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Occurrence and First Molecular Identification of Sarcocystis spp. in Egyptian Domestic Dogs

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ABSTRACT

Sarcocystis species are protozoan parasites with complex life cycles that require both predator and prey hosts. Despite their significance, little is known about the prevalence and diversity of Sarcocystis in dog feces worldwide. This study aimed to determine the presence of Sarcocystis in domestic dogs in Egypt and to identify the species involved using a combination of parasitological and molecular approaches. Fecal samples from 100 domestic dogs were collected and examined for Sarcocystis oocysts or sporocysts using a sugar flotation method. DNA was extracted from 30 of these samples, and a fragment of the 18S rRNA gene was amplified through PCR, followed by sequencing to confirm species identity. Microscopic examination detected Sarcocystis in 4% of samples. PCR and sequence analysis confirmed infection in 16 out of 30 dogs (53.3%). There was no significant association between infection prevalence and the age or sex of the animals. Sporocysts measured 13.2–16.0 × 9.4–11 µm. Sequence comparison revealed 99.82% similarity with Sarcocystis tenella previously reported in sheep from Iraq and Iran. This study provides the first molecular evidence that domestic dogs in Egypt can serve as definitive hosts for S. tenella. These results contribute important information for future epidemiological research and may aid in developing control strategies against sarcocystosis in livestock and dogs.

Keywords: Domestic dogs, *Sarcocystis tenella*, Molecular identification, PCR, Fecal samples

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Introduction

Sarcocystis species are intracellular protozoan parasites that rely on a two-host life cycle, involving an intermediate herbivorous host and a definitive carnivorous host. Infection of the definitive host occurs when it ingests tissue cysts containing bradyzoites from raw or undercooked meat. Inside the intestine, the parasites undergo sexual reproduction, producing oocysts or sporocysts that are excreted in feces, completing the cycle [1, 2].

Canids are particularly important in the epidemiology of parasitic infections, acting as both hosts and reservoirs for a variety of parasites with implications for animal health and zoonotic transmission. Through the shedding of eggs, larvae, and oocysts, they can contribute to the spread of enteric and respiratory parasitic diseases, which in some cases can have serious health consequences [3, 4]. Domestic dogs (*Canis familiaris*) are recognized as key definitive hosts for several *Sarcocystis* species, including *S. cruzi*, *S. levinei*, *S. tenella*, *S. arieticanis*, *S. cameli*, *S. capracanis*, *S. hircicanis*, and *S. miescheriana*, which utilize a wide variety of livestock as intermediate hosts, such as cattle, sheep, goats, camels, buffaloes, and pigs [2, 5, 6]. In addition, dogs can serve as intermediate hosts

for *S. caninum* and *S. svanai*, which have been linked to muscular sarcocystosis in canines [7]. While infections are generally mild in carnivores, they can occasionally lead to diarrhea, whereas infections in herbivores are often associated with severe tissue damage, increased mortality, and economic losses [8, 9].

Historically, *Sarcocystis* species have been identified through the examination of sarcocysts in intermediate host tissues using light or electron microscopy, or via experimental infection of definitive hosts [10]. The microscopic detection of sporulated oocysts or sporocysts in fecal samples remains the traditional diagnostic method for definitive hosts. However, the morphological similarity of sporocysts among species makes species-level identification by microscopy unreliable. In contrast, molecular approaches, including PCR amplification of species-specific DNA markers followed by sequencing, have become the gold standard for accurate identification [11–13].

Despite these advances, most molecular studies have focused on cysts from intermediate hosts, and genetic information on *Sarcocystis* oocysts and sporocysts in dog feces is limited. To address this gap, the present study investigates the prevalence and molecular characteristics of *Sarcocystis* species in domestic dog fecal samples from the Aswan governorate in Upper Egypt, using a combination of parasitological and molecular methods.

Materials and Methods

Study area and sample collection

This investigation was carried out in Aswan Governorate, located in southern Egypt (24°5′20.18″ N, 32°53′59.39″ E). The area is known for extensive livestock rearing, particularly cattle and sheep, which contribute significantly to the local economy. Its consistently warm climate also supports a substantial population of free-roaming dogs, particularly near slaughterhouses.

