

Prevalence and distribution patterns of *Sarcocystis* spp. in buffaloes in Beni-Suef, Egypt

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Abstract This study was performed for the purpose of investigating the prevalence of *Sarcocystis* spp. in buffaloes in Beni-Suef Governorate, Egypt. Both macroscopic (*Sarcocystis fusiformis*) and microscopic (*Sarcocystis levinei*) cysts were recognized, and were differentiated by their morphological features and location in the tissues. Of 379 buffaloes examined in abattoirs in Beni-Suef, 299 were found to be infected, with an overall prevalence of 78.9%. Depending on age, three categorized groups of naturally infected buffaloes were examined: male buffalo calves aged 1.5–2 years, adult females aged 2–5 years, and females older than 5 years. Among these groups, infection rates were 74.5%, 82.3%, and 81.2%, respectively. Organs examined included esophagus, tongue, and heart. Macroscopic cysts were examined by the naked eye through meat inspection in abattoirs, while the pepsin-digestion method and the histological technique were applied to detect microscopic cysts. It has been found that esophagus showed the highest rate of infection among the infected organs, with both macroscopic and microscopic cysts seen in the infected buffaloes.

Moreover, results of the pepsin-digestion method proved more accurate than those produced by the histological technique in terms of infection rates for the microscopic cysts. Our findings indicated that infected buffaloes aged 2–5 years showed the highest mixed infection rate (82.3%) for both types of cysts. The high prevalence of microscopic *Sarcocystis* spp. in Beni-Suef Governorate reflects a significant role played by stray dogs, rather than cats, in the transmission of these parasites.

Keywords *Sarcocystis* · Buffaloes · Prevalence · Egypt

Introduction

Sarcocystis spp. is a cyst-forming coccidian parasite which needs two obligatory hosts during its life cycle, including a carnivorous final host and a herbivorous intermediate host (Soulsby 1982). Each host may be infected with more than one *Sarcocystis* spp. (Dubey et al. 1996; Bhatia 2000).

Sarcocystosis is a zoonotic and parasitic disease commonly seen in domestic animals such as buffaloes, cattle, and pigs. Among these, *Sarcocystis suihominis* is important in terms of public health, as meat and meat products are the main source of infection in human beings, who become infected when ingesting well-developed tissue cysts containing bradyzoites (Juyal and Bhatia 1989). In animals, infection by some of the *Sarcocystis* spp. can lead to anemia, loss of weight, abortion, and even death in cases of severe infections (Dubey et al. 1989). Sarcocystosis is found worldwide. Most investigations have concerned the prevalence of *Sarcocystis* spp. infection in esophagus, heart, tongue, diaphragm, and intercostal muscles of slaughtered cattle and buffaloes.

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Table 1 The overall prevalence of *Sarcocystis* spp. in muscular organs categorized by age and gender of the examined buffaloes in Beni-Suef Governorate

Age (years)	Number examined (gender)	Infected buffaloes	
		Number	Percentage (%)
1.5–2	153 (males)	114	74.5
2–5	141 (females)	116	82.3
Older than 5	85 (females)	69	81.2
Total	379	299	78.9

In this paper, we focus on the prevalence of both macroscopic and microscopic *Sarcocystis* spp. cysts in organs of slaughtered buffaloes in Beni-Suef Governorate, Egypt.

Materials and methods

Study area and animals

A total of 379 buffaloes (classified into three groups categorized by age; the first included 153 young male buffalo calves aged 1.5–2 years, the second 141 adult female buffaloes aged 2–5 years, and the third 85 females aged more than 5 years) were surveyed for the presence of sarcocysts. Animals slaughtered in abattoirs in Beni-Suef

Governorate, Egypt (coordinates: 29°04'N, 31°05'E) were collected from different areas of the province, and sent to the laboratory of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

Tissue samples from esophagus, tongue, and heart were taken from each freshly slaughtered animal and preserved in properly labeled ice bags that were transported from the slaughterhouse as early as possible to the Parasitology Laboratory for further investigation (Huong 1999). Specimens were kept refrigerated prior to examination.

