Sarcocystis hominis and Other *Sarcocystis* Species Infecting Cattle in Sharkia Province, Egypt

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Abstract: Bovine sarcocystosis is an economically important disease worldwide caused by the apicomplexan protozoan, *Sarcocystis* species. There are three main species of cattle *Sarcocystis; S. cruzi* (*S. bovicanis*), *S. hirsuta* (*S. bovifelis*) and *S. hominis* (*S. bovihominis*). The current study was conducted to identify *Sarcocystis* species infecting slaughtered cattle at Sharkia province, Egypt using light and electron microscopy, as well as random amplified polymorphic DNA (RAPD-PCR) technique. Both thin-walled and thick-walled microscopic sarcocysts were observed in 29.6 % (24 out of 81) of the examined cattle. Thin-walled sarcocysts were observed in the 24 infected animals **(**29.6 %), while thick-walled sarcocysts were only detected in three samples (4.9 %). Large macroscopic cysts were not observed by the naked eyes. The identified microscopic cysts measured 198-1050 µm (average 624 µm) in length and 72-150 µm (average 111 µm) in breadth. Thin-walled microscopic sarcocysts were identified as *S. cruzi*, while thick-walled sarcocysts were confirmed as *S. hominis* using light microscopy, transmission electron microscopy and RAPD-PCR technique. Thick-walled *S. hirsuta* was suspected only at light microscopic level. This is the first report and molecular confirmation of *S. hominis* in slaughtered cattle at Sharkia province, Egypt.

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1. Introduction

Sarcocystis species are cyst-forming intracellular protozoan parasites with an obligate two host's life cycle between predators as final hosts and their prey animals as intermediate hosts (Jehle *et al.,* 2009). *Sarcocystis* species are highly prevalent in livestock animals and are considered to be very host specific. *Sarcocystis cruzi, S. hirsute* and *S. hominis* have canids, felids and humans as definitive hosts respectively, and can affect bovines as intermediate hosts producing muscle cysts (Dubey *et al.,* 1989a).

Although *Sarcocystis* species are generally considered non-virulent for cattle, *S. hominis* infection was occasionally associated to eosinophilic myositis (Wouda *et al.,* 2006) and condemnation of beef due to the presence of *S. hirsute* macroscopic cysts was reported (Dubey *et al.,*1990). The symptoms of infection in cattle, when apparent, involve reduced weight gain, anorexia, fever, muscle weakness and eosinophilic myositis, reduced milk yield, abortion and death (Dubey *et al.,* 1989a; Vangeel *et al.,* 2012).

The relevance of *S. hominis* infection lies in its zoonotic character (Fayer 2004). Human infection occurs mainly through consumption of raw or insufficiently cooked cattle meat or meat products containing tissue cysts of the parasite. Acquiring the infection in humans results in intestinal sarcocystosis which is manifested by nausea, vomiting, abdominal pain and diarrhea (Soulsby 1986; Fayer 2004; Dubey and Lindsay, 2006).

In Egypt, *Sarcocystis* species were previously detected in cattle using light and electron microscopy (Khalifa *et al.,* 2008). The aim of the present study was to detect and differentiate *Sarcocystis* species in the oesophagus of slaughtered cattle in Sharkia province using the light and electron microscopy, as well as random amplified polymorphic DNA (RAPD-PCR) technique.

2. Material and Methods

Samples

Eighty one slaughtered fattened and emaciated cattle carcasses at Zagazig and Menya El-Kamh abattoirs for human consumption were investigated for the presence of sarcocysts. The age of the animals ranged from 1-2 years.

Fresh samples in the size of walnut from oesophagus were collected from each examined animal and transported to the laboratory of Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University.

Macroscopic and microscopic examination

Naked eye inspection was carried out for the investigated slaughtered cattle carcasses for the presence of macroscopic *Sarcocystis* cysts.

Small pieces of muscles (about 5x5 mm dimensions, 1-2 mm thickness) were compressed between two glass slides and examined microscopically for microscopic *Sarcocystis* cysts.

Histopathological examination

Histopathological examination was performed for microscopically positive samples. About 0.5-1.0 cm from the oesophgeal samples were fixed in 10% neutral buffered formalin, processed by the standard histological techniques and stained with hematoxylin and eosin (Bancroft and Stevens, 1993).

Electron microscopy

The thick-walled *Sarcocystis* cysts were processed for examination by transmission electron microscopy in order to differentiate *S. hominis* from *S. hirsuta.* Samples from the oesophageal muscles (2 mm x 2 mm) were fixed in 2.5% glutaraldehyde. Semi-thin sections were made and stained with toluidine blue, while ultra thin sections were stained with urinyl acetate and lead citrate and examined with the electron microscope (Jeol, JXA, 840A electron probe microanalyzer, Japan) at the national research center, Dokki, Egypt.