For this study, fecal samples were obtained from 100 domestic dogs, mostly strays living around abattoirs. Each sample was collected and transported to the Parasitology Laboratory at the Faculty of Veterinary Medicine, South Valley University. Information regarding the age and sex of each dog was recorded to allow assessment of potential epidemiological patterns.

Microscopic examination

Fresh fecal specimens were screened for *Sarcocystis* oocysts and sporocysts using a sugar flotation procedure. Approximately 3 g of feces was mixed thoroughly with 16 ml of Sheather's sucrose solution (prepared from 454 g sugar, 355 ml water, and 6 ml of 37% formaldehyde; specific gravity = 1.27) [14]. The mixture was passed through a 0.6 mm sieve while stirring to remove debris, then transferred to a 15-ml centrifuge tube and centrifuged at 2000 × g for 3 minutes. A small amount of the supernatant was placed onto a glass slide and examined at 100× magnification with an Olympus optical microscope for the presence of *Sarcocystis* oocysts/sporocysts, following the protocol described by Dryden *et al.* [15].

Molecular analysis

DNA extraction

Thirty of the 100 fecal samples were selected for molecular testing. Genomic DNA was extracted using the Stool DNA Kit (D4015-01, Omega Bio-TEK, USA) according to the manufacturer's instructions. The extracted DNA was stored at –20 °C until PCR amplification.

PCR amplification

PCR was performed to amplify a fragment of the 18S rRNA gene using *Sarcocystis*-specific primers (Sar-F1: 5′-GCACTTGATGAATTCTGGCA-3′; Sar-R1: 5′-CACCACCCATAGAATCAAG-3′) as reported by Bahari *et al.* [16]. Each 50 µL reaction contained 50 ng of DNA, 50 pmol of each primer, 200 µM dNTPs, 5 µL of 10× PCR buffer (100 mM Tris-HCl, pH 9, 15 mM $MgCl_2$, 500 mM KCl), and 1 U Taq DNA polymerase (Promega). The PCR conditions included an initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of 94 °C for 45 seconds (denaturation), 55 °C for 1 minute (annealing), and 72 °C for 1 minute (extension), with a final elongation at 72 °C for 7 minutes [17].

Visualization of PCR products

PCR amplicons were analyzed by electrophoresis on a 1.5% agarose gel (peq GOLD, Peqlab, Germany) prepared in 1× TBE buffer. Approximately 20 µL of each PCR product was loaded into the wells alongside a 100 bp DNA ladder (peqGOLD 2 kb DNA Ladder, Peqlab, VWR) to determine fragment size. Gels were stained with 0.5 µg/mL ethidium bromide and visualized under ultraviolet light using a DigiDoc-It® Imaging System (UVP, UK). Gel images were recorded and analyzed with Totallab software.

Genomic DNA sequencing and phylogenetic analysis

PCR products were purified with the E.Z.N.A. Gel Extraction Kit (Omega Bio-TEK, USA) and submitted to Macrogen Inc., Korea, for sequencing. Purification was performed using micro spin columns, and sequencing was conducted on an ABI PRISM 3100 Genetic Analyzer (Micron-Corp., Korea). The resulting sequences were compared against existing sequences in the NCBI database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) following Altschul *et al.* [18]. The sequences obtained in this study were deposited in GenBank under accession number ON421649. Phylogenetic relationships were inferred using Maximum Likelihood, Neighbor-Joining, and Maximum Parsimony methods based on the 18S rRNA sequences, implemented in MEGA7 software [19].

Statistical analysis

The association between *Sarcocystis* prevalence and epidemiological factors (age and sex) was evaluated using the chi-square (χ^2) test in IBM SPSS Statistics for Windows, version 21. A p-value ≤ 0.05 was considered statistically significant [20].