Techniques

For macroscopic cysts, examination was done by the naked eye through meat inspection at the abattoir by the

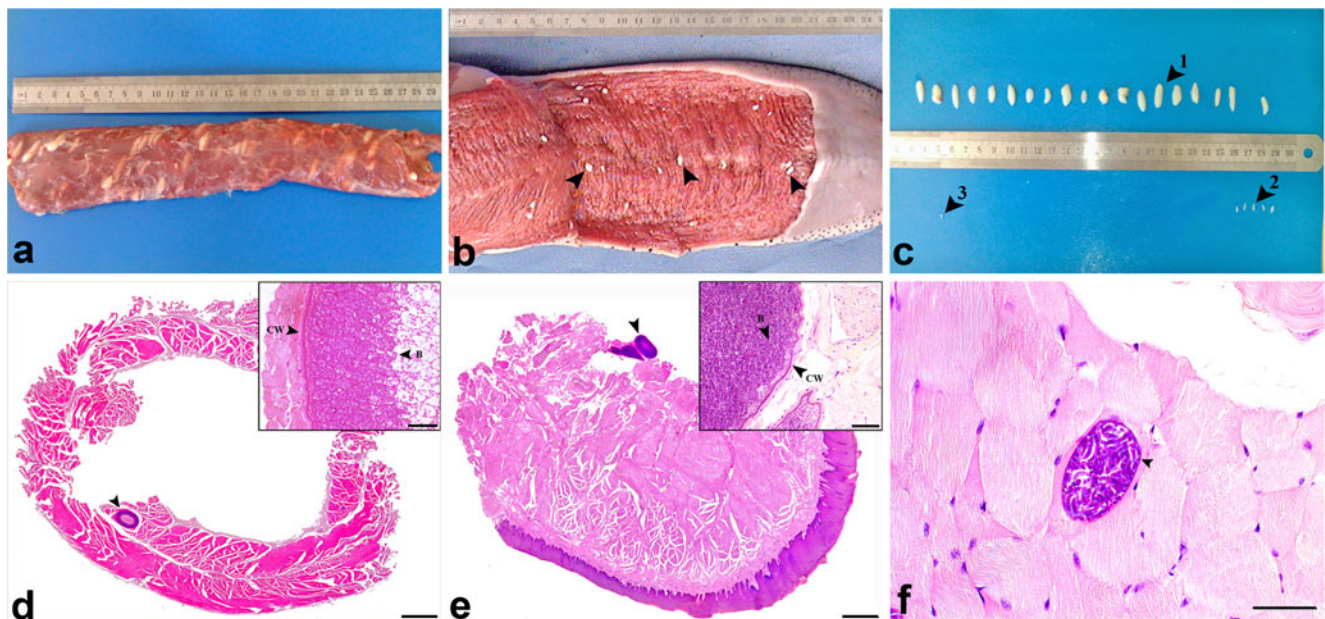


Fig. 1 **a** An esophagus of an elderly buffalo infected with numerous macroscopic *S. fusiformis* cysts. Note that they are distributed mainly under the serosal membrane. **b** Tongue of an elderly buffalo infected with large number of macroscopic cysts between the muscle bundles (arrowheads). **c** Individual macroscopic cysts isolated from both esophagus (1, 2) and tongue (3) of infected buffaloes. **d** Esophagus of an infected buffalo showed macroscopic cyst (arrowhead) (Scale bar=2 mm). *Inset*, higher magnification of the cyst denotes a very thick cyst

wall (CW) and bradyzoites (B). The latter were found condensed at the periphery then decrease towards the lumen (Scale bar=100 μ m). **e** Tongue of an infected buffalo showed macroscopic cyst (arrowhead) (Scale bar=1 mm). *Inset*, higher magnification of the cyst denotes a thick cyst wall (CW) and bradyzoites (B) (Scale bar=100 μ m), and **f** esophagus of a *Sarcocystis*-infected buffalo showing a microscopic cyst. Notice the thin cyst wall (arrowhead) (Scale bar=50 μ m)

Table 2 Distribution of macroscopic cysts in different organs using the naked eye

Tissue	Esophagus		Tongue		Heart		Total	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
1.5–2 (<i>n</i> =153)	2	1.3	0	0	0	0	2	1.3
2–5 (<i>n</i> =141)	6	4.6	4	2.8	0	0	9	6.4
Older than 5 (<i>n</i> =85)	11	12.9	6	7.1	0	0	15	17.7
Total							26	6.9

veterinarians. Microscopic cysts were examined by two methods:

Pepsin-digestion method

Approximately 10 gm of each tissue organ of the examined animals was minced and digested for 30 min at 40°C in 50 ml of digestion solution containing 1.3 gm pepsin, 3.5 ml HCl, and 2.5 gm NaCl in 500 ml of distilled water. The digested solution was poured through a fine-mesh sieve into a beaker and the filtrate was allowed to settle for 30 min. The supernatant was discarded and the sediment was microscopically examined at ×400 magnification (Latif et al. 1999; Florencia and Mary 2000; Saeid et al. 2009).

