SARCOCYSTIS INFECTION IN CATTLE AT ASSIUT ABATTOIR: MICROSCOPICAL AND SEROLOGICAL STUDIES

Fatma G. Sayed*, Maha S. I. Shaheen*, Mohsen I. Arafa** and Hoda M. Koraa**

*Dept. of Parasitology, Faculty of Medicine, Assiut University
**Animal Health Research Institute Assiut

ABSTRACT:

The present work was conducted to study Sarcocystis infection in cattle by microscopical and serological examinations. Samples from the ocular muscle, oesophagus, diaphragm and heart of 100 cattle slaughtered at Assiut abattoir were examined grossly and microscopically. The total infection rate of the examined cattle was found to be 94%. The infection rate in different organs was 89% in ocular muscles, 84% in oesophageal muscles, 51% in cardiac muscles and 30% in diaphragm. Serological examination of sera of the same examined animals by enzyme linked immuno-sorbent assay (ELIZA) revealed that the infection rate was 98%. The maximum antibody level of the examined cattle by ELIZA was associated with highly infected oesophageal muscle with Sarcocystis cysts.

Two types of cysts were detected in the present work: microscopic thin–walled and macroscopic thick -walled cysts. Microscopic thin–walled cysts were recovered in all positive animals. Their cyst wall was narrow and homogenous. The accurate identification of microscopic cysts as Sarcocystis cruzi has been completed after the success of experimental infection in puppies. They began to shed sporocysts after seven days from infection and remained till the end of the experiment. Macroscopic thick –walled cysts were recovered in four cases only. Their cyst wall was composed of long striated protrusions in a palisade-like arrangement. It could not be identified as Sarcocystis hirsuta or Sarcocystis hominis by light microscope, where differentiation between them need another investigation by electron microscope. Certain pathological changes were associated only with heavy infection with microscopic cysts (S. cruzi) infection. These changes included muscular degeneration and focal leukocytic infiltration composed of eosinophils, macrophages and lymphocytes.

INTRODUCTION:

Sarcocystis is one of the most prevalent parasites of the livestock. In some hosts such as domestic cattle, all adult animals may be infected. It is economically important and pathogenic to livestock. It may causes abortion, acute fatal illness and poor growth in cattle (Dubey, 1976; Dubey et al., 1989). It is an intracellular protozoan parasite. It has an obligatory prey–predator two host life cycle, that has a carnivorous predator hosts (dogs, cats and man) and a wide variety of prey hosts (sheep, cattle, buffalo, pig, camels, birds, fish...
and man). Species of *Sarcocystis* are generally more specific for their prey hosts than for their predator hosts (Collier et al., 1998).

There are three species of *Sarcocystis* in cattle: *Sarcocystis cruzi*, *Sarcocystis hirsuta* and *Sarcocystis hominis*. *Sarcocystis cruzi* is the most common and important species affecting cattle (Heydorn et al., 1975).

The clinical signs of infected cattle with *Sarcocystis* differ according to the amount of sporocysts inoculated and these included fever, anorexia, anaemia, diarrhea, cachexia, weight loss, accelerated heart rate, abortion, myositis, neurological signs, and occasionally may lead to death (Meads, 1976 and Dubey et al., 1982).


The aim of the present work was to study the prevalence of *Sarcocystis* in slaughtered cattle at Assiut abattoir by using microscopical examination and ELIZA. In addition to try to identification of the detected species by microscopical examination and experimental infection.

**MATERIALS AND METHODS:**

I-Collection of samples:

Samples were collected from 100 cattle (less than two years old) slaughtered in Assiut city abattoir. These samples included oesophagus, heart, diaphragm and ocular muscles.

II-Examination of muscle samples:

1-Macroscopic examination:

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

2- 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).

III-Histopathologicel studies:

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

IV-Experimental work:

-Recently weaned 3 puppies and 3 kittens were used (parasitic free) one from each was used as control. Each animal was fed 250 gm of raw infected meat from naturally infected cattle in a divided doses (Latif et al., 1999).

-After a day of infection, faeces was regularly collected twice daily and thoroughly examined for 60 days post inoculation.

-Fecal samples were examined for the presence of sporocysts by direct smear and the centrifugation flotation technique (Soulsby, 1982).

V-Serological diagnosis (ELIZA):

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

Serum samples: One hundred of venous blood samples were taken from the same examined animals at the time of slaughterring. Sera were separated by centrifugation at 1500 r/min after being kept in the refrigerator for overnight. Sera were kept at -20°C until used.

ELIZA: ELIZA 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:
Frequency of occurrence:

Gross and microscopical examination of muscle samples of one hundred (100) cattle slaughtered in Assiut abattoir revealed that the infection rate of *Sarcocystis* was 94%. All infected animals had microscopic cysts, while four cases (4%) had mixed infection with macro and microscopic cysts (Table 1).

Concerning the infection rate of different organs, the highest infection rate was detected in ocular muscles (89%) followed by oesophageal muscles (84%), cardiac muscles (51%) and lastly diaphragmatic muscles (30%) (Table 2).

Morphological studies:
1- Microscopic cysts:

Table (1): Prevalence of *Sarcocystis* in the examined cattle in Assiut

<table>
<thead>
<tr>
<th>Examined animals</th>
<th>Infected animals</th>
<th>Microscopic cysts</th>
<th>Macroscopic cysts</th>
<th>Mixed infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>100</td>
<td>94</td>
<td>94%</td>
<td>94</td>
<td>94%</td>
</tr>
</tbody>
</table>

Table (2): *Sarcocystis* infection in different organs of examined cattle

<table>
<thead>
<tr>
<th>Organs</th>
<th>Ex. samples</th>
<th>Infected samples</th>
<th>Macroscopic cyst</th>
<th>Microscopic cyst</th>
<th>Mixed infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Ocular m.</td>
<td>100</td>
<td>89%</td>
<td>3</td>
<td>3%</td>
<td>89</td>
</tr>
<tr>
<td>Oesophageal m.</td>
<td>100</td>
<td>84%</td>
<td>1</td>
<td>1%</td>
<td>84</td>
</tr>
</tbody>
</table>

Fresh cysts were seen as fusiform-shaped microscopic cysts, parallel to muscle fibers, their measurements ranged from 114.2-643.05x 45.68-200.06 µm (378.63×122.87 µm). In histopathological section, the cyst wall was seen as narrow homogenous wall less than 0.