Results

Prevalence of Sarcocystis spp. in dog feces

Coproscopic examination detected *Sarcocystis* stages in 4 out of 100 dogs (4%). In contrast, PCR analysis of 18S rRNA revealed infection in 16 out of 30 dogs (53.3%), showing a statistically significant difference between the two diagnostic methods ($\chi^2 = 21.572$, $P < 0.0001$) **(Table 1)**. Age-specific prevalence indicated that older dogs (21.95%) were more likely to harbor *Sarcocystis* than younger dogs (4.2%), although this difference was not statistically significant ($\chi^2 = 3.678$, $P = 0.05$). Similarly, male dogs exhibited a higher infection rate (19.05%) compared to females (8.7%), but the association was not significant ($\chi^2 = 1.223$, $P > 0.05$).

Table 1. Occurrence percentages of *Sarcocystis* infection relative to age and gender of dogs by coprological and PCR analysis

Variables		Coproscopy		PCR on genomic DNA extracted directly from feces		Total	
		Examined	Infected (%)	Examined	Infected (%)	Examined	Infected (%)
Sex	Male	66	4 (6.06)	18	12 (66.66)	84	16(19.05)
	Female	34	0 (0)	12	4 (33.33)	46	4(8.7)
Age	< 2 years	38	0 (0)	10	2(20)	48	2(4.2)
	≥ 2 years	62	4 (6.45)	20	14(70)	82	18(21.95)
Total		100	4 (4)	30	16 (53.33)	100	20(20)
P				< 0.0001			

Overall, the detection of *Sarcocystis* spp. was significantly higher when using molecular techniques compared to conventional coprological analysis ($\chi^2 = 5.4$, $p = 0.020$, $r = 0.6$, Fisher's $Zr = 0.693$, 95% CI: 0.1085–1.277). These findings indicate that PCR provides a more sensitive approach for identifying *Sarcocystis* stages in dogs than traditional fecal examination. In this study, coproscopy identified 4 of the 16 PCR-positive samples, resulting in a sensitivity of 25%. Conversely, 2 out of 14 PCR-negative samples were detected as positive by coproscopy, yielding a specificity of 85.7%. The agreement between the two diagnostic methods was low, as indicated by a Kappa value of 0.103 **(Table 2)**.

Table 2. Diagnostic performance of coproscopical analysis and PCR tool for detecting *Sarcocystis* stages in dog feces

Coproscopy	PCR		Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (95% C.I.)	NNP (95% C.I.)	^{K} values (95% C.I.)	SE of kappa
	+Ve (n = 16)	-Ve (n = 14)						
+ Ve	4(a)	2(b)	0.250 (0.319—to 0.651)	0.857 (0.421—0.996)	0.667 (0.185 - 0.946)	0.500 (0.377-0.623)	0.103 (0.279–0.484)	0.195
- Ve	12(c)	12(d)						

[a]true positive, [b]false positive, [c]false negative, [d]true negative

Morphologically, the recovered sporocysts were oval in shape and measured 13.2–16.0 × 9.4–11 µm **(Figure 1)**.

