribosomal RNA gene				
Accession	**Country**	**Isolation source**	**Date of registration**	**Compatibility**
ID: MH790139.1	China	Dog	2018	99%
ID: KT184362.1	Canada	Dog	2015	99%
ID: KT184360.1	Canada	Dog	2015	99%
ID: KT184368.1	Canada	Dog	2015	99%
ID: KT184361.1	Canada	Dog	2015	99%
ID: KT184363.1	Canada	Dog	2015	99%

Table 7: The NCBI-BLAST homology sequence identity (%) between local *Cystoisospora ohioensis* in dog stool isolates and NCBI-BLAST deposited strains.

Source: *Cystoisospora ohioensis*; 5.8S ribosomal RNA gene				
Accession	**Country**	**Isolation source**	**Date of registration**	**Compatibility**
ID: OP837796.1	China	dogs	2022	99%
ID: OP837793.1	China	dogs	2022	99%
ID: EU124688.1	Australia		2007	99%
ID: GU292307.1	USA	dogs	2009	99%
ID: GU292306.1	USA	dogs	2009	99%
ID: OM870403.1	Australia	fox	2022	93%
ID: 0Q473126.1	S. Korea		2023	91%

DISCUSSION

This study is proposed as the first contribution to the knowledge of the attendance and prevalence of *Cystoisospora* species in dogs in the Babylon area, Iraq. The initial examination of faecal samples showed that most positive cases occurred in dogs with watery diarrhoea. Mitchell *et al.* (2007) reported that *Cystoisospora* infections in immunocompromised humans and dogs involve extra-intestinal stages linked to watery diarrhoea and weight loss. This supports the current findings, which indicate that cases of watery diarrhoea are associated with the highest infection rates in dogs.

Oocyst morphology has been the standard method for identifying *Cystoisospora* species to date. However, molecular confirmation using DNA analysis has been infrequently applied. As a result, the GenBank database contains limited 18S rRNA sequence data for *Cystoisospora* spp.

The current data revealed that the overall prevalence of *Cystoisospora* spp., as determined by conventional PCR, was 10%. This finding agreed with a study in Canada, which revealed the infection rate was 10.4% (Villeneuve *et al.*, 2015), and it was also consistent with a study in Germany (7.3%) (Murnik *et al.*, 2023). However, research conducted in South Korea found no such thing (Liyanagama *et al.*, 2024), which reported an infection rate of *Cystoisospora* of 4.9%. Various geographical climates, host distribution, and other variables likely contributed to the disparity in prevalence rates of this parasite among nations or areas within a country (Confalonieri *et al.*, 2014).

According to sex, the infection rate in dogs was higher in males compared to females, with no significant differences shown in the infection rate between males and females. This result was consistent with a study in the southern part of Iraq. Basrah, during which it was discovered that the prevalence of *Cystoisospora* infection was greater in male canines (6.6% vs. 6.3%) (Al-Jassim *et al.*, 2017). However, it has issues with the findings of Jarad *et al.* (2025) in Diwaniyah city, Iraq, which revealed a higher contagion rate of *C. canis* in female dogs (22.34%) compared to male dogs (16.03%), as well as a variation in infection rates of *Cystoisospora* spp. When it comes to canines, this discrepancy may be due to variations in research sites, study periods, sample sizes, and diagnostic methods. Another possible factor is the existence of conditions that allow oocysts to survive for an extended period (Arsalan *et al.*, 2006).

The PCR results revealed that the infection rate was highest in puppies under six months and lowest in dogs older than one year in this research. These results agreed with those of previous research that also considered animal age. Taking Germany as an example, Murnik *et al.* (2023) reported an infection rate of 14.0%, which exceeds the indicated prevalence. Villeneuve *et al.* (2015) found that canines less than one year old had the highest infection rate (12.6%). In France, Osman *et al.* (2015) found a significantly higher infection rate in puppies under one year (28.2%), in contrast to a significantly lower prevalence (15.6%) in dogs older than one year. In a similar vein, research in Diwaniyah city, Iraq, found that the infection rate was 38.02% in young pups and 8.52% in mature dogs, which aligns with the results of the present investigation (Jarad *et al.*, 2025). The prevalence of parasite infection is significantly impacted by age. This finding aligns with Abere *et al.* (2013), who noted that the frequency of parasites was significantly linked to age. In addition, the same researcher reported that the parasite prevalence was higher in young dogs than in adults (Abere *et al.*, 2013). Furthermore, Ahmed *et al.* (2014) noted that the prevalence of the infection was greater in pups compared to adults. This can be due to a lack of immunity during the subject's youth, or it may be the result of a single or recurrent exposure (Ramírez-Barrios *et al.*, 2004).

