

Meat sarcocystosis: a critical meat-borne parasite impacting carcasses in abattoirs

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Abstract

One of the most commonly occurring foodborne, tissue cyst-forming protozoan zoonotic parasites with public health and veterinary relevance is *Sarcocystis* species. This research was conducted using traditional and molecular techniques to identify the incidence of *Sarcocystis* spp. in 750 slaughtered bovine animals of varying age and sex (375 cattle and 375 buffalo) at various abattoirs in Aswan Governorate, Egypt. The overall occurrence of macroscopic lesions of *Sarcocystis* spp. in cattle and buffaloes was 20.5% and 38.4%, respectively, while the occurrence of microscopic infection was 30.4% and 70.1%, respectively. Furthermore, the disease strongly correlated with the measured variables, such as the animal's age and sex. Animals older than 5 years had the highest infection rate, and females had higher infection rates than males. The esophagus, tongue, and diaphragm had the highest rates of sarcocyst compared to other organs. Histopathology studies of sarcocyst in various tissues revealed encased, circular to elongated, basophilic sarcocysts with numerous bradyzoites embedded in muscle fibers. PCR-RFLP reports that *S. cruzi*, *S. hominis*, *S. hirsuta*, *S. buffalonis*, and *S. fusiformis* were the species most often found. Moreover, such frequency highlights the pressing need for an effective disease control strategy and a systematic surveillance system among bovine populations. We strongly encourage the One Health approach to reduce zoonotic spread to humans and financial losses in the livestock industry.

Keywords: abattoirs, meat, postmortem inspection, PCR-RFLP, *Sarcocystis*

Introduction

Meat is the primary source of animal protein for a variety of consumers, leading to a global increase in the demand for red meat consumption (FAO/WHO, 2014). As a result, abattoirs play a crucial role in managing and controlling various zoonotic infections, including *Sarcocystis*, a parasite responsible for foodborne illnesses that humans can contract by consuming undercooked or raw meat (Albayati et al., 2023). *Sarcocystis* species, typical intracellular coccidian foodborne parasites of the phylum Apicomplexa, undergo a required two-host life cycle, with sexual development in the intestinal tract of the definitive host and asexual growth in many tissues of the intermediate host (Elshahawy et al., 2022). Cysts of this species are detected in the hearts, esophagus, diaphragms, tongues, masseter muscles, and other skeletal muscles of herbivores, their intermediate hosts, following multiple growth phases (Oğuz et al., 2020).

Many types of *Sarcocystis* species, including *S. cruzi* (which needs canids as its main host), *S. bovifelis*, *S. bovis*, and *S. hirsuta* (which need felids as their main host), *S. hominis*, and *S. heydorni* (which need humans as their main host), and *S. bovis* and *S. sigmoideus* (the definitive hosts are unknown), use cattle as their main intermediate host (Gjerde, 2016; Gupta et al., 2024). *Sarcocystosis* commonly causes eosinophilic myositis and myocarditis in bovines, leading to financial losses due to reduced milk production, miscarriages, and newborn deaths (Elshahawy et al., 2022). Similarly, human sarcocystosis can manifest in two distinct forms as a result of the ingestion of raw and/or inadequately cooked beef. The most well-known form is intestinal sarcocystosis caused by zoonotic species, which results in symptomatic stomach disruption (Dubey, 2015). The second case includes muscular involvement, which occurs when humans become accidental hosts as a result of the consumption of contaminated food by livestock *Sarcocystis* spp. fecal sporocyst stage (Murata et al., 2018). *Sarcocystis* secretory toxins have recently elevated the importance of this issue (Ota et al., 2019).

Concerns about meat-borne parasites are growing for several reasons, including the increasing consumption of raw and/or lightly cooked foods, which may increase exposure to parasites, and the growing importation of food, some of which comes from countries lacking advanced hygiene standards and testing procedures (CDC, 2000). *Sarcocystosis* in meat significantly diminishes the quality, and a high meat rating renders meat and offal unpalatable to consumers. *Sarcocystosis* is one of the zoonotic foodborne diseases that affect both humans and animals, in addition to the substantial financial losses that result from the condemnation

of carcasses and/or offal in slaughterhouses. *Sarcocystis* infections in intermediate hosts are frequently asymptomatic, but serious cases have been reported, causing weariness, lack of appetite, diarrhea, weight loss, muscular convulsions, and, in extreme cases, death. Muscle tissue cysts containing bradyzoites distinguish *sarcocystosis*, a degenerative change in the intermediate host (Albayati et al., 2023).

This research aims to investigate the incidence of *Sarcocystis* infection in cattle and buffalo, which are strategically valuable and economically significant animals slaughtered at various central abattoirs in Aswan Governorate, Egypt, through macroscopic and microscopic investigation, as well as molecular recognition of *Sarcocystis* spp. by PCR-RFLP of the 18S rRNA gene to reduce the zoonotic risk posed by this parasite.

Materials and Methods

Research period and area

A cross-sectional investigation was conducted in Aswan, which is located in southern Egypt and experiences the warmest, hottest summers. Its 62,726 km² region is bounded by latitude 24° 5' 20.18" N and longitude 32° 53' 59.39" E. To ascertain the occurrence of *Sarcocystis* spp. and their monetary effect on slaughtered bovines (cattle and buffalo), the present retrospective analysis was carried out in several different abattoirs in Aswan Province, Egypt, between April and October 2024. The chosen abattoirs were the central slaughterhouses selected for their high annual animal throughput and varied geographic locations for the cattle. We reasoned that samples from these abattoirs would provide a thorough understanding of the prevalence of sarcocystosis in the research region because they receive slaughtered animals from different places. A comprehensive antemortem clinical assessment and postmortem investigation were performed on the slaughtered animals. Daily records for animals designated for slaughter were obtained from the general veterinary services' archives.