Histomorphological studies

Freshly collected muscle samples from *Sarcocystis*-infected buffaloes were fixed in 10% neutral buffered formalin solution, dehydrated in graded ethyl alcohol, embedded in paraffin, cut at 5-µm thickness and processed routinely for hematoxylin and eosin staining (Avapal et al. 2004; Mahran 2009).

Results

The present study revealed that 299 buffaloes examined in Beni-Suef abattoirs had sarcocysts, indicating an overall infection rate of 78.9% (*n*=379). Macroscopic and microscopic cysts were present either in single or mixed infections. Sarcocysts were seen in infected buffaloes of all ages (Table 1).

The present study revealed that macroscopic cysts were found in both esophagus and tongue. The cyst was spindle or fusiform in shape and consisted of opaque bodies, milky white in color, lying between muscle bundles parallel to the longitudinal axis of the muscle mass. In these organs, macrocysts were found either just beneath the serosal surface, as in esophagus (Fig. 1a and c), or deep in the muscular layer, as in esophagus and tongue (Fig. 1b and c). Examination of slaughtered animals clarified that esophagus was the organ most frequently found to be infected, followed by the tongue, while the heart showed no infection in any of the three categorized groups of buffaloes (Table 2). A total of 26 animals (6.9%) were found to be infected with macroscopic cysts. All of these were also infected with microscopic cysts. Macroscopic cysts ranged in size from 1.27 to 19.0 mm×0.7 to 7.0 mm, with very thick cyst walls ranging from 2.6 to

Table 3 Distribution of microscopic cysts in organs of the infected buffaloes using the histological technique

Age group per year	Examined buffaloes					
	1.5–2 (<i>n</i> =153)		2–5 (<i>n</i> =141)		Older than 5 (<i>n</i> =85)	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
Esophagus	107	69.9	109	77.3	63	74.1
Tongue	11	7.2	15	10.6	13	15.3
Heart	6	3.9	3	2.1	6	7.1
Esophagus and tongue	4	2.6	8	5.7	7	8.2
Esophagus and heart	0	0	2	1.4	3	3.5
Tongue and heart	6	3.9	3	2.1	6	7.1
All organs	0	0	2	1.4	3	3.5
Total	114	74.5	116	82.3	69	81.2

14.5 μm . In all of the macrocysts, bradyzoites tended to be overcrowded at the periphery of the cyst and decrease in number towards the center. Bradyzoites ranged in size from 8.6 to 15.8 \times 2.5 to 5.5 μm . Based on cyst size, wall thickness and bradyzoite size, macrocysts were identified as *Sarcocystis fusiformis* (Fig. 1d and e).

In the present investigation, the histological technique revealed microscopic cysts from esophagus, tongue, and heart in the three naturally infected groups (Table 3). Our results showed that esophagus was the most frequently infected organ. To confirm the presence of microcysts, given that sections taken for histological analysis did not necessarily contain cysts, the pepsin-digestion method was used to detect bradyzoites in the digested muscular tissue. This method produced more accurate results than the histological technique in detecting microcysts (Table 4). The present investigation showed that the size of globular to oval-shaped microcysts ranged from 19.1 to 95.9 \times 10.2 to 68.9 μm , and revealed a slightly compartmentalized arrangement of tightly packed zoites with fine septal partitions. Walls of these cysts were thin and ranged from 0.04 to 0.8 μm , while bradyzoites measured 6.6–13.2 \times 1.3–4.2 μm in size. Based on cyst size, wall thickness, and bradyzoite size, microcysts were identified as *Sarcocystis levinei* (Fig. 1f).

Results also indicated mixed infections with both macroscopic and fusiform and/or globular to oval microscopic cysts, and mixed infections with both types of microscopic cysts.

Discussion

In the present investigation, esophagus, tongue, and heart were found to be the most common sites for *Sarcocystis* spp. infection in buffaloes (Oryan et al. 2010). Results of the present work indicated a high prevalence of *Sarcocystis* spp. infection among slaughtered buffaloes in the region of Beni-Suef, Egypt, and this suggests that buffaloes are frequently exposed to infection due to their close relationship with dogs, cats, and even wild animals that act as final hosts for these protozoa. Similar results were obtained by Said (1996), who found an infection rate of 76.8% in Assiut Governorate. He added that elderly buffaloes were more commonly exposed to infection. Similar results were obtained by Fawaz (1998), who detected an infection rate of 72.6% in examined buffaloes in Qena Governorate, Egypt. Fatma et al. (2008) reported an infection rate of 94% among cattle in Egypt. Higher infection rates have also been recorded in other countries that have similar climatic conditions, such as 87% in India (Mohanty et al. 1995), 79% in Vietnam (Huong 1999), and 82.9% in Iraq (Latif et al. 1999). However, there have also been reports indicating