Regarding the DNA sequencing analysis, thirteen PCR products of the protozoan *Cystoisospora* isolated from dogs' faeces were genetically analysed using partial 18S rRNA sequencing, identifying *C. canis* and *C. ohioensis*. Similar to previous studies, this investigation also recorded *Cystoisospora* spp. based on 18S rRNA sequencing, with the obtained sequences submitted to GenBank. In line with these efforts, in Diwaniyah city, Iraq, Jarad *et al.* (2025) reported a 19% prevalence of *Cystoisospora* spp. in stray dogs, as determined by microscopy. PCR and sequence analysis revealed parasite DNA in 56% of samples, showing 100% identity with *C. canis* strains from Canada. While in Basrah, Iraq, Jassim *et al.* (2024) recorded a 9.5% infection rate with *C. canis* in dogs, confirmed by PCR and DNA sequencing. The genetic results matched *C. canis,* confirming its presence and host specificity.

Globally, *Cystoisospora* spp. have been reported in dogs, with *C. ohioensis* identified as a common species. In Indonesia, Tokiwa *et al.* (2018) established the presence of *C. ohioensis* in dogs, and molecular analysis revealed 99.9% similarity with *C. ohioensis* based on the *18S rRNA* gene. In Australia, Samarasinghe *et al.* (2008) conducted phylogenetic analysis of ITS1 orders from *C. ohioensis* -like oocysts. They found that they cluster within the family Sarcocystidae, rather than Eimeriidae, which supports their close evolutionary relationship to other tissue-cyst-forming coccidia, such as *Toxoplasma* and *Neospora.*

Based on the phylogenetic tree analysis. The Iraqi isolates of *C. canis* and *C. ohioensis* showed a high level of similarity to gene sequences deposited in the GenBank database. The *C. canis* isolates shared 99% sequence similarity with strains from Canada and China based on the 18S ribosomal RNA gene. Similarly, *C. ohioensis* isolates from Iraq showed close genetic relationships with strains from China, the USA, Australia, and South Korea, with sequence similarity ranging from 91% to 99%. According to the phylogenetic study, there were a few genetic variations between the Iraqi isolates and those from other nations. Differences in reference sequence length or sample origins, particularly in terms of geography, are potential causes of these variances. Several molecular methods were employed, including PCR and sequencing of partial or full-length gene fragments. According to Matsubayashi *et al.* (2011), Dogs infected with *Cystoisospora* species structurally and genetically identical to *C. ohioensis* have been identified through molecular and morphological investigations. Dog samples were genetically similar to those of raccoons, which may indicate that the two carnivorous hosts share a similar life cycle or mode of transmission.

Furthermore, the DNA sequences were analyzed using phylogenetic methods, which revealed that the raccoon and dog isolates belonged to the same clade as additional types of *Cystoisospora*, including *C. ohioensis*. According to these results, *C. ohioensis* is the most commonly shared type of canine infection. Using 18S rRNA and ITS-1 gene sequences, *Cystoisospora* isolates collected from dogs in Changchun, China, were subjected to a phylogenetic study. The results showed that one of the isolates, Chang Chun 2, was genetically identical to *C. ohioensis*, and another, Chang Chun 1, clustered separately but exhibited substantial genetic similarity to *C. suis*. These findings suggested either the presence of a unique, yet undiscovered species or the possibility of intra-species genetic variation (He *et al*., 2012).

Reiterating the dependability of molecular analysis, particularly when morphological traits alone are insufficient for species classification, our results align with comparable studies from Japan and Korea that employed the same target genes (*18S rRNA* and *ITS-1*). The data also show the importance of revising the genetic classification of *Cystoisospora* species found in dogs.