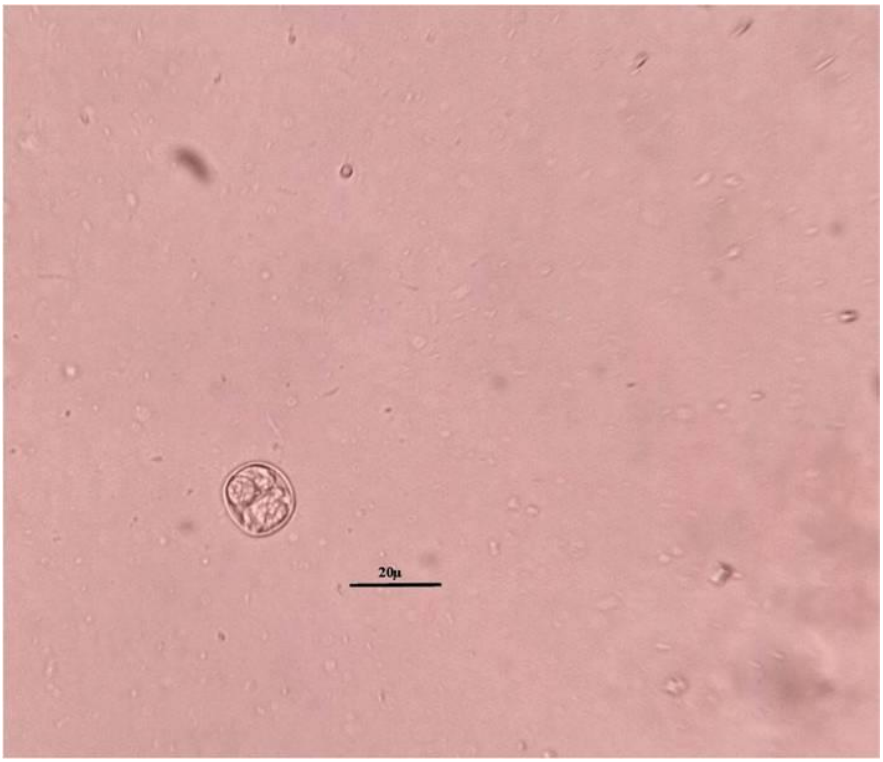

Figure 1. *S. tenella* sporocyst

Molecular and phylogenetic description

Sixteen out of the 30 PCR-tested samples yielded homogenous electrophoretic bands of 600 bp **(Figure 2)**.

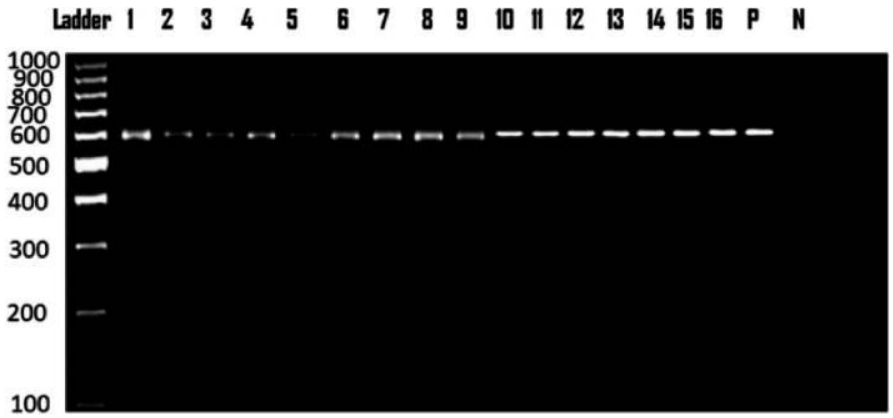

Figure 2. PCR products of the 18S rRNA gene visualized on agarose gel, showing bands at approximately 600 bp corresponding to *Sarcocystis tenella*. Lanes 1–16 represent fecal samples, P is the positive control, N is the negative control, and L denotes the 1 kb DNA ladder

Sequence analysis of the amplified 18S rRNA fragments (GenBank accession ON421649) revealed complete identity among all isolates. Comparative BLAST analysis indicated the closest match with *S. tenella* sequences

from sheep (MT569891, LC364052, OP302809, MH236177) at 99.82% similarity, while the sequence showed 99.64% similarity with a cattle-derived *S. cruzi* isolate (LC214880). Further comparison with Egyptian sheep isolates yielded 99.44% (MG515213, MG515220, MG515221) and 99.10% (MH413034) similarity. A single nucleotide deletion at position 81/566 distinguished our isolates from these references. In addition, sequence similarity with *S. tenella* from a Pampas fox (KY614537) was 99.15%, and 98.92% similarity was observed when compared with *S. capracanis* from goats and sheep (MW832482), as illustrated in **Figure 3**.