Sample size

Thrusfield (2017) computed the sample size using a 95% confidence interval (CI) and 5% absolute precision. Thus, Elshahawy et al. (2022) calculated the anticipated prevalence of *Sarcocystis* spp. to be 92.5%.

$$n = \frac{Z^2 \times P_{\text{exp}}(1 - P_{\text{exp}})}{d^2}$$

n = requisite sample size, Z = appropriate percentage for the standard deviation for the expected confidence = 1.96, P_{exp} = predictable occurrence, and d = anticipated absolute accuracy (usually 0.05).

$$n = \frac{1.96^2 \times 0.925(1 - 0.925)}{0.05^2} = 106.6 \text{ (Minimum sample size)}$$

In contrast, 750 samples from cattle and buffaloes were inspected for the occurrence of *Sarcocystis* spp., with the larger sample size boosting the likelihood of discovering positive cases.

Study animals

According to the legal requirements of Egyptian abattoirs, routine daily antemortem and postmortem inspections examined about 750 slaughtered bovines of various ages and sexes, consisting of 375 cattle (237 male and 138 female) and 375 buffaloes (158 male and 217 female). Animal ages were determined by dental eruptions and categorized as <2 years (young), 2–5 years (adult), and >5 years (old).

Antemortem and postmortem checkup

Every animal underwent a physical clinical checkup before slaughter, adhering to the antemortem assessment protocols under the Egyptian Guidelines for Cattle Inspection under Law 517 (GOVS, 1986). Specifically, superficial lymph nodes, visible mucous membranes, and body condition were examined. The postmortem assessment for *Sarcocystis* spp. was carried out under Egyptian regulations (GOVS, 1986). The gross inspection involved slicing muscle masses from the esophagus, heart, tongue, masseter muscles, and skeletal muscles, which were meticulously examined and palpated with the naked eye for the detection of macroscopic sarcocysts. Upon the detection of a cyst, the sample was regarded as positive macroscopically; otherwise, it was subjected to microscopic analysis using the digestion method.

Samples collection

From each apparently *Sarcocystis*-free carcass, samples (75 g) were collected from the esophagus, heart, tongue, diaphragm, masseter muscles, and skeletal muscles of the fore and hind quarters to detect the incidence of microscopic bradyzoites. All samples were labeled, transported to Aswan University, Faculty of Veterinary

Medicine, Food Hygiene Laboratory, and stored at 4°C until analysis.

Microscopy identification of *Sarcocystis* bradyzoites

Oguz *et al.* (2021) employed the pepsin digestion method for the identification of microcysts. In summary, 5 g of samples were placed in a 50 cc trypsin solution (1.3 g pepsin, 3.5 ml 25% HCl, 2.5 g NaCl, and 500 ml distilled water), thawed, and chopped for 30 minutes at 40 °C, and then homogenized using a tissue homogenizer. The filtrate was placed into a tube after the broken-down content was passed through a smooth mesh filter. Before processing each tissue, the homogenizer was washed with boiling water. The filtrate was centrifuged for ten minutes at 3500 rpm. A small amount of sediment at the bottom was then removed using a pipette, stained with Giemsa staining, and morphologically analyzed for *Sarcocystis* bradyzoites using a light microscope (x40).

Histopathological inspection

Tissue samples from positive microcyst samples were preserved in 10% neutral buffered formalin, dried in a series of graded alcohols, cleaned in xylene, embedded in paraffin, cut into 5 µm thick sections, and then placed on slides (Elbarbary *et al.*, 2024). The samples were subsequently stained with hematoxylin and eosin and observed under a microscope. Highly qualified staff members from the Pathology Department, Faculty of Veterinary Medicine, New Valley University, thoroughly inspected every slide for microscopic *Sarcocystis*. The results were captured and documented using a Canon digital camera (Canon Powershot A95) attached to a Leitz Dialux 20 Microscope (Germany).

Sarcocystis species DNA identification

Getting DNA out

The Quick-gDNA™ MiniPrep reagent (Catalog No. D3024, Zymoresearch, USA) was used to extract DNA from microscopically positive samples from each category and preserved at -20°C until PCR analysis (Khairy *et al.*, 2024).

PCR amplification

PCR was used to identify the 18S rRNA gene of *Sarcocystis* species specific for cattle and buffalo using the primers (Willowfort Company, United Kingdom), sarF-5'CGTGGTAATTCTATGGCTAATACA'3 and sarR-5'TTTATGGTTAAGACTACGGGTA'3, at 900 bp (Hooshyar *et al.*, 2017). Each 25 µL PCR reaction

included 5 µL of genomic DNA (~25 ng) and 1 µL of each primer at a 20 pmol concentration, mixed with 12.5 µL of EmeraldAmp Max PCR Master Mix (Takara, Japan) and 5.5 µL of nuclease-free water. Denaturation was performed at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 sec, 57.5 °C for 45 sec (annealing), and 72 °C for 60 sec (extension), with a final extension at 72 °C for 5 min. Extracted DNA samples were electrophoresed in a 1.5% agarose solution in 1x TBE electrophoresis buffer at 80 V for 100 min; the gel was stained with ethidium bromide and then photographed on a UV transilluminator. The fragment sizes at 900 bp were confirmed using a 100 bp plus DNA ladder (Qiagen, Germany, GmbH).

