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Microscopical and serological studies on *Sarcocystis* infection with first report of *S. cruzi* in buffaloes (*Bubalus bubalis*) in Assiut, Egypt

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Abstract This study was performed for the purpose of investigating the prevalence and the species composition of *Sarcocystis* spp. in buffaloes in Assiut province, Egypt. Macroscopically we reported the infection of buffaloes with *Sarcocystis fusiformis*, while microscopically three *Sarcocystis* species (*Sarcocystis cruzi*, *Sarcocystis levinei* and *Sarcocystis hominis)* cysts were recognized, and were differentiated by their morphological features using both histopathological sections and electron microscope scanning. Regarding the prevalence of *Sarcocystis* species among buffaloes in Assiut province, we reported that, using gross examination of 90 buffaloes' esophagus, only 23 samples out of 90 (25.5 %) were found to be infected; on the other hand, by using microscopical examination, the prevalence was 27.7 % (25 samples out of 90 samples were found to be infected). Using ELISA, 85 samples out of 90 (94.4 %) were found positive, an overall prevalence of 94.4 %. In this work we concluded that customary meat inspection methods in abattoirs in Egypt are insufficient for detecting *Sarcocystis* infection. Due to the presence of hidden or microscopic cysts, we strongly recommend the use of combined microscopical examination and ELISA for *Sarcocystis* diagnosis, to avoid human infection of such zoonotic parasite and to control the consequent disease. In addition, this study introduced the first report of *S. cruzi* in buffaloes in Egypt, and proved the hypothesis that *S. cruzi* is able to use animals such as water buffalo as intermediate hosts.

Keywords Buffaloes · *Sarcocystis* · ELISA · Assiut · *S. cruzi*

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Introduction

Sarcocystis is one of the most prevalent parasites in livestock. In some hosts such as domestic cattle, all adult animals in a herd may be infected. It is economically important that increased surveillance methods be found to control this pathogen in livestock. Species of *Sarcocystis* are generally more specific for their prey hosts than for their predator (It may cause abortion, and fetal malformation in man) hosts (Collier et al. 1998).

Sarcocystis spp. normally develop in 2-host cycles consisting of an intermediate host (prey) and the final host (predator). Each host may be infected with more than one *Sarcocystis* spp. (Dubey et al. 1996; Bhatia 2000). Life cycles have been demonstrated for cattle-dog (*S. cruzi*), cattle-cat (*S. hirsuta*), cattle-human (*S. hominis*), sheep-dog (*S. capracanis*, *S. hircicanis*), sheep-cat (*S. gigantea*, *S. medusiformis*), goat-dog (*S. capracanis*, *S. hircicanis*), goat-cat (*S. moulei*), pig-dog (*S. meischeriana*), pig-human (*S. suihominis*), pig-cat (*S. porcifelis*), and others. Some wildlife may serve as intermediate hosts (such as raccoons) or final hosts (coyotes) for some species of *Sarcocystis* (Soulsby 1982). Sarcocystosis is a zoonotic and parasitic disease commonly seen in domestic animals such as buffaloes, cattle, and pigs. Among these, *Sarcocystis hominis* has a significant impact on public health. Meats 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). El-Dakhly et al. (2011) reported the infection of Egyptian buffaloes with 2 *Sarcocystis* spp. (*S. fusiforms* and *S. levinei*), with an overall prevalence of 78.9 %. The same work conducted in Sohag, Egypt by (Khalifa et al. 2008), who reported three *Sarcocystis* spp., the prevalence rate in the herd was 28 %. Only the macroscopic fusiform-shaped species was detected (*Sarcocystis fusiformis*).

In fact corresponds to *S. cruzi*, occupying an intermediate host range that is larger than previously understood. Very recently, a report employing genetic and ultrastructural methods to investigate the parasites of cattle and water buffalo in Vietnam concluded that certain parasites are shared by water buffalo and cattle (Jehle et al. 2009).

The current consensus is that all *Sarcocystis* species found in livestock show high specificity at the level of the intermediate host (Dubey et al. 1989). For example, those species infecting cattle (including *S. cruzi*) are not supposed to occur in water buffalo and vice versa. In support of this, (Jain and Shah 1985) performed the first cross-transmission studies of *S. cruzi* from cattle and were unable to infect water buffalo. But Wang et al. (1992) and Xiao et al. (1993) reported cross transmission of *Sarcocystis* species between water buffalo and cattle and demonstrated the infection of water buffalo with *S. cruzi.*

The present work is an attempt to study the prevalence of the different *Sarcocystis* spp. infecting slaughtered buffaloes at Assiut, Egypt, using combined microscopical and serological examinations.