Table 4 Comparison of histological analysis and pepsin-digestion method for detecting microscopic cysts in different organs

Age (years)	Esophagus						Tongue						Heart					
	Histology			Pepsin-digestion			Histology			Pepsin-digestion			Histology			Pepsin-digestion		
	Number	Percentage (%)		Number	Percentage (%)		Number	Percentage (%)		Number	Percentage (%)		Number	Percentage (%)		Number	Percentage (%)	
1.5–2 (n=153)	104	68		107	69.9		10	6.5		11	7.2		6	3.9		6	3.9	
2–5 (n=141)	109	77.3		109	77.3		15	10.6		15	10.6		3	2.1		3	2.1	
Older than 5 (n=85)	63	74.1		63	74.1		13	15.3		13	15.3		6	7.1		6	7.1	

lower prevalences, among them 65% in the Philippines (Claveria et al. 2000) and 57% in Iran (Gharbanpoor et al. 2007). It has been found that the esophagus was the organ most frequently infected with either macroscopic or microscopic sarcocysts, while the heart had the lowest rate of infection (Fayer and Dubey 1986; Haddadzadeh et al. 2004). However, Daryani et al. (2006) found that the abdominal muscles of infected buffaloes were more frequently infected than the esophagus. Evidently, distribution of sarcocysts does not follow a specific pattern in most of the infected organs in buffaloes, with the exception of macroscopic cysts, which tend to be located in the esophagus. In addition, hearts of these animals do not usually contain any macroscopic forms.

Felids are known to be the definitive hosts of macroscopic sarcocysts, whereas canids are considered to be the final host of microscopic forms (Dubey et al. 1989). In the present study, macroscopic cysts were found to be far less prevalent than microscopic cysts, which were probably due to infrequent opportunities for cats to be in contact with buffaloes; the latter are commonly found on farms or even in village houses, while cats are viewed as pets and are usually found in cities. On the other hand, canids play a wide spectrum of roles in transmitting the infection to a number of animals. This occurs because dogs are commonly found in close contact with buffaloes and other ruminants. The frequent contact between buffaloes and household and/or stray dogs, together with the feeding habits of dogs that allow the ingestion of infected raw offal from the slaughtered animals in abattoirs, keep the life cycle active and consequently promote the spread of infection. In this manner, the presence of infected dogs causes the dissemination of large numbers of sporocysts via feces, thus increasing the exposure of buffaloes to infection. Contamination of feed, drinking water, and pastures by disseminated *Sarcocystis* spp. sporocysts is the main source of infection for buffaloes. Thus, infection of intermediate hosts plays an important role in inducing a high prevalence of microscopic cysts. Humans also can act as intermediate hosts, and are thus at risk when eating raw or improperly cooked meat from infected animals. This can result in intestinal sarcocystosis, and is potentially important in terms of public health (Bunyaratvej et al. 2007).

We have reported here that all of examined buffaloes that were infected with macroscopic sarcocysts were also infected with the microscopic forms; this implies that these animals were in contact with both felids and canids. It has been stated that infected dogs can shed millions of sporocysts daily, and these stages will be infective just after dissemination with feces of the final host. This explains the high prevalence of microscopic sarcocysts in areas where dogs are present, denoting the epidemiology of sarcocystosis (Oryan et al. 2010). Our findings revealed the

possibility of infection in buffaloes aged 1.5 years and older, with some variations in groups under 2 years and those above, but with no significant differences. We were unable to use animals younger than 1 year old in the present study, as regulations governing abattoirs in Egypt do not allow slaughtering before this age. Moreover, it has been suggested that geoclimatic conditions in Beni-Suef area, including high temperatures and high relative humidities, may increase the survival and persistence of sporocysts shed from infected dogs. This finding is consistent with that reported by Gharbanpoor et al. (2007).

The obtained results confirmed that Egyptian domestic buffaloes are widely infected with *Sarcocystis* spp. This high infection rate may be due to the abundance of final hosts, especially dogs and cats, that encourage the spreading of infection by this protozoan. Authors concluded that microscopic sarcocysts, *S. levinei*, were more prevalent than macroscopic cysts, *S. fusiformis*. Although sarcocysts present in livestock are host specific, advanced techniques such as PCR analysis reveal certain degrees of homology between different *Sarcocystis* spp. even between cattle and buffaloes (Jehle et al. 2009), so further investigation must be done to elucidate the homology of different *Sarcocystis* spp. in our domestic animals.

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