Restriction fragment length polymorphism (RFLP)

To differentiate distinct *Sarcocystis* species, PCR products were digested with the restriction endonucleases *BslI* and *SspI* (FastDigest, Thermo Scientific) according to Jehle *et al.* (2009) and Hamidinejat *et al.* (2015). With the *BslI* enzyme, the specific fragment sizes of RFLP digestion produce 343 and 513 bp for *S. cruzi*, 110, 242, and 525 bp for *S. buffalonis*, and 335 and 532 bp for *S. fusiformis*. *SspI* enzyme-assisted RFLP digestion yields 260 and 647 bp for *S. hirsuta* and 233 and 637 bp for *S. hominis*. In a 50 µL reaction volume, PCR products were directly subjected to RFLP analysis, which included 5 µL of reaction buffer and 1 µL (10 U/µL) of restriction enzyme (*SspI* and *BclI*) combined with 10–20 µL of PCR product. The restricted mixture was incubated for 16 hours at either 37 °C (*SspI*) or 55 °C (*BslI*). As directed by the manufacturer, enzymes were inactivated for 20 min at 65 °C (*SspI*) and 80 °C (*BslI*). After separation on 2% agarose gels and staining with ethidium bromide, the resulting restriction fragments were examined under ultraviolet light. A 100 bp DNA ladder was used as a scale marker.

Analyzing data

Microsoft Excel was used for the computations, and SAS (2004) Version 5 was used to apply the chi-square test to determine statistical significance. Branger (2013) discussed the analytic and prognostic utilities of microscopic examination.

Results

Incidence of *Sarcocystis* spp. in inspected carcasses and related major risk factors

Table 1 displays the characteristics of animals with suspicious *Sarcocystis* found during postmortem investigations; out of 750 examined slaughtered cattle and buffaloes, 20.5% (77/375) and 38.4% (144/375),

respectively, were found to have macroscopic sarcocyst lesions, while the infection with microscopic sarcocyst was 30.4% (114/375) and 70.1% (263/375), respectively. Additionally, the findings showed that older cattle and buffaloes had a greater prevalence of macroscopic sarcocyst (41.5% and 52.3%) and microscopic sarcocyst (51.5% and 83.5%) than younger ones. According to the current findings, the young calf carcasses did not appear to have any apparent cyst infections. Both macroscopic cysts (29% and 51.6%) and microscopic cysts (52.2% and 88%) were highly prevalent in female cattle and buffaloes, respectively. Also, the majority of the bovine carcasses positive for *Sarcocystis* were discovered at the Aswan and Kom Ombo slaughterhouses. The age and sex of the slaughtered animals were found to be significantly linked to the occurrence of *Sarcocystis* ($p \leq 0.05$).

Distribution of *Sarcocystis* spp. in different tissues

Localized sarcocyst lesions, mostly restricted to one or a few organs, were present in the majority of positive carcasses (Table 2). Macroscopic cysts were found more frequently in cattle and buffalo tissues, including the esophagus (19.5% and 36.5%), heart (15.5% and 25.9%), tongue (19% and 33.9%), diaphragm (17.6% and 35%), masseter muscles (16.5% and 32.2%), forequarters (13% and 22.6%), and hindquarters (10.4% and 17.9%), respectively. Microscopic cysts were found in the following tissues in cattle and buffalo: esophagus (28.5% and 66.4%), heart (23.5% and 46.7%), tongue (26.1% and 59.5%), diaphragm (27.5% and 63.5%), masseter muscles (27.2% and 62.1%), forequarters (19.2% and 31.7%), and hindquarters (18.1% and 23.7%), respectively.

Morphological identification of isolated macroscopic *Sarcocystis*

The macroscopic sarcocysts cysts that have been found can be fusiform, oval, spindle, elongated, cucumber, or rice-seed shaped and consist of opaque bodies that are milky white in color, located between muscle bundles along the longitudinal axis of the muscle mass. Macrocyts were found in various organs, either just beneath the serosal surface, as in the esophagus, or deep within the muscular layer, as in the diaphragm, tongue, and masseter muscles (Figures 1 and 2).

Histopathological findings

The recovered microcysts were mostly fusiform, with an oval shape. The cell wall was thin and smooth, with a granular layer immediately underneath. There were septa that extended from the granular layer, splitting the

Table 1. Prevalence of sarcocyst infection in the examined bovine carcasses.

Characteristics	Examined animal (n=750)				Positive for sarcocyst							
	Cattle		Buffalo		Macroscopic				Microscopic			
					Cattle		Buffalo		Cattle		Buffalo	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total No.	375	50	375	50	77	20.5	144	38.4	114	30.4	263	70.1
Age												
young	103	27.5	87	23.2	0	0	17	19.5	6	5.8	44	50.6
adult	142	37.9	112	29.9	23	16.2	35	31.3	41	28.9	72	64.3
old	130	34.6	176	47	54	41.5*	92	52.3*	67	51.5*	147	83.5*
Chi ² *					6.35		9.27		8.41		11.74	
Sex												
Male	237	63.2	158	42.1	37	15.6	32	20.3	42	17.7	72	45.6
Female	138	36.8	217	57.9	40	29*	112	51.6*	72	52.2*	191	88*
Chi ² *					5.83		8.26		10.62		12.35	
Slaughterhouse												
Aswan	124	33.1	111	29.6	37	29.8*	63	56.8*	53	42.7*	96	86.5*
Daraw	62	16.5	56	14.9	6	9.7	12	21.4	13	21	28	50
Kom Ombo	106	28.3	117	31.2	23	21.7	48	41*	31	29.2	84	71.8*
Edfu	83	22.1	91	24.3	11	13.3	21	23	17	20.5	55	60.4
Chi ² *					7.48		10.61		10.32		12.74	

* = significantly different by Chi-square statistics at ($p < 0.05$) for each character.