Materials and methods

I- Study area and animals

A total number of 90 buffaloes were surveyed for the presence of *Sarcocystis* during the period from February to June 2010. Samples from slaughterhouses belong to Assiut Governorate, Egypt (27°3′0″N, 31°1′0″E) were sent to the laboratory of Parasitology, Faculty of Veterinary Medicine, at Assiut University, Egypt. Tissue samples taken from the esophagus of each freshly slaughtered animal were preserved in labeled ice bags and transported from the slaughterhouse in a timely manner to the Parasitology Laboratory for further investigation (Huong 1999). Specimens were kept refrigerated prior to examination.

II- Examination of muscle samples

Macroscopic examination

Fresh muscle samples were examined macroscopically for the presence of macroscopic *Sarcocystis* cysts.

Microscopic examination

For detection of microscopic *Sarcocystis* cysts, small pieces of fresh muscle were compressed between two slides and examined microscopically according to Mowafy (1993).

Histopathological studies

Specimens from positive muscular samples were fixed in 10 % formalin. Sections of muscle samples were stained by Ehrlich's Hematoxylin and Eosin (Bancroft and Stevens 1993) and examined histopathologically.

Serological diagnosis (ELISA)

Antigen: *Sarcocystis* cystizoite antigen was prepared from *S. fusiformis* as described by Morsy et al. (1994).

Serum samples

Blood samples for separation of serum were collected from the jugular vein in plain tubes (Coles 1986), kept at room temperature for 30 min, centrifuged at 3,000 rpm for 15 min. The obtained serum samples were transferred to Eppendorf tubes and kept at −20 °C until used.

ELISA

ELISA was done according to Morsy et al. (1994). Antigen was diluted 1:1 in carbonate buffer and all serum samples were diluted 1:100. Peroxidase-conjugated rabbit anti-bovine IgG (h&L) (Sigma Chemical Co., USA) was diluted 1:250 and Tetramethyl benzidin and ureamderoxide (TMB) was used as substrate. The optical density (OD) was measured at 450.

Results

In the present study, we examined 90 buffaloes for *Sarcocystis*, found an infection rate of 25.5 %, using macroscopical examination, and only *S. fusiformis* were reported. Microscopical examination revealed that 27.7 % of the examined samples tested positive for the parasite. Results of ELISA testing showed that 94 % of the examined animals were actually infected (Table 1).

Macroscopic and microscopic cysts were present either in single or mixed infections. *Sarcocystis* were seen in infected buffaloes of all ages. 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.

Table 1 Prevalence of *Sarcocystis* in the examined buffaloes in Assiut

Examined buffaloes $N = 90$	Macroscopic examination		Microscopic examination		ELISA examination	
	no	%	no	%	no	%
Positive animals	23	25.5	25	27.7	85	94.4

Fig. 1 An esophagus of a buffalo infected with numerous Macroscopic. *S. fusiformis* cysts. Note that they are distributed mainly, under the serosal membrane

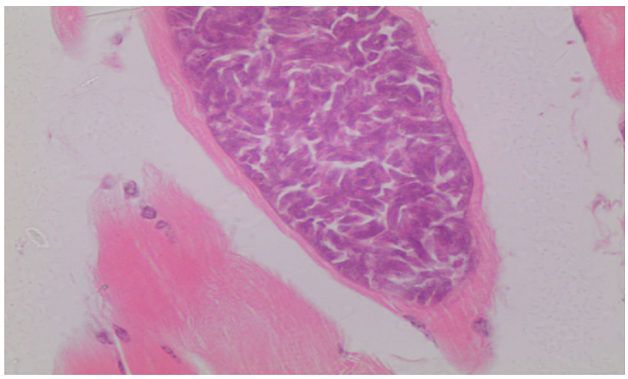

Fig. 2 Microscopic cyst (*S. cruzi*): showing spindle shaped cyst filled with bradyzoites, showing thin cyst wall (*arrow*) followed by large groups of bradyzoites without separating septa H&E ×400

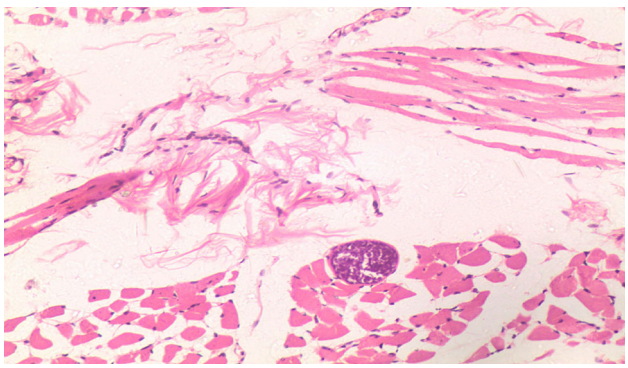

Fig. 3 Esophagus of a *Sarcocystis*-infected buffalo showing a microscopic cyst. (*S. levinei*) Notice the thin cyst wall (*arrowhead*) (×100)

The macrocysts were found either just beneath the serosal surface, as in (Fig. 1), or deep in the muscular layer. Macroscopic cysts ranged in size from 1.27 to 22.0 × 0.5 to 8.0 mm. 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 8.4 to 15.6 × 2.5 to 5.4 µm. Based on cyst size, wall thickness and bradyzoite size, macrocysts were identified as *S. fusiformis* (Fig. 1).