Tokiwa *et al.* (2018) initially documented an infection of Asian small-clawed otters (*Aonyx cinereus*) with *Cystoisospora*, a parasite often seen in cats and dogs; molecular investigation confirmed a high degree of genetic similarity. According to phylogenetic analysis, the otter isolate is closely related to *C. rivolta*, suggesting a strong evolutionary connection. *Cystoisospora* spp. may infect more carnivorous hosts than previously thought, according to this finding. This highlights the need for genetic surveillance and cross-species monitoring, especially in exotic animal populations. Previous investigations have indicated minimal genetic diversity among *Cystoisospora spp*., particularly *C. canis* and *C. ohioensis*. The substantial genetic similarity between the Iraqi isolates and those from other countries confirms this finding. It also shows potential for cross-species transmission.

CONCLUSION

Canines from Babylon province, Iraq, were tested for *Cystoisospora* species for the first time in this revolutionary investigation. Through phylogenetic analysis and sequencing of the 18S rRNA gene, two species were identified: *C. canis* and *C. ohioensis*, with *C. canis* being the more prevalent species. The study demonstrates that molecular methods offer greater accuracy than traditional morphological identification. Furthermore, the high genetic similarity between local and international isolates confirms the conserved nature of the 18S rRNA gene. These findings underscore the importance of incorporating molecular diagnostics into parasitological studies to enhance the understanding of parasite epidemiology, transmission, and species identification.

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NOVELTY STATEMENT

This paper presented novel findings for the first time in Babylon province, Iraq where dogs were found to be infected by the protozoan Cystoisospora, as well as two species of this protozoan (C. canis and C. ohioensis) that have been identified in this region and recorded in the NCBI database. These findings are useful for authorities concerned in veterinary hospitals and private veterinary sectors in order to aware owners about this infection and its distribution among domestic and stray dogs of Babylon province.

AUTHOR'S CONTRIBUTION

Mohammad H. Al-Hasnawy: ideas, design the study and analyzed the results. Abdullah Riyadh Abd Alhamza: data collection, analysis, draft writing, and interpretation. Both authors have read, reviewed, and approved the final manuscript.

Conflict of interest

The authors have declared that they have no conflict of interests in the publication of this research.

REFERENCES

Abd Alhamza AR, Al-Hasnawy MH (2025). First microscopic detection of Cystoisospora protozoan infecting dogs in Babylon province. Acta Entomol. Zool., 6(1): 150–155. https://doi.org/10.33545/27080013.2025.v6.i1b.202

Abed DA, Hamzah KJ, Abdul-Kareem S, Mehrzad J (2024). Bacteriological and molecular study of some urinary tract infections bacteria in human and cows in Babylon Province. Pak. Vet. J., 44(4).

Abere T, Bogale B, Melaku A (2013). Gastrointestinal helminth parasites of pet and stray dogs as a potential risk for human health in Bahir Dar town, north-western Ethiopia. Vet. World, 6(7): 388–392. https://doi.org/10.5455/vetworld.2013.388-392

Ahmed WM, Mousa WM, Aboelhadid SM, Tawfik MM (2014). Prevalence of zoonotic and other gastrointestinal parasites in police and house dogs in Alexandria, Egypt. Vet. World, 7(5): 275–280. https://doi.org/10.14202/vetworld.2014.275-280

Al-Asady SIJ, Al-Hasnawy MH (2024). Investigation of *Cryptosporidium* species infecting dogs. J. Anim. Health Prod., 12(s1): 174–181. https://doi.org/10.17582/journal.jahp/2024/12.s1.174.181

Al-Jassim KB, Mahmmod YS, Salem ZM, Al-Jubury A (2017). Epidemiological investigation of gastrointestinal parasites in dog populations in Basra province, Southern Iraq. J. Parasit. Dis., 41: 1006–1013. https://doi.org/10.1007/s12639-017-0926-2

Arsalan S, Daham E, Al-Obaidi Q, Sulaiman E (2006). Study of the percentage of infection with endo- and ectoparasites in dogs in Mosul, Iraq. Iraqi J. Vet. Sci., 20: 125–137. https://doi.org/10.33899/ijvs.2006.62475

Barta JR, Schrenzel MD, Carreno R, Rideout BA (2005). The genus Atoxoplasma (Garnham 1950) as a junior objective synonym of the genus Isospora (Schneider 1881) species infecting birds and resurrection of Cystoisospora (Frenkel 1977) as the correct genus for Isospora species infecting mammals. J. Parasitol., 91(3): 726–727. https://doi.org/10.1645/GE-3341.1