Table 2. Prevalence of sarcocyst in different tissues of slaughtered bovine.

Affected organ	Macroscopic				Microscopic			
	Cattle		Buffalo		Cattle		Buffalo	
	No.	%	No.	%	No.	%	No.	%
Esophagus	73	19.5*	137	36.5*	107	28.5*	249	66.4*
Heart	57	15.2	97	25.9*	88	23.5	175	46.7*
Tongue	71	19	127	33.9	98	26.1	223	59.5
Diaphragm	66	17.6	131	35	103	27.5	238	63.5
Masseter muscles	62	16.5	121	32.2	102	27.2	233	62.1
Forequarter muscles	49	13*	85	22.6	72	19.2*	119	31.7
Hindquarter muscles	39	10.4*	67	17.9*	68	18.1	89	23.7*
Chi ² *		5.43		8.25		9.63		11.22

Positive samples % was calculated from the total examined carcasses ($n = 375$ of each).

Chi²* Significantly different at ($p < 0.05$).

cysts into sections with many banana-shaped bradyzoites inside. The histopathological analysis revealed that deteriorated cysts were located in areas of tissue necrosis. Additionally, there was an absence of an inflammatory response in the majority of the examined slides, and, to some extent, there was inflammation surrounding the muscle fibers and among the cysts, where neutrophils, lymphocytes, eosinophils, and plasma cells were present (Figure 3).

PCR-RFLP findings

The PCR investigation of all microsarcocyst samples indicated that 89 (78.1%) out of 114 cattle carcasses and 217 (82.5%) out of 263 buffalo carcasses had positive diagnostic bands at 900 bp on gel electrophoresis, demonstrating the occurrence of the *Sarcocystis* spp. 18S rRNA gene (Figures 4). On the other hand, Figures 5 & 6 display gel electrophoresis of the PCR-RFLP by amplification of the

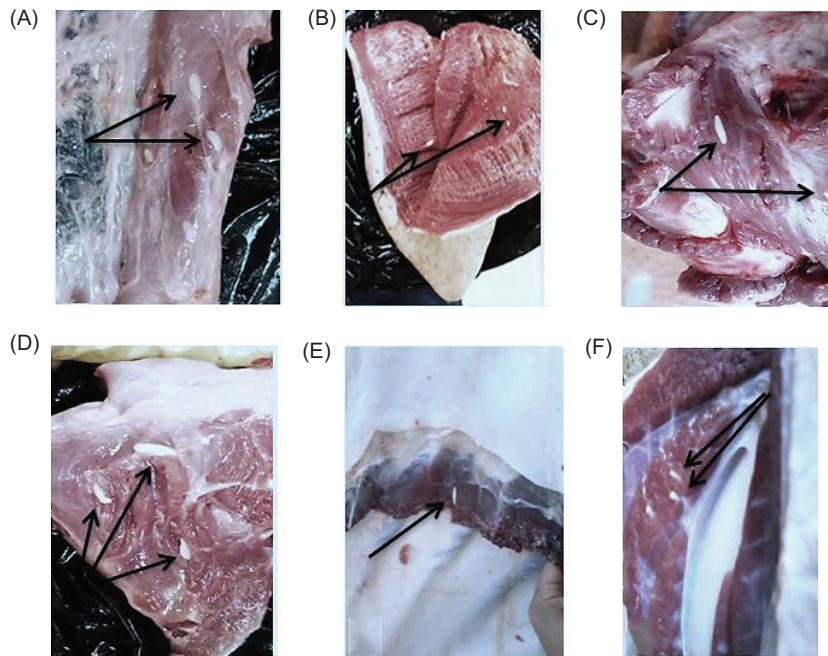


Figure 1. Macroscopic appearance of sarcocysts (indicated by black arrows) in different tissues of cattle carcass: esophagus (A), tongue (B), skeletal muscles (C), heart (D), diaphragm (E), and masseter muscles (F).

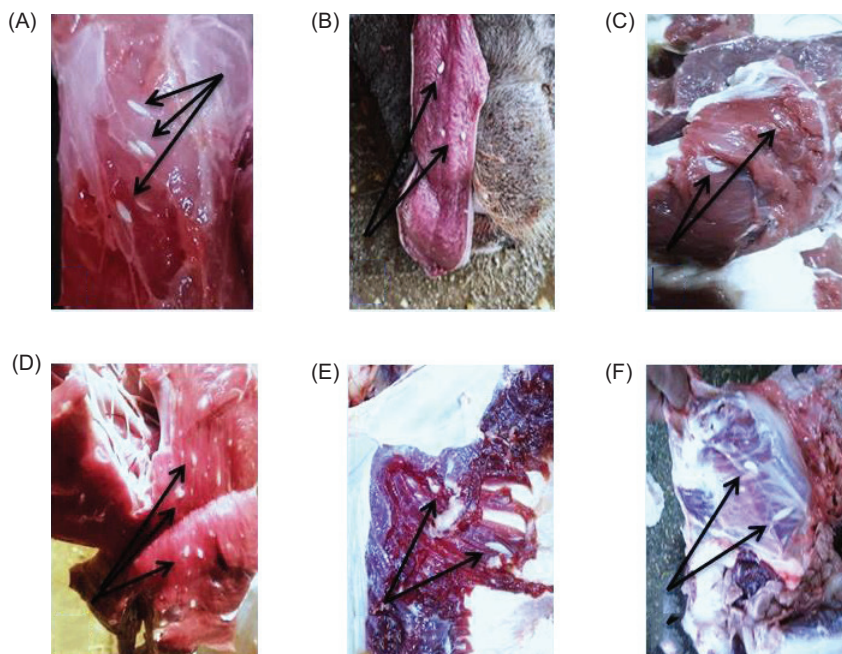


Figure 2. Macroscopic appearance of sarcocysts (indicated by black arrows) in different tissues of buffalo carcass: esophagus (A), tongue (B), skeletal muscles (C), heart (D), diaphragm (E), and masseter muscles (F).