In the present investigation, the histological technique revealed microscopic cysts from the esophagus, and three different microscopic species of *Sarcocystis* in buffaloes, zoonotic (*S. hominis*), *S. cruzi*, and *S. levinei*.

1- *S. cruzi*, seen as fusiform-shaped microscopic cysts, parallel to muscle fibers. Their measurements ranged from 140.5 to 450 × 55.6 to 170.6 µm. In histopathological section, the cyst wall was seen as a narrow, homogenous wall, less than 0.55 µm. The cyst was filled with bradyzoites, while the dividing septa were not clear (Fig. 2). This is the first report of *S. cruzi* in buffaloes at Egypt (Also it is the first report of natural infection of buffaloes with *S. cruzi* allover the world as far as we knows).

2- *S. levinei*, characterized by a slightly compartmentalized arrangement of tightly packed zoites with fine septal partitions. Walls of these cysts were thin and ranged from 0.05 to 0.9 µm, while bradyzoites measured 6.4–13.4 × 1.2–4.3 µm in size. Based on the cited criteria, the cysts were identified as *Sarcocystis levinei* (Figs. 3, 4).

3- *S. hominis,* characterized by a thick cyst wall (2–7u) consisting of cylindrical, fingerlike, villar protrusions and having microfilaments. The protrusions containing microfilaments were perpendicular to the cyst surface with broad tips. Bradyzoites, present in packets separated by septa, were up to 9.0 mm long and up to 2.5 mm wide (Figs. 5, 6).

Serological diagnosis (ELISA)

Out of 90 serum samples of slaughtered buffaloes, 85 samples tested positive for *Sarcocystis* infection (94.5 %). Most of the positive samples 60 (70.5 %) were considered moderately positive, in which the density values ranged between 2.6 and 3, while 10 cases (8.5 %) had the lowest positive reading with an optical density below 2.6. Highly positive samples were detected in 15 cases (17.6 %) in which the optical density was above 3.0. The highly

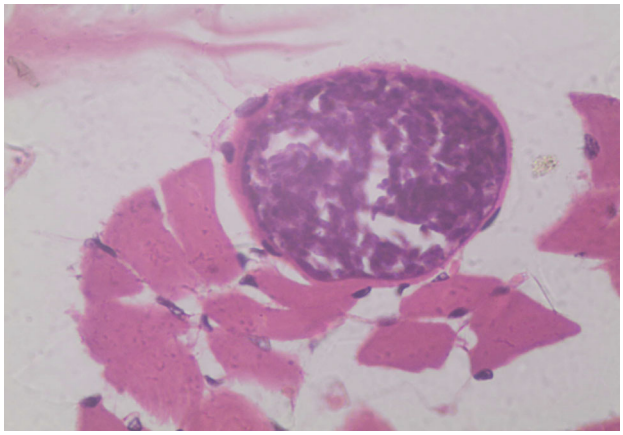

Fig. 4 Esophagus of a *Sarcocystis*-infected buffalo showing a microscopic cyst. (*S. levinei*) Notice the thin cyst wall (*arrowhead*) (×400)

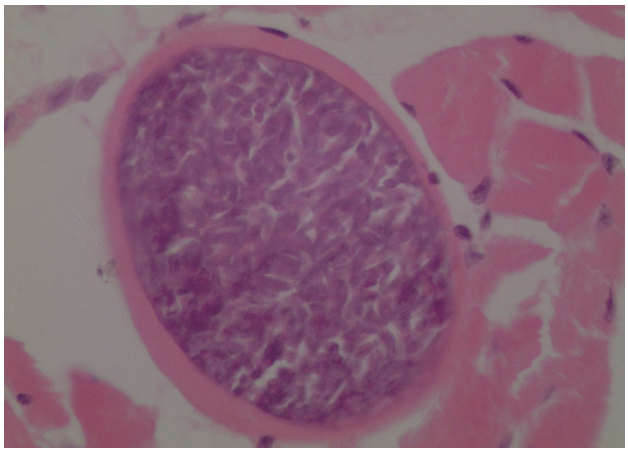

Fig. 5 *S. hominis*, characterized by a thick cyst wall (2–7u) (H&E ×400)