RAPD-PCR

DNA was extracted from ethanol preserved heavily infested oesophageal samples proved positive for sarcocysts by light microscopy using Gene JET^{TM} according to the manufacturer's instructions. OSA-04 primer with the sequence of 5'-CCAGGGGAAGAGGCAT-3' (manufactured locally by Sigma, Egypt) was used to distinguish *S. hominis* from other *Sarcocystis* species of cattle (Güclü, et al. 2004). The RAPD-PCR protocol of Williams *et al.* (1990) was followed with minor modifications to optimize PCR conditions of the current study. PCR was performed in a total volume of 50 µl in a sterile 0.2 ml RNase free PCR tubes contained 25 µl of 2x reaction mix (Dream Taq^{TM} PCR Master Mix, Fermentas), 40 pmole of the primer and 2 µl of template DNA, the reaction was then completed to 50 µl using nuclease free water. DNA was amplified using 45 cycles with a denaturation step at 94^{o}C for one minute, primer annealing at 42^{o}C for 1.5 minute and an extension step at 72^{o}C for two minutes. Amplified PCR products were analyzed by electrophoresis in 1.5% ethidium bromide stained agarose gels at 100 volts for 40 minutes and visualized under UV transluminator.

3.Results

The prevalence of sarcocysts in slaughtered cattle carcasses used for human consumption in Zagazig city, Sharkia province was determined in the current study using light and electron microscopy. RAPD-PCR technique was also used for the differentiation of *S. hominis* which has zoonotic importance.

Macroscopic examination of the investigated samples could not detect large macroscopic cysts. Light microscopy revealed both thin-walled and thick-walled microscopic sarcocysts in 29.6% (24 out of 81) of the examined samples. Most of the examined carcasses were emaciated and showed muscle weakness. Thin-walled sarcocysts (Fig.1) were observed in the 24 infected animals **(**29.6%), while thick-walled sarcocysts were only detected in three samples (4.9%). The measurements of the observed sarcocysts ranged from 198-1050 µm (average 624 µm) in length and 72-150 µm (average 111 µm) in breadth.

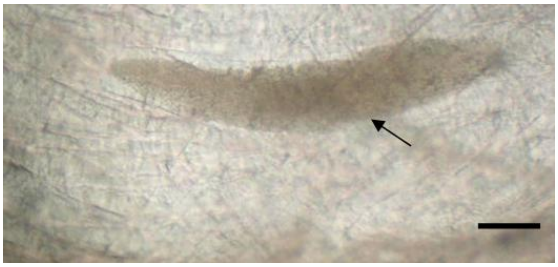

Fig.1. Light micrograph showing fresh microscopic thin-walled sarcocyst from oesophageal muscles of cattle (Arrow, Bar = 80 µm).

In addition to the compression method, histopathological sections stained with hematoxylin and eosin stain revealed both thin-walled and thick-walled sarcocysts indicating the presence of different types of *Sarcocystis* species. Thin walled cysts suggesting *Sarcocystis cruzi* (Fig. 2, A) measured 198-720 µm (average 459 µm) in length and 72-108 µm (average 90 µm) in breadth. The cyst wall thickness measured less than 1 µm. Higher magnification of sarcocysts wall showed hair-like villar protrusions in some specimens (Fig. 2, B). Thick walled cysts measured 525-1050 µm (average 787.5 µm) in length and 105-150 µm (average 127.5 µm) in breadth. The cyst wall thickness measured 7-9 µm (average 8 µm) and showed, palisade-like villar protrusions suggesting *S. hirsuta* or *S. hominis* (Fig. 2, C). Typical nearly perpendicular, cylindrical villar protrusions of *S. hominis* were also observed (Fig. 2, D).

Transmission electron microscopy detected only thick walled sarcocysts of *S. hominis*. The characteristic thick cyst wall was provided with nearly perpendicular, cylindrical villar protrusions with many longitudinal microfilaments and few dense granules (Fig. 3).

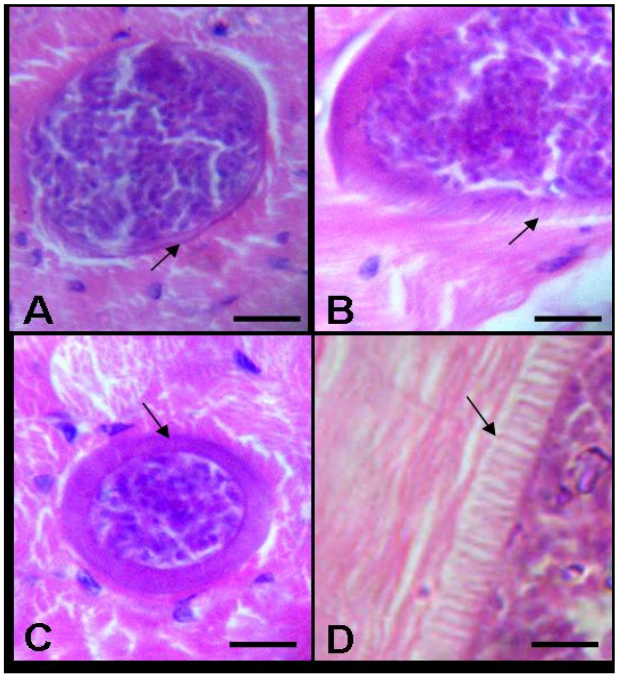

Fig. 2. Histopathological sections of the cattle oesophagus showing different types of sarcocysts wall (Arrows, Hematoxylin and eosin stain). (A) Thin-walled sarcocyst of *S. cruzi* (Bar = 20 µm); (B) Higher magnification of thin-walled *S. cruzi* sarcocyst showing cyst wall with hair-like villar protrusions (Bar = 18 µm); (C) Thick-walled sarcocyst suggesting *S. hominis* or *S. hirsuta* (Bar = 20 µm); (D) Higher magnification of Thick-walled sarcocyst with nearly perpendicular cylindrical villar protrusions characteristic for *S. hominis* (Bar = 1.0 µm).

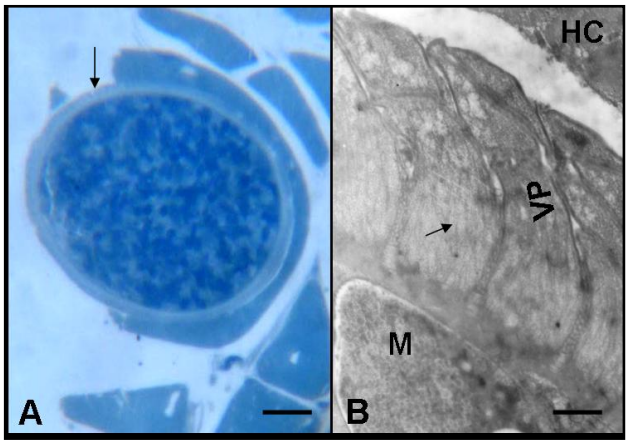

Fig. 3. Transmission electron micrograph of *S. hominis*. (A) Toluidine blue stained section showing thick cyst wall (arrow, Bar= 20 µm); (B) TEM image showing the villar protrusions (VP), microfilaments (arrow), Metrocyte (M) and the host cell (HC) (Bar= 1.6 µm).

One sample that revealed thick walled cysts was further investigated using RAPD-PCR to differentiate *S. hominis* that can infect humans. Using a duplicate of the sample, RAPD-PCR technique showed only one DNA fragment at about 290 base pairs (Fig. 4) which is characteristic for *S. hominis*.

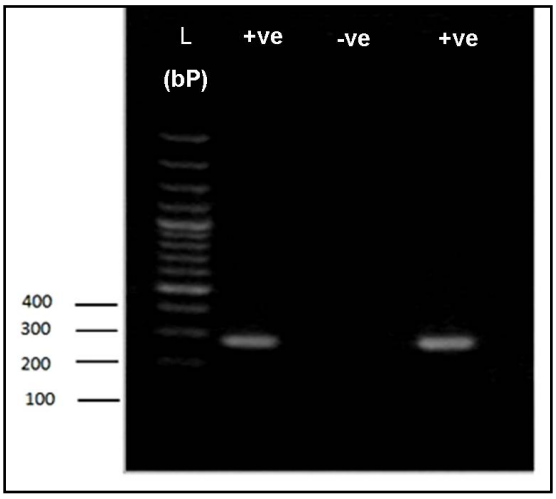

Fig. 4. RAPD-PCR using OSA-04 primer showing a characteristic single band of *S. hominis* at about 290 base pairs (L: Ladder; bp: base pairs; +ve: positive samples; -ve: negative control).

4. Discussion

The current study aimed to identify *Sarcocystis* species infecting cattle slaughtered at two abattoirs in Sharkia province, Egypt, using light microscopy, electron microscopy and RAPD-PCR technique.

The results showed that microscopic sarcocysts were prevalent in examined animals with a percentage of 29.6%, while large macroscopic sarcocysts could not be detected. In Assiut, similar results were reported by Sayed *et al.* (2008) who found that microscopic sarcocysts were more prevalent in cattle with 94% percentage than macroscopic cysts (4%). Also, investigations by Nourollahi Fard *et al.* (2009) and Nourani *et al.* (2010) detected microscopic sarcocysts in 100% (Kerman abattoir) and 89% microscopic *S. cruzi* (Fesaran abattoir) in the examined cattle in Iran, respectively; while macroscopic cysts were not found in any of the examined animals in both investigations.

The high frequency of microscopic sarcocysts in cattle may be attributed to the fact that cattle are in more close contact to sporocysts shed from infected stray dogs infected with the microscopic cysts of *S. cruzi* than that shed from infected cats with the macroscopic cysts of *S. hirsuta* where cats are almost bred inside houses.

There are three main species of cattle *Sarcocystis; S. cruzi* (*S. bovicanis*) of which canides are definitive hosts; *S. hirsuta* (*S. bovifelis*) transmitted by cats and *S. hominis* (*S. bovihominis*) transmitted by humans (Tenter, 1995; Dubey and Lindsay, 2006). In the current investigation, compression technique and histopathology revealed both thin-walled and thick-walled microscopic sarcocysts in infected cattle. Measurements and the characteristic hair-like villar protrusions of the cyst wall of thin-walled sarcocysts indicated *S. cruzi* infection of the examined cattle. Similar earlier descriptions of *S. cruzi* were also stated elsewhere (Sayed *et al.,* 2006; Khalifa *et al.,* 2008; Xiang *et al.,* 2011). *S. cruzi* was also detected with high percentage in infected cattle reared in different localities in Egypt (Sayed *et al.,* 2006; Khalifa *et al.,* 2008; Sayed *et al.,* 2008). These findings confirm that *S. cruzi*, the most pathogenic *Sarcocystis* species of cattle, is prevalent in cattle in Egypt.

Sarcocystis species can be differentiated on the basis of their specific cyst wall structure as well as by molecular methods (Dubey *et al.,* 1989 a, b; Tenter, 1995; Jehle *et al.,* 2009). Concerning the findings of electron microscopy, only thick-walled *S. hominis* could be detected in the examined sections. The structure of *S. hominis* was described in the current study to be provided with nearly perpendicular, cylindrical villar protrusions with many longitudinal microfilaments and few dense granules. Similar characteristic features of *S. hominis* were also reported (Dubey *et al.,* 1989c; Saito *et al.,* 1999; Moré *et al.,* 2011). RAPD-PCR results using OSA-04 primer revealed a characteristic one band at about 290 base pairs which differentiate *S. hominis* from other types of *Sarcocystis* infecting cattle as previously stated by Güçlü *et al.* (2004). *S. hirsuta* could not be confirmed neither by electron microscopy nor by RAPD-PCR in the conduced study.

Based on detection by light microscopy only, not by naked eye inspection, *S. hominis* was categorized under microscopic sarcocysts in the current study. However, *S. hominis* sarcocysts detected in cattle were previously described by some authors as macroscopic cysts, measured from 1.2-7 mm in maximum length (Saito *et al.,*1999; Dubey and Lindsay, 2006). This variation in size could be attributed to the age of sarcocysts. This argument is supported by the findings of Böttner *et al.* (1987), who concluded that the structure and morphology of sarcocysts differ with its age.

The identification of *S. hominis* in the current study has public health significance due to the zoonotic potential of this species. Of fecal specimens examined from children in Poland and Germany, 10.4 and 7.3% were found positive, respectively. Of 1,228 apprentices in Central Slovakia in 1987 to 1989, 14 (1.1%) were positive (Straka *et al.,* 1991). After raw beef containing *S. hominis* was prepared and fed to seven human volunteers, six excreted sporocysts and two developed diarrhea (Pena *et al.,* 2001). After eating raw beef, a patient in Spain with abdominal discomfort and loose stools was diagnosed with *S. hominis* oocysts in his feces (Clavel *et al.,* 2001). In Tibet, Sarcocystis was detected in 42.9% of beef specimens examined from the marketplace, and *S. hominis* was found in stools from 7% of 926 persons (Yu, 1991).

In conclusion, the results of the present study revealed that pathogenic microscopic sarcocysts of *S. cruzi* are prevalent in cattle slaughtered at Sharkia province. Also, the prevalence of *S. hominis* infection in slaughtered cattle has a potential of public health importance because humans can be infected through consumption of raw or insufficiently cooked meat. Further control measures should emphasize awareness in farmers and all the beef production chain to prevent sarcocysts infections, especially by *S. hominis*. Moreover, more efforts in excluding dogs from animal houses and facilities are strongly needed.

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