18S rRNA gene from all positive isolates of microsarcocysts by PCR, revealing a unique fragment pattern for *Sarcocystis* spp. with the restriction endonucleases *SspI* enzyme (*S. hominis*, *S. hirsuta*) and *BslI* enzyme (*S. cruzi*, *S. buffalonis*, *S. fusiformis*).

Discussion

Sarcocystis, an intracellular protozoan that infects a diverse array of vertebrates, livestock, and humans, is a parasite that contributes to the development of

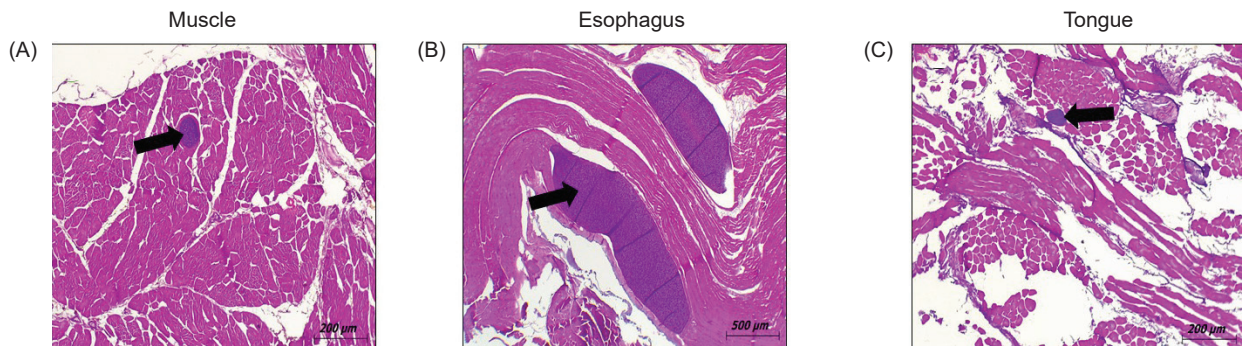


Figure 3. Histopathological observation of sarcocysts in different tissues stained with H&E stain. Encapsulated, circular to elongated basophilic sarcocysts filled with numerous bradyzoites, inserted into muscle fibers (indicated by black arrow) (A,C). B: large fusiform shaped, thin-walled, banana-like bradyzoites.

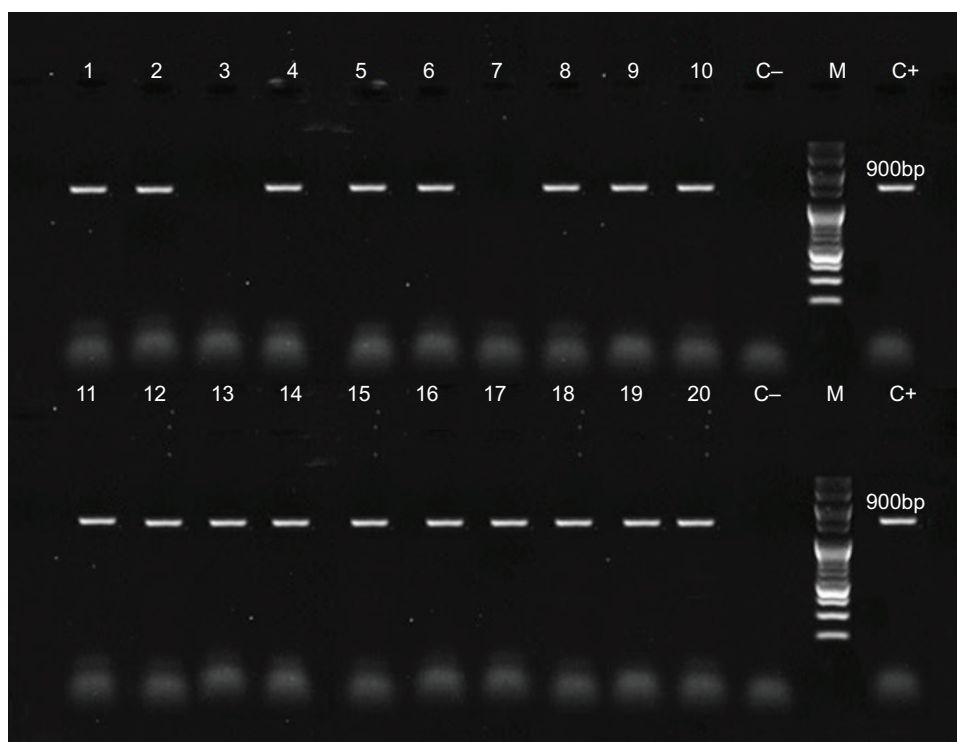


Figure 4. Agarose gel showing PCR amplification pattern for the 18S rRNA gene of *Sarcocystis* spp. from examined cattle (lane 1 to lane 10) and buffalo (lane 11 to lane 20) at 900 bp. CN: control negative, CP: control positive, M= Marker (100 bp).

food-borne diseases (Hussein *et al.*, 2023). This makes food safety measures crucial. Additionally, *Sarcocystis* is considered one of the primary contributors to economic losses in the livestock industry and may result in health issues for consumers. As part of the meat assessment procedure, split carcasses, organs, and lymph nodes are checked to ensure they are safe for human consumption and to collect global epidemiological information about zoonotic and parasitic infections like *Sarcocystis* (Lawan *et al.*, 2020). The Egyptian General Organization of Veterinary Services employs visual, palpation, and incision assessments to conduct slaughterhouse monitoring

to identify the presence of disease in slaughtered animals for consumption (Khairy *et al.*, 2024).