Buehl IE, Prosl H, Mundt HC, Tichy AG, Joachim A (2006). Canine isosporosis–epidemiology of field and experimental infections. J. Vet. Med. B, 53(10): 482–487. https://doi.org/10.1111/j.1439-0450.2006.00973.x

Carreno RA, Schnitzler BE, Jeffries AC, Tenter AM, Johnson AM, Barta JR (1998). Phylogenetic analysis of coccidia based on 18S rDNA sequence comparison indicates that Isospora is most closely related to Toxoplasma and Neospora. J. Eukaryot. Microbiol., 45: 184–188. https://doi.org/10.1111/j.1550-7408.1998.tb04523.x

Confalonieri UE, Margonari C, Quintão AF (2014). Environmental change and the dynamics of parasitic diseases in the Amazon. Acta Trop., 129: 33–41. https://doi.org/10.1016/j.actatropica.2013.09.013

Dubey JP (2018). A review of Cystoisospora felis and *C. rivolta* induced coccidiosis in cats. Vet. Parasitol., 263: 34–48. https://doi.org/10.1016/j.vetpar.2018.09.016

Franzen C, Muller A, Bialek R, Diehl V, Salzberger B, Fatkenheuer G (2000). Taxonomic position of the human intestinal protozoan parasite Isospora belli as based on ribosomal RNA sequences. Parasitol. Res., 86: 669–676. https://doi.org/10.1007/PL00008550

Garcia LS (2016). Diagnostic medical parasitology, 6th Ed., ASM Press, Washington, DC, p. 64. https://doi.org/10.1128/9781555819002

He P, Li J, Gong P, Huang J, Zhang X (2012). Cystoisospora spp. from dogs in China and phylogenetic analysis of its 18S and ITS1 gene. Vet. Parasitol., 190: 254–258. https://doi.org/10.1016/j.vetpar.2012.05.025

Imran A, Alsayeqh A (2022). Anticoccidial efficacy of Citrus sinensis essential oil in broiler chicken. Pak. Vet. J., 42(4). https://doi.org/10.29261/pakvetj/2022.082

Jarad NI, Al-Difaie RS, Mohammed NQ (2025). Molecular recognition and phylogenetic tree analysis of *C. canis* in stray dogs in Diwaniyah city, Iraq. Iraqi J. Vet. Sci., 39(2): 307–312.

Jassim E, Al-Emarah Ghazi, Mustafa J (2024). Microscopic and molecular diagnosis of *Isospora* spp. parasites in cats and dogs. Assiut. Vet. Med. J., 70(183): 413–420. https://doi.org/10.21608/avmj.2024.318475.1402

Kadhim WN, Ameer QG (2025). Diagnosis of *Sarcocystis* spp. in slaughtered cattle by molecular technique in Babylon Province in Iraq. Sarhad J. Agric., 41(1): 330–339. https://doi.org/10.17582/journal.sja/2025/41.1.330.339

Lappin MR (2010). Update on the diagnosis and management of *Isospora* spp. infections in dogs and cats. Topics Compan. Anim. Med., 25: 133–135. https://doi.org/10.1053/j.tcam.2010.07.001

Liberato CD, Berrilli F, Odorizi L, Scarcella R, Barni M, Amoruso C, Scaramozzino P (2018). Parasites in stray dogs from Italy: prevalence, risk factors and management concerns. Acta Parasitol., 63(1): 27–32. https://doi.org/10.1515/ap-2018-0003

Lindsay DS, Dubey JP, Blagburn BL (1997). Biology of *Isospora* spp. from humans, nonhuman primates, and domestic animals. Clin. Microbiol. Rev., 10(1): 19–34. https://doi.org/10.1128/CMR.10.1.19

Liyanagama I, Oh S, Choi JH, Yi MH, Kim M, Yun S, Kang D, Kim SL, Ojeda Ayala MG, Odua F, Yong TS, Kim JY (2024). Metabarcoding study of potential pathogens and zoonotic risks associated with dog feces in Seoul, South Korea. PLoS Neglect. Trop. Dis., 18(8): e0012441. https://doi.org/10.1371/journal.pntd.0012441

Matsubayashi M, Carreno RA, Tani H, Yoshiuchi R, Kanai T, Kimata I, Sasai K (2011). Phylogenetic identification of *Cystoisospora* spp. from dogs, cats, and raccoon dogs in Japan. Vet. Parasitol., 176: 270–274. https://doi.org/10.1016/j.vetpar.2010.11.008

