SARCOCYSTIS SPECIES PREVALENT AMONG SLAUGHTERED BUFFALOES AT DAKAHLIA PROVINCE, EGYPT

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ABSTRACT

This study was carried out in order to investigate the prevalence of different Sarcocystis species in slaughtered water buffaloes at Dakahlia Province, Egypt. Muscle samples from esophagus, heart, tongue, diaphragm and throat muscles were recovered from 550 water buffaloes slaughtered at Mansoura abattoir, Dakahlia Province, Egypt. Sarcocysts were detected in 68.18 % of examined water buffaloes. Aged buffaloes (n=400) were more infected than younger ones (n=150). Our findings showed that macroscopic sarcocysts were more prevalent than microscopic ones and this is evoking the great role played by cats in transmission of Sarcocystis spp more than dogs. Four Sarcocystis species were identified. They are: S. fusiformis which was the most prevalent one (58.72 %), S. levinei (47.45 %), S. buffalonis (8.72 %) and finally S. dubeyi (3.45 %).

Keywords: *Sarcocystis, Egypt, Buffalo, Identification, Prevalence.*

INTRODUCTION

Sarcocystis species represent one of the most frequently prevalent protozoons of livestock animals. They are cyst-forming coccidian parasites with obligatory heteroxenous two-host life cycle involving carnivores as definitive hosts, in which sexual stages take place, while herbivores or omnivores animals act as intermediate hosts where tissue cysts are formed. **(Dubey et al., 1989 a)**. Each intermediate and definitive host may harbor more than one Sarcocystis species. Some species cause economic losses from clinical and subclinical disease **(Solusby, 1982)**, condemnation and downgrading of carcasses and decrease quality of meat **(Mostafa and Yasein, 2010)**.

The population of water buffaloes (Bubalus bubalis) in Egypt was recorded approximately 3,977,000 head in 2009 (FAO, 2009). They considered as an important source for meat and milk production. Buffaloes are frequently affected with Sarcocystis species. The incidence of infection was discussed in many countries recording 100% in Indonesia **(Holz 1957)**, 69.7% in India **(Juyal et al., 1982)**, 33% in Italy **(Camisasca et al., 1996)**, 39.2% in Turkey **(Dundar and Ozer, 1996)**, 64.8% in Philippine **(Clevaria and Cruz, 1999)**, 79% in Vietnam **(Huong, 1999)**, 68.54% in Egypt **(Abass, 2008)**, 83% in Iran **(Oryan et al., 2010)**.

Four Sarcocystis species were reported in buffaloes. Two of them had macroscopically

visible sarcocysts: S. fusiformis **(Railliet, 1897)** and S. buffalonis **(Huong et al., 1997b)**, both with felines as definitive hosts. While the other two species are microscopic forming sarcocysts: S. levinei with canines as definitive hosts **(Dissanaike and Kan 1978, Huong et al., 1997a)** and S. dubeyi **(Huong and Uggla 1999)**, the definitive host of which has not yet been identified **(Chen et al., 2011)**. Differentiation between Sarcocystis species is based upon size, shape and location of the sarcocysts as well as the structure of the cyst wall **(Huong, 1999)**. Studying the morphological features of the sarcocyst wall was done by light microscopy which considered as cheap and feasible way for diagnosis, however fine details of the cyst wall can not be studied due to the low resolution of the light microscope **(Dubey et al., 1989 a)**. Moreover, the use of transmission electron microscopy (TEM) is the ultimate tool for perfect and sharp characterization of the sarcocysts **(Mehlhorn et al., 1976)** but it is a highly costive method for diagnosis.

Therefore, this study was conducted in order to investigate the prevalence, distribution and identification of Sarcocystis species infecting water buffaloes slaughtered at Dakahlia Province, Egypt.

MATERIAL AND METHODS

Muscle samples from esophagus, heart, tongue, diaphragm and throat muscles were collected from 550 water buffaloes slaughtered at Mansoura abattoir at Dakahlia Province, Egypt, at the period between July 2009 and June 2010. Animals under investigation were assigned into two age groups by visual inspection of both horns and teeth, the first one was over five years of age (n=400), while the other group was 2-3 years old (n=150).