Visual evaluation of muscle tissues in this study revealed macroscopic cysts as elongated, ovoid, and cigar-shaped, with rounded tips. A thorough postmortem investigation revealed a total rate of 20.5% and 38.4% macroscopic cysts and 30.4% and 70.1% microscopic cysts among slaughtered cattle and buffaloes, respectively. However, the incidence of *Sarcocystis* differs among the analyzed abattoirs. The Aswan and Kom Ombo abattoirs show a statistically significant greater frequency than others in the studied area, implying

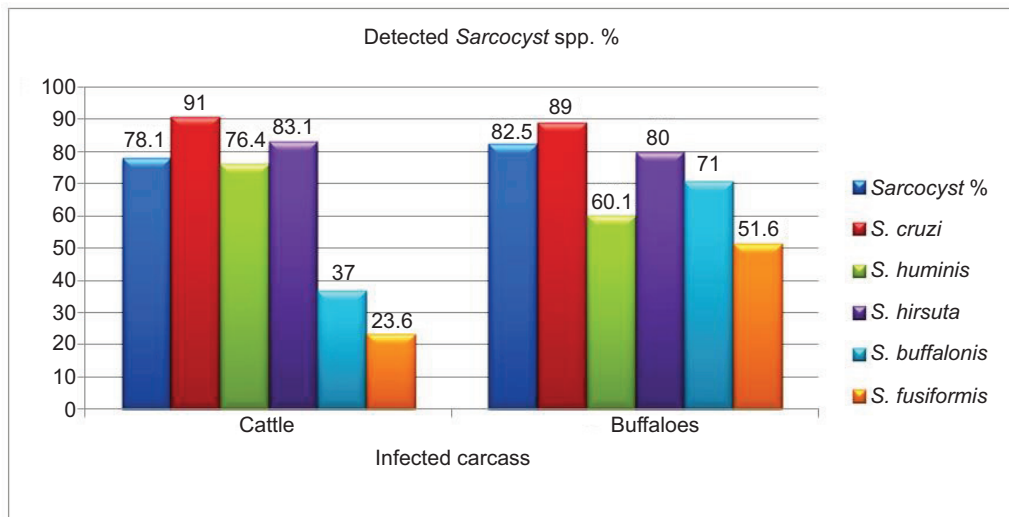


Figure 5. Identification of *Sarcocystis* spp. in slaughtered food animals using PCR-RFLP.

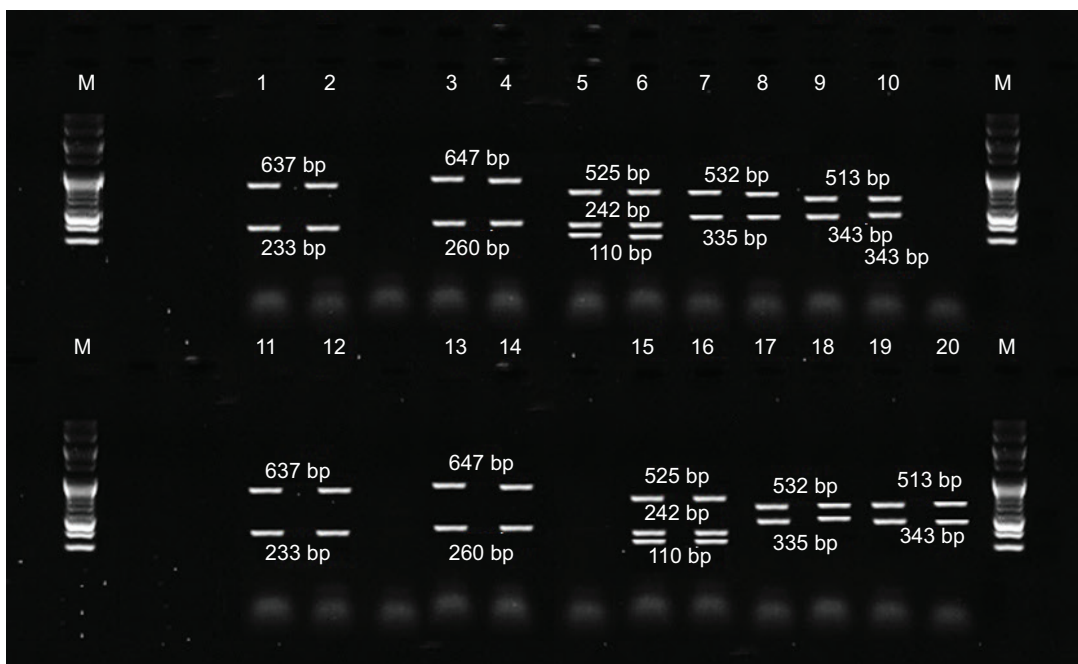


Figure 6. PCR-RFLP analysis of PCR products of the 18S rRNA gene of *Sarcocystis* spp. digested by the *Ssp1* enzyme, resulting in 233 and 637 bp for *S. hominis* (Lanes 1&2 and 11&12), 260 and 647 bp for *S. hirsuta* (Lanes 3&4 and 13&14), and by the *Bsl1* enzyme, resulting in 110, 242, and 525 bp for *S. buffalonis* (Lanes 5&6 and 15&16), 335 and 532 bp for *S. fusiformis* (Lanes 7&8 and 17&18), and 343 and 513 bp for *S. cruzi* (Lanes 9&10 and 19&20) from examined cattle and buffalo, respectively.

a highly contaminated environment. A thorough postmortem investigation revealed a total frequency of 20.5% and 38.4% macroscopic cysts and 30.4% and 70.1% microscopic sarcocyst in slaughtered cattle and buffaloes in this study, respectively. Nonetheless, the incidence of *Sarcocystis* differs among the analyzed abattoirs ($p \leq 0.05$). The Aswan and Kom Ombo abattoirs show a statistically significant greater

frequency than others in the studied area, implying a highly contaminated environment. The high infection rate in this investigation might be because the survival and viability of sporocysts in the environment are affected by seasonal changes in the infection, which are influenced by climate conditions like high temperature and humidity that characterize the studied area (Elshahawy *et al.*, 2022).