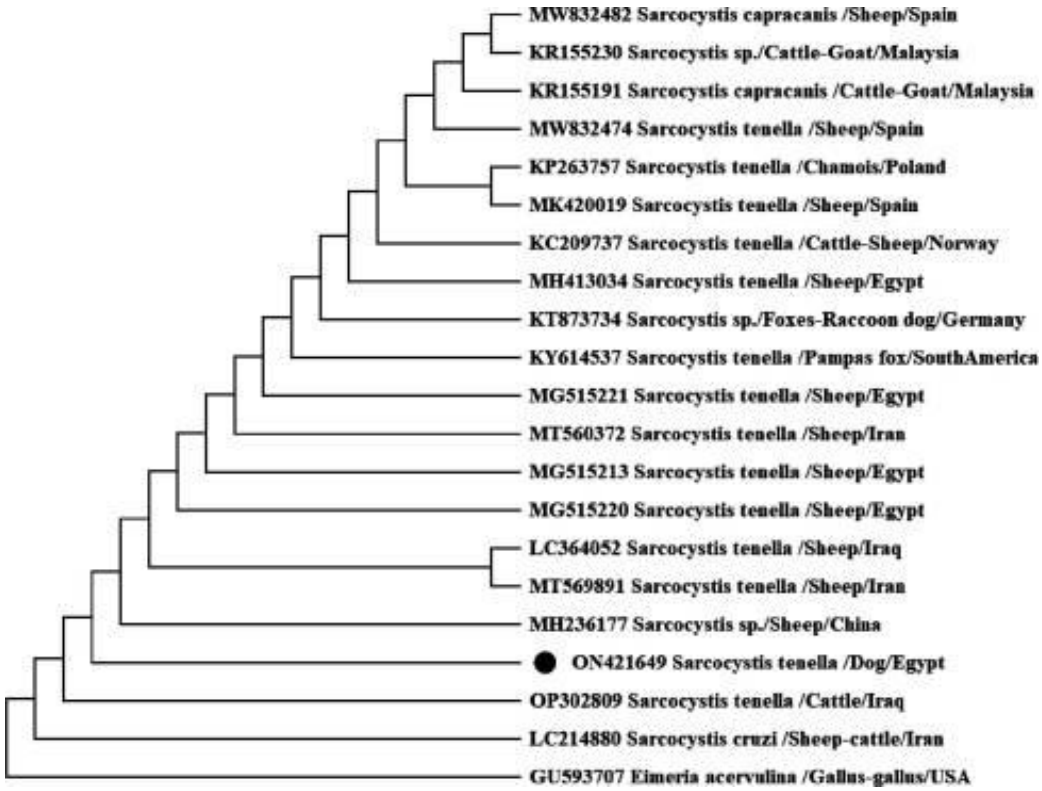

Figure 3. Phylogenetic relationships among selected *Sarcocystidae* members based on 18S rDNA sequences, with *Eimeria acervulina* serving as the outgroup. The tree was constructed using the Maximum Likelihood method in MEGA 7 software. GenBank accession numbers are provided alongside each taxon. The sequence obtained in this study (ON421649) is highlighted with a black circle

Discussion

Sarcocystis species are significant cyst-forming coccidian parasites with diverse life cycles and variable pathogenic effects on their hosts. Despite their veterinary and zoonotic importance, information regarding the prevalence and diversity of *Sarcocystis* species in Egyptian dogs has been limited. This study contributes to filling this knowledge gap by providing molecular and epidemiological data on canine sarcocystosis in Egypt.

The infection pattern observed in the present survey aligns with previous findings. For instance, Nathalia *et al.* [21] reported a 17.6% prevalence of *Sarcocystis* in Pampas fox intestinal and fecal samples. Globally, reported prevalence in dogs ranges from 2.2% to 9% [22–24]. In Egypt, Abbas *et al.* [25] found *Sarcocystis* oocysts in 29 out of 1126 dog fecal samples, corresponding to a pooled prevalence of 2.0%, while El Menyawe and Abdel Rahman [26] and Sabry and Lotfy [27] reported prevalence rates of 1.8% and 14.8% in Cairo and Giza, respectively. Other regions have shown both very low (0.3% in Calgary, Canada [28]) and much higher prevalence, such as 28.5% in Ethiopian domestic dogs [29] and 42–72% in sheepdogs from Peru [30].

Such variation in prevalence likely reflects multiple factors, including geographic location, dog ownership status, sampling methodology, population demographics, use of anthelmintics, and the sensitivity of diagnostic approaches. In Egypt, dogs often have access to tissues from infected livestock, particularly around slaughterhouses, where they may consume organs, aborted fetuses, or placentas. This exposure increases the risk of acquiring infections with cyst-forming parasites, including *Sarcocystis* and *Neospora* species [31, 32].