Mitchell SM, Zajac AM, Charles S, Duncan RB, Lindsay DS (2007). *C. canis* Nemeséri, 1959 (syn. Isospora canis), infections in dogs: Clinical signs, pathogenesis, and reproducible clinical disease in beagle dogs fed oocysts. J. Parasitol., 93: 345–352. https://doi.org/10.1645/GE-1024R.1

Mohsen AK, Jassim SE, Alaridhi TT, Al-Erjan AM, Lahhob QR, Mudhafar M (2024). Revolutionizing infectious disease management in animals through advanced phage therapy: A comprehensive strategy for the eradication of multidrug-resistant bacterial pathogens. J. Anim. Health Prod., 12(s1): 157-167. https://doi.org/10.17582/journal.jahp/2024/12.s1.157.167

Murnik LC, Daugschies A, Delling C (2023). Gastrointestinal parasites in young dogs and risk factors associated with infection. Parasitol. Res., 122(2): 585–596. https://doi.org/10.1007/s00436-022-07760-9

Ortuño A, Scorza V, Castellà J, Lappin M (2014). Prevalence of intestinal parasites in shelter and hunting dogs in Catalonia, northeastern Spain. Vet. J., 199: 465–467. https://doi.org/10.1016/j.tvjl.2013.11.022

Osman M, Bories J, El Safadi D, Poirel MT, Gantois N, Benamrouz-Vanneste S, Delhaes L, Hugonard M, Certad G, Zenner L, Viscogliosi E (2015). Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France and evaluation of zoonotic transmission risk. Vet. Parasitol., 214(1–2): 167–170. https://doi.org/10.1016/j.vetpar.2015.09.015

Papazahariadou M, Founta A, Papadopoulos E, Chliounakis S, Antoniadou-Sotiriadou K, Theodorides Y (2007). Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece. Vet. Parasitol., 148(2): 170–173. https://doi.org/10.1016/j.vetpar.2007.05.013

Ramírez-Barrios RA, Barboza-Mena G, Muñoz J, Angulo-Cubillán F, Hernández E, González F, Escalona F (2004). Prevalence of intestinal parasites in dogs under veterinary care in Maracaibo, Venezuela. Vet. Parasitol., 121(1–2): 11–20. https://doi.org/10.1016/j.vetpar.2004.02.024

Raza A, Rand J, Qamar AG, Jabbar A, Kopp S (2018). Gastrointestinal parasites in shelter dogs: Occurrence, pathology, treatment and risk to shelter workers. Animals, 8(7): 108. https://doi.org/10.3390/ani8070108

Samarasinghe B, Johnson J, Ryan U (2008). Phylogenetic analysis of *Cystoisospora* species at the rRNA ITS1 locus and development of a PCR-RFLP assay. Exp. Parasitol., 118(4): 592–595. https://doi.org/10.1016/j.exppara.2007.10.015

Sneath PHA, Sokal RR (1973). Numerical taxonomy. WH Freeman and Co., San Francisco, pp. 1–573.

Tamura K, Nei M, Kumar S (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci., 101(30): 11030–11035. https://doi.org/10.1073/pnas.0404206101

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evolut., 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Tokiwa T, Ohnuki A, Kubota R, Tamukai K, Ike K (2018). Morphological and molecular characterization of *Cystoisospora* sp. from Asian small-clawed otters *Aonyx cinereus*. Int. J. Parasitol. Parasit. Wildl., 7(3): 268–273. https://doi.org/10.1016/j.ijppaw.2018.07.001

Villeneuve A, Polley L, Jenkins E, Schurer J, Gilleard J, Kutz S, Conboy G, Benoit D, Seewald W, Gagné F (2015). Parasite prevalence in fecal samples from shelter dogs and cats across the Canadian provinces. Parasit. Vectors, 8: 281. https://doi.org/10.1186/s13071-015-0870-x

Yun CS, Moon BY, Lee K, Kang SM, Ku BK, Hwang MH (2023). The detection and phylogenetic characterization of *Cryptosporidium, Cystoisospora*, and *Giardia duodenalis* of cats in South Korea. Front. Cell. Infect. Microbiol., 13: 1296118. https://doi.org/10.3389/fcimb.2023.1296118