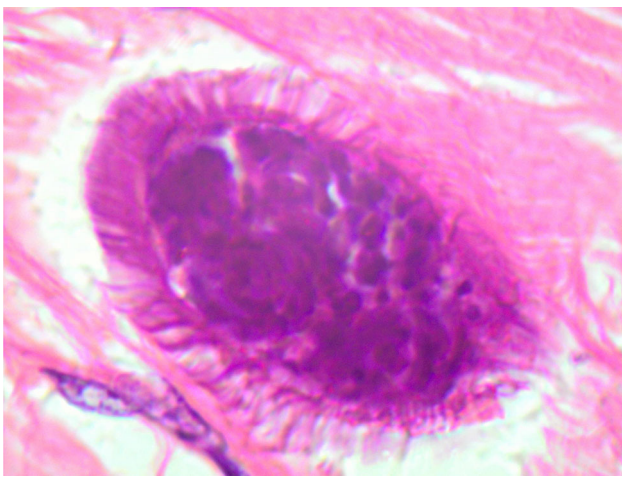

Fig. 6 Higher magnification of macroscopic cyst (*S. hominis*) showing characteristic. Consisting of cylindrical finger-like villar protrusions and having microfilaments. H&E ×1,000

positive cases were associated with highly infected esophageal muscle with *Sarcocystis* cysts. The sensitivity of the macroscopic method was 27 %. Specificity was 100 %. Positive predictive value was 100 %, and negative predictive value was 7.46 % (Table 2).

Discussion

In the present study, macroscopical, microscopical and serological examinations (ELISA) were used for diagnosis of *Sarcocystis* infection in buffaloes at Assiut abattoir. The prevalence of the parasite was 25.5 % using gross examination, and 27.7 % using microscopical examination. Using serological diagnosis, the prevalence rate was 94 %. Accordingly, the present study strongly recommends the use of ELISA in *Sarcocystis* diagnosis, due to the low sensitivity of both macroscopical and microscopical examinations. In the serological diagnosis, the *S. fusiformis* was used in the present work as a source of antigen to diagnose the *Sarcocystis* infection in cattle by ELISA. Fatma et al. (2008); Habeeb et al. (1996) and El-Nazer and Abdel-Azem (2000) used *S. fusiformis* antigen in ELISA diagnosis of sarcocystosis in humans. Also, Abdel Rahman (2001) used the same antigen in ELISA and Western blot for diagnosis of *Sarcocystis* infection in cattle.

The present study indicated a high prevalence of *Sarcocystis* spp. infection among slaughtered buffaloes in Assiut abattoir using ELISA. 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. (Collier et al.1998) cited a variety of conditions that permit such a high prevalence of *Sarcocystis*: many definitive hosts are involved in transmission, the shedding of a large number of sporocysts (as infective form) over many months, the resistance of oocysts or sporocysts in the external environment for a long period, the role of invertebrate transport hosts in the spreading of infection, in addition to little or no immunity to the reshedding of sporocysts after each meal of infected meat. Similar results were obtained by Fatma et al. (2008), which reported the high prevalence (94 %) of *Sarcocystis* infection in cattle, at Assiut, Egypt. The reported species were *S. fusiformis* and *S. cruzi*. El-Dakhly et al. (2011) reported the high infection rate of buffaloes in Beni-suef, Egypt, where the overall prevalence was 78.6 % and the reported species were *S. susiformis* and *S. levinei*. Said (1996) found an infection rate of 76.8 % in the Assiut Governorate. He noted 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. Higher infection rates have been recorded in other

Table 2 Evaluation of macroscopic method for diagnosis of *Sarcocystis* against ELISA in buffaloes

	Test results				Evaluation parameters (%)			
	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
Macroscopic method	23	5	0	62	27	100	100	7.46

ELISA finding was considered as the golden test

TP true positive, *TN* true negative, *FP* false positive, *FN* false negative, *PPV* positive predictive value, *VPN* negative predictive value

countries that have similar climatic conditions, for example, 87 % in India (Mohanty et al. 1995) and 82.9 % in Iraq (Latif et al. 1999).

In conclusion, the present study reported three *Sarcocystis* spp. infecting buffaloes in Egypt. In addition, this study is the first report for the infection of Egyptian buffaloes with *S. cruzi*. Our findings prove the hypothesis that *S. cruzi* is able to use animals such as water buffalo as intermediate hosts. Finally, it is strongly recommend the use of microscopical examination at postmortem and serological test (ELISA) for routine examination for Sarcocystosis in Egypt.

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