This study revealed an age-related trend in *Sarcocystis* infection among dogs, with older animals (>2 years) exhibiting the highest prevalence. Similar observations were reported by Katagiri and Oliveira-Sequeira [33], who noted increased sarcocystosis in older dogs. The higher infection rates in mature dogs may be associated with their use as guard animals, which increases their roaming behavior and likelihood of exposure to infected tissues. In contrast, puppies, which are less active hunters, are presumably at lower risk. However, some earlier studies reported higher parasitic burdens in younger dogs, suggesting that immature immunity and reduced passive protection from maternal antibodies may predispose puppies to protozoan and helminth infections [34–40].

In this survey, sex did not significantly influence *Sarcocystis* infection, although male dogs showed slightly higher prevalence than females. This finding contrasts with the work of Zelalem and Mekonnen [41], who observed higher infection rates in females, potentially due to reproductive stress compromising immune function.

The current results also highlight the limitations of light microscopy alone for routine detection of *Sarcocystis*. While traditional coproscopic methods provide preliminary information, molecular tools such as PCR offer higher sensitivity and specificity for species identification. Nonetheless, combining both approaches remains valuable for comprehensive screening [42–44]. Morphometric analysis of recovered *S. tenella* sporocysts in this study corresponded with previous reports by Saito *et al.* [45] but differed from measurements reported for related species, including *S. gracilis* (red deer origin), *S. gigantea*, and *S. medusiformis* (sheep-cat origin) [2, 45], as well as *S. arieticanis* from sheep-dog hosts. According to Levine [46], no *Sarcocystis* species naturally parasitizes more than one genus of intermediate host.

Importantly, this investigation provides the first molecular confirmation of domestic dogs acting as definitive hosts for *S. tenella* in Egypt. Prior to this study, no experimental evidence had documented this role, although closely related sequences have been reported from other carnivores, such as the Pampas fox (*Lycalopex gymnocercus*, 99.15% identity). The detection of *S. tenella* in dog feces from Upper Egypt suggests that these animals likely ingested tissues from small ruminants or prey harboring related *Sarcocystis* species.

Globally, few studies have examined carnivores as definitive hosts for *Sarcocystis* using combined molecular and phenotypic analyses. Nathalia *et al.* [21] identified the Pampas fox as a definitive host for *S. cruzi*, *S. tenella*, and possibly other species utilizing birds as intermediate hosts. Similarly, More *et al.* [13] reported multiple *Sarcocystis* species in fox intestinal mucosa, including *S. tenella* or *S. capracanis* (10%), *S. miescheriana* (8%), *S. gracilis* (8%), and species using birds as intermediate hosts (6%). In raccoon dogs, sequences with ≥99% identity were detected for *S. miescheriana* (18.4%), *S. gracilis* (13.1%), *Sarcocystis* spp. with avian intermediate hosts (10.5%), *S. tenella* or *S. capracanis* (2.6%), and *S. capreolicanis* (2.6%). In Hungary, dog fecal isolates matched 100% with *S. morae* previously reported from cervids in Lithuania and Spain [47]. The *S. tenella* isolates from Egyptian sheep (MG515213, MG515220, MG515221, MH413034) shared 99.44% and 99.10% nucleotide identity with the dog isolate identified here (ON421649) [48, 49].

Conclusions

This study establishes a molecular framework for detecting and characterizing *Sarcocystis* infections in dog fecal samples from Aswan, Egypt. The combination of 18S rRNA gene amplification, sequencing, and phylogenetic analysis proved effective for identifying definitive hosts and confirming species-level infections. Future research should focus on elucidating the complete life cycle of *Sarcocystis*, assessing the health impacts on definitive hosts, and monitoring epidemiological and taxonomic patterns in both livestock and carnivore populations using multiple genetic markers.

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Conflict of Interest: None

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Ethics Statement: The current study was approved by the Ethics Committee, Faculty of Veterinary Medicine, South Valley University, Egypt according to the ethical principles of animal research and regulations (under

permit number: VM/SVU/23(2)-23). Oral consent was achieved from each owner of inspected dog. Furthermore, the owners of dogs involved in the current study were informed about the objectives of the study and their contact details was also recorded to obtain the results of the survey.

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