Additionally, bovines are commonly susceptible to infection due to their frequent breeding in regions where stray canines and cats are present or environmental pollution caused by infectious species (El-Sayad *et al.*, 2023).

Previous indigenous investigations revealed a wide range of infection rates in various Egyptian provinces: 7.5% in the New Valley city (Ahmed *et al.*, 2016), 26.5% in Tanta (El-Sayad *et al.*, 2023), 28% in Sohag (Khalifa *et al.*, 2008), 88% in Menoufia (Mousa *et al.*, 2021), and 92.5% in Aswan (Elshahawy *et al.*, 2022). Similarly, researchers worldwide have documented the highest peak occurrences between 91.0% and 100%, including Argentina (More *et al.*, 2008), the Southwest of Iran (Hamidinejat *et al.*, 2010), Southern Italy (Bucca *et al.*, 2011), Karnataka, India (Dafedar *et al.*, 2011), Italy (Chiesa *et al.*, 2013), Yazd and Hungary (Hornok *et al.*, 2015), Iran (Sarafraz *et al.*, 2020), Belgium (Zeng *et al.*, 2021), Iraq (Albayati *et al.*, 2023), and Iran (Dalir *et al.*, 2023). Variances can be attributable to sample size, examined organs, diagnostic procedures, and, most importantly, the existence of infectious stages in the environment. A high frequency of sarcocystosis is associated with close interaction between the final and intermediate hosts (Dong *et al.*, 2018).

The current study found a statistically significant correlation between the age and sex of the animal and the frequency of infection in older female animals, specifically buffaloes. Earlier investigations in Egypt (El-Sayad *et al.*, 2023; Elshahawy *et al.*, 2022; Gareh *et al.*, 2020; Mousa *et al.*, 2021) and other countries (Fayer *et al.*, 2015; Imre *et al.*, 2019; Mounika *et al.*, 2018; Zeng *et al.*, 2021) have also found a steady increase in infection with age. The animal management organization in Egypt may be responsible for the low ratio of infected bulls, as the majority of bulls are confined to the fattening system and are slaughtered at approximately two years of age, while cows are reserved for extended periods for milk production (Elrais *et al.*, 2022). The higher infection rate may be explained by the fact that every female infected in our study was older than five years, which corresponds to the age at which the legislation of the Egyptian Ministry of Agriculture (laws 53/1966 and 207/1980) compels female slaughter. The results did not match those of Mousa *et al.* (2021), who found that microscopic cysts were more common in bulls (92% vs. 84%) in Egypt; El-Kady *et al.* (2018) found that they were 76.2% more common in bulls than in cows (9.5%) in Qena, Egypt; and Obijiaku *et al.* (2013) found 40% in cows and 47.7% in bulls in Nigeria. In young cattle, microcysts were discovered, but no macroscopic cysts were seen. Shekarforoush *et al.* (2013) attribute the scarcity of macroscopic cysts to their feline origin and the unusual cattle-cat contact in the study area. Likewise, Elshahawy *et al.* (2022), Mousa *et al.* (2021), and Yang and Dong (2018) found no macroscopic *Sarcocystis* cysts in the investigated cattle.

According to the findings of the present investigation, there was a statistically significant connection between the percentage of infection and the tissues that were examined, with the esophagus being the organ most frequently infected. Visual inspection of organs showed that *Sarcocystis* macrocysts preferentially localized to the esophagus, followed by the tongue and diaphragm, while the hindquarter muscles had the fewest macrocysts. It appears that the esophagus was an earlier stop on *Sarcocystis*' migration route. Accordingly, future studies on the occurrence of *Sarcocystis* in cattle should focus on the esophagus, as it may be more useful in recognizing the infection at an earlier stage. A previous study that tracked the esophagus as the organ most frequently affected supported this outcome (Ahmed *et al.*, 2016; El-Sayad *et al.*, 2023; Elshahawy *et al.*, 2022; Elrais *et al.*, 2022; JyothiSree *et al.*, 2017; Mousa *et al.*, 2021). In contrast, numerous countries, including Argentina (Moré *et al.*, 2011), Italy (Bucca *et al.*, 2011), Brazil (Ferreira *et al.*, 2018), Iran (Shahraki *et al.*, 2018), the Netherlands (Hoeve-Bakker *et al.*, 2019), and Belgium (Zeng *et al.*, 2021), have recorded high incidence rates in the hearts and diaphragms of cattle, respectively, ranging from 58% to 99.5%, indicating that the heart and diaphragm are target colonizing sites for *Sarcocystis*. In addition to the distinctions between the aforementioned organs and muscles, variations in detection and identification methods (such as morphological and molecular recognition) and the research populations (such as age, sex, and breed) can lead to differences in the occurrence rates reported in the studies.