Detection of macroscopic sarcocysts was done by visual inspection of the muscular tissues, whereas muscle squash technique was used for the detection of microscopic sarcocysts **(Huong, 1999)**. Samples were then cut off into about 1 cm^3 thick specimens, fixed in neutral buffered formalin 10% and processed for histopathological technique **(Bancroft and Stevens, 1996)** through dehydration in graded ethanol, embedded in Paraffin wax, sectioned at 5µm in thickness, stained by hematoxylin and eosin and examined under ordinary light microscope.

RESULTS AND DISCUSSION

Examination of the collected muscle specimens revealed that 375 (68.18%) out of 550 slaughtered water buffaloes had sarcocysts. This relatively high prevalence of Sarcocystis infection was coincided with that reported by **Juyal et al. (1982)** in India (69.7%), and **Claveria and Cruz (1999)** in Philippine (64.8%). Other studies showed higher incidences like **Huong (1999)** in Vietnam (79%), 100% in Indonesia **(Hole, 1957)**, while lower incidences was noted by **Dundar and Oxer (1996)** in Turkey (39.2%). In Egypt, studies in different provinces showed varied degrees of infection from high 97.7% in Cairo **(Nassar, 1982)** and 63.7% in Dakahlia (Abu El-wafa and Alaraby, 2008), to low 28% in Sohag **(Khalifa et al., 2008)** and 36.66% in Assiut **(Arafa et al., 2003)**. The significant difference in prevalence between the northern moderate climatic **(Cairo and Dakahlia)** and southern hot climatic

(Sohag and Assiut) Provinces may be attributed to the difference in ambient temperature and humidity as reported by **Savini et al. (1996)** who found that the infectivity of Sarcocystis cruzi sporocysts is likely to be maintained longer in cool climates and is reduced by fluctuation of aridity and ambient temperatures.

In the present investigation, the macroscopic species (63.45%) were more common than microscopic ones (48.36%), Table 1. An inverse incidence was found by **Latif et al. (1999)** and **Oryan et al. (2010)** who explained that microscopic sarcocysts were more prevalent than macroscopic cysts. **Dubey et al. (1989a)** reported that dogs had a great role for transmission of Sarcocystis species more than cats due to their close live with buffalo herds. In this regard, our results evoked the significant role of felines more than canines in transmission of Sarcocystis species, meaning cats may an important role for increasing the prevalence of macroscopic than microscopic species. This may attributed to that most slaughtered buffaloes at Mansoura abattoir were delivered from villages more than farms and reared at villager houses in which feral cats are kept frequently as dogs. Also, may referred to the longer period of sporocyst shedding in cats **(Huong et al., 1995)** than dogs **(Huong et al., 1997b)**.

Four Sarcocystis species were recognized in the investigated samples, two of them had macroscopic sarcocysts and the remaining two species forming microscopic sarcocysts.

The macroscopic species were S. fusiformis which was the most prevalent one (58.72%) and S. buffalonis (8.72%), while the microscopic species were S. levinei (47.45%) and S. dubeyi (3.45%), Table 2. This is the first study that involves all the four Sarcocystis species infecting buffaloes in Egypt. **El-Morsy (2010)** reported three species only: S. fusiformis (68.1%), S. buffalonis (13.2%) and S. dubeyi (30%) in aged animals. **Huong (1999)** found the four species in Vietnamese buffaloes: S. fusiformis (41%), S. levinei (74%), S. buffalonis (33%) and S. dubeyi (12%). The definitive host of S. dubeyi has not been identified up till now, but it thought to be primates other than human being **(Chen et al., 2011)**. Therefore, the significant difference in the prevalence of S. dubeyi between the three studies may depend on the presence or absence of primates in areas of study; although there's no wild life present in Dakahlia Province (This may need further studies). S. buffalonis prevalence was studied by **Oryan et al. (2010)** in Iran (3%). Also, the great variety in the incidence of S. buffalonis may be attributed to the miss non-experienced diagnosis during naked eye examination because of its nearly similar shape to S. fusiformis sarcocysts in early formed stage.

The prevalence was found to be increased with age with an infection rate of 72.75% among aged animals and 56% in younger ones (Table, 3). This finding is most likely due to a longer exposure of aged animals to the sporocyst infection, which is coincided with **Nassar (1982)., Huong (1999)** and **El-Morsy (2010)**.

In the present study, the esophagus, heart, tongue, diaphragm and throat muscles were examined, as recommended by previous in-

vestigations, to be the most common sites of Sarcocystis infection in the water buffaloes (**Huong, 1999**). These results showed that esophagus was the most infected tissue in all the four species of Sarcocystis. **Nassar, (1982). Claveria** and **Cruz (1999)** and **Huong (1999)** stated that S. fusiformis cysts were common in the esophagus. Moreover, S. levinei was the only species found in the heart as reported by **Houng et al. (1997a)**. S. buffalonis was not found in heart or tongue which harmonized with **Huong et al. (1997a)**, but conflicted with **Huong (1999)** who noted that S. buffalonis was found in 8 % of investigated buffalo tongues. Furthermore, S. dubeyi was never found in Tongue, heart or diaphragm. This is agreed with that reported by Huong and **Uggla (1999)**, Table 4.

Based upon size, shape and location of the sarcocyst as well as the structure of the cyst wall, four morphologically distinct Sarcocystis species were found in Egyptian water buffaloes. Two of them were macroscopic sarcocysts forming species: S. fusiformis and S. buffalonis. The differentiation between the previously named species was easily done by their gross appearance. S. fusiformis (Fig. A1) was milky white spindle-shaped cysts, not embedded deeply in host tissues and measured 4-35 X 1-8mm, while S. buffalonis cyst (2-10 X 0.5-1.0mm) appeared as white threads under the connective tissue layer of the muscular tissue and sometimes twisted due to the postmortem contractions of the underlying muscles (Fig C1).

Histologically, the differentiation between Sarcocystis species depends on the thickness of the cyst wall and the appearance of the villar protrusions (**Dubey et al., 1989 a**). **Dubey, 1982** mentioned that the thickness of the cyst wall depend on the stage of development of the cyst, therefore confusion may occur when light microscope is used in identification of Sarcocystis species. This obstacle was avoided by selecting fully mature cysts for studying. The wall of S. fusiformis was thin (1-2 µm) and had highly branched villar protrusions (Fig. A2), while that of S. buffalonis was thick (3.5-7 µm), striated and had conical villar protrusions with constricted base, laterally expanded mid region and tapered tips (Fig. C2). The previous characters of S. buffalonis were coincided with **Huong et al. (1997a), Jehle et al. (2009)** and **Oryan et al. (2010)**. Moreover, the description of S. fusiformis is closely similar to that of **Ghaffar et al. (1978)., Kan** and **Dissanaike (1978).. Dubey et al. (1989 b)., Claveria and Cruz (1999)., Huong (1999)., Khalifa et al. (2008)** and **Jehle et al. (2009),** but conflicted with **El-Dakhly et al. (2011)** who found that S. fusiformis with thick cyst wall (2.6-14.5 µm), but other characters were nearly similar.

Dealing with the microscopic cyst forming species, S. levinei and S. dubeyi. S. levinei cyst (Fig. B1) was narrow spindle, oval or round shaped and reaches up to 0.9 X 0.2 mm in size. Histological sections of the cyst wall appeared to be very thin (≤ 1 µm) and smooth with hair like villar protrusions (Fig. B2). These morphological features agreed with that reported by **Wang et al. (1991), Huong et al. (1997b), Claveria** and **Cruz (2000)** and **Xiang et al. (2010),** but conflicted with Kan and **Dissanaike (1978)** who described S. buffalonis in Malaysian buffaloes as S. levinei.

Moreover, S. dubeyi cysts were spindle shaped, sometimes with undulating edges and measured up to 0.7 x 0.2 mm (Fig. D1). Histologically, the striated cyst wall was thick (4.5-8 µm) and emanating cylindrical villar protrusions with uniform length and width and flattened ends (Fig. D2). These results are found in harmony with that mentioned by **Huong** and **Uggla (1999)** and **Chen et al. (2011)**.

Table (1) : Total prevalence of macroscopic and microscopic sarcocysts in slaughtered buffaloes.

Sarcocyst / Total No.	Macroscopic sarcocysts only		Microscopic sarcocysts only		Mixed infection with both types	
	+ve	%	+ve	%	+ve	%
550	109	19.81	26	4.72	240	43.63

Table (2) : Total prevalence of different Sarcocystis species in slaughterd buffaloes.

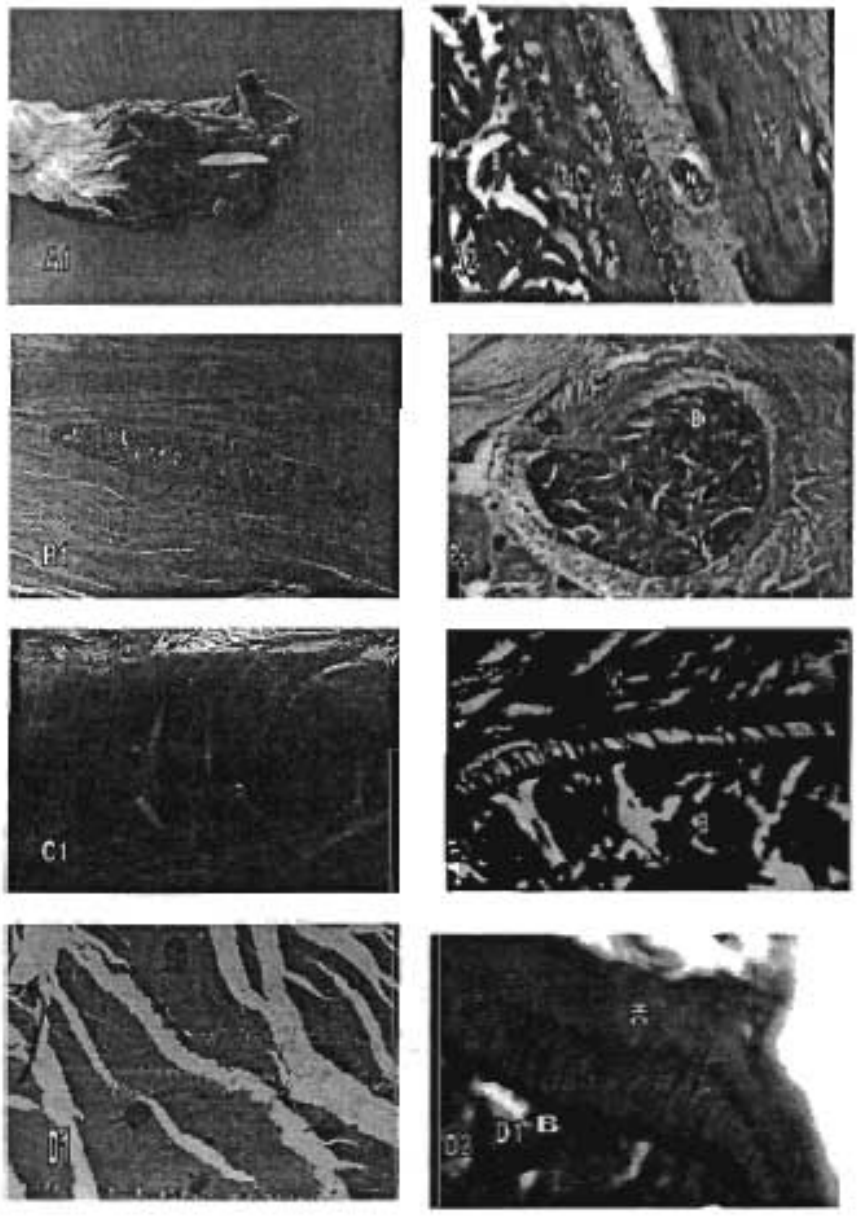
species	S. fusiformis		S. levinei		S. buffalonis		S. dubeyi	
	+ve	%	+ve	%	+ve	%	+ve	%
550	323	58.72	48	8.72	261	47.45	19	3.45

Table (3): Prevalence of different Sarcocystis species in different age groups:

Age group / Species	Over 5 years old (n=400)		2-3 years old (n=150)	
	+ve	%	+ve	%
S.fusiformis	270	67.5	53	35.33
S.levinei	198	49.5	63	42
S.buffalonis	40	10	8	5.33
S.dubeyi	15	3.75	4	2.67

Table (4) : Prevalence of different Sarcocystis species in different tissues of slaughtered buffaloes:

Species / Tissue	S.fusiformis		S.levinei		S.buffalonis		S.dubeyi	
	+ve	%	+ve	%	+ve	%	+ve	%
Esophagus	323	100	240	91.95	43	89.58	19	100
Heart	0	0.00	185	70.88	0	0.00	0	0.00
Tongue	130	40.25	73	27.97	0	0.00	0	0.00
Throat muscles	182	56.35	70	26.82	15	31.25	2	10.52
Diaphragm	48	14.86	15	5.75	4	8.33	0	0.00



HC: host cell, B: bradyzoites, N: nucleus, GS: ground substance, M: metrocytes

Fig. A1: large spindle-shaped macroscopic S. fusiformis (arrow) in the diaphragm

Fig. A2: High power view of S. fusiformis. Note the thin cyst wall (arrow) and branched VP (arrow head)

Fig. B1: Low power view of S. levinei. Note the thin cyst wall (arrow).

Fig. B2: high power view of S. levinei. Note the thin cyst wall (arrow head) and hair like VP (arrow).

Fig. C1: Macroscopic S. buffalonis (arrow) in esophageal muscles. Appeared as twisted white lines under the connective tissue.

Fig. C2: High power view of S. buffalonis. Note the striated thick cyst wall (between arrow heads) and the conical VP with tapered tips (arrow).

Fig. D1: Low power view of S. dubeyi (arrow). Note the thick cyst wall. Arrow head refers to S. levinei.

Fig D2: High power view of S. dubeyi. Note the striated thick cyst wall (between arrow heads) and cylindrical VP with blunt tips (arrow).

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الملخص العربى

المتكيسات العضلية المنتشرة بين الجاموس المذبوح بمحافظة الدقهلية ,مصر

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قسم الطفيليات -كلية الطب البيطري -جامعة المنصورة

أجريت هذه الدراسة لتحديد مدى انتشارية أنواع المتكيسات العضلية المختلفة التي تصيب الجاموس المذبوحة بمحافظة الدقهلية ,مصر. وتم أخذ العينات من أنسجة كل من المريء ,القلب ,اللسان ,الحجاب الحاجز و عضلات الحلق من عدد 550 جاموسة ذبحت بمجزر المنصورة التابع لمحافظة الدقهلية. وقد وجد أن 68.18 % من الجاموس المفحوصة مصاب بالمتكيسات العضلية. كما ثبت أن الحيوانات المتقدمة في العمر أكثر إصابة من الحيوانات الصغيرة وأيضا المتكيسات العضلية المرئية بالعين المجردة كانت أكثر انتشارا من المتكيسات العضلية المجهرية. كما تم تحديد أربعة أنواع من المتكيسات العضلية وهم: ساركوسيستس قيوزيفورميس ,(58.72%) ساركوسيستس باقلونيز ,(8.72%) ساركوسيستس ليفيتى (47.45%) وأخيرا ساركوسيستي دوبى ,(3.45%)

الكلمات الدالة: المتكيسات العضلية. انتشارية. الجاموس. مصر.