Improvements in livestock diagnosis techniques are crucial (Fotouh *et al.*, 2024). This study subjected cysts recovered from slaughtered carcasses to a variety of diagnostic techniques beyond visual assessment. In this respect, the process of digestion and histological examination was applied to investigate microscopic cysts. Microscopic analysis revealed microcysts within the tissues of several organs, including the esophagus, heart, tongue, diaphragm, masseter muscles, forequarters, and hindquarters. These findings were consistent with other studies conducted on different hosts, which found minute cysts in various bovine tissues (Elrais *et al.*, 2022; El-Sayad *et al.*, 2023; Zeng *et al.*, 2021). While buffaloes serve as intermediate hosts for *S. fusiformis* and *S. buffalonis*, the primary species that infect cattle are *S. cruzi*, *S. hominis*, and *S. hirsuta* (Lindsay and Dubey, 2020). The thickness and form of the cyst wall are used to categorize *Sarcocystis* species (El-Sayad *et al.*, 2023). Some species, like *S. cruzi*, have thin walls, while others, such as *S. hominis* and *S. hirsuta*, have thick walls (Elshahawy *et al.*, 2022). The histopathological analysis of the animals under investigation revealed the presence of morphologically distinct *Sarcocystis* species, including thick-walled species that form macrocysts

and microcysts with distinct chambers, as well as thin-walled species. Comparable results were published by Ayazian *et al.* (2020), Elshahawy *et al.* (2022), and Ibrahim *et al.* (2018). Additionally, the tissue surrounding the cysts showed no signs of inflammation. Protozoa are found in cysts inside muscle fibers, providing defense from host immunity—a theory that has been validated for many other parasites—thus explaining the apparent absence of an inflammatory response (Nance *et al.*, 2012; Shosha *et al.*, 2024). Our findings align with the fact that inflammatory cells are not commonly observed in tissues affected by *Sarcocystis* infection (Gareh *et al.*, 2020; Italiano *et al.*, 2014).

Molecular techniques that rely on the recognition of the 18S rRNA gene use genetic markers for the species-specific differentiation of *Sarcocystis*. These approaches do not amplify other host or environmental DNA, as previously reported (Hajimohammadi *et al.*, 2014). Thus, in the current investigation, PCR amplification of the 18S rRNA gene confirmed infection with *Sarcocystis* spp. in 78.1% and 82.5% of the analyzed microcysts in cattle and buffalo carcasses, respectively. This highlights the need for molecular diagnostics to distinguish *Sarcocystis* spp. from other cyst-forming diseases. The 18S rRNA fragment is considered a suitable tool for distinguishing *Sarcocystis* spp. on a global scale (Elshahawy *et al.*, 2022). In the current study, the identification of *Sarcocystis* species using PCR-RFLP amplification of the 18S rRNA gene with restriction endonuclease enzymes (*Ssp1* and *Bsl1*) demonstrated that *S. cruzi* and *S. hirsuta* are the most frequently identified species in *Sarcocystis* spp. in the examined bovine carcasses, followed by *S. hominis*, *S. buffalonis*, and *S. fusiformis*. Previous research published by El-Sayad *et al.* (2023), Elshahawy *et al.* (2022), Mousa *et al.* (2021), Prakas *et al.* (2020), Portella *et al.* (2021), and Oğuz *et al.* (2021) supported these findings. This result suggests that dogs and cattle often interact on Egyptian farms, supporting the parasite life cycle (Ferreira *et al.*, 2018). Infected carnivores are thought to shed *Sarcocystis* sporocysts into the environment in very high quantities. For many reasons, including high temperatures, these sporocysts can remain viable for extended periods. Pastoral cattle easily consume these contaminated feces, which then contribute to further environmental contamination (Oğuz *et al.*, 2021).

However, the authors recognize a significant gap in the available information regarding zoonotic foodborne parasites, particularly sarcocystosis, which pose a serious threat to human health and major livestock species, especially bovines, in Upper Egypt. Additionally, this issue remains underexplored in the farming sector, particularly concerning the often undetected *Sarcocystis*. The limitations of this research include the fact that PCR-RFLP was

applied only to the samples that tested positive microscopically, and the absence of DNA sequencing techniques or phylogenetic studies for molecular validation of *Sarcocystis* species following PCR-RFLP analysis. We recommend further research to determine the actual prevalence of sarcosporidiosis in other herbivores across various regions of Egypt.

Conclusion

The current investigation has highlighted a significant incidence of bovine *Sarcocystis* in slaughtered cattle and buffalo intended for human consumption in Aswan abattoirs, Egypt. The most commonly identified *Sarcocystis* species in the bovine carcasses examined were *S. cruzi*, *S. hirsuta*, *S. hominis*, *S. buffalonis*, and *S. fusiformis*. To ensure the accurate identification of suspected cysts, traditional morphological techniques should be combined with modern molecular diagnostic methods in slaughterhouses. To mitigate the spread of the parasite and its associated economic and zoonotic risks, it is crucial for farmers and veterinarians to implement strategies that disrupt the parasite's life cycle. Further molecular and biochemical research is required to differentiate between *Sarcocystis* spp. recovered from various hosts in different geographical regions of Egypt.

Ethics Statement

All technique used in this investigation were conducted in accordance with the relevant rules and guidelines. Approval was obtained from the Faculty of Veterinary Medicine, Research Ethics Committee, New Valley University (No. 02/3/3-2024/15).

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Data Availability Statement

The article contains all the data.

Author Contributions

N.E., A.G., and A.F.: involved in conceiving the research idea, sampling, methodology, and writing—original draft

preparation. M.B. and W.D.: investigation, supervision and interpretation. M.A., M.D. and M.A.: participated in methodology, data analysis and contributed their scientific advice. M.S., M.D., and N.A.: data analysis drafted and prepared the manuscript for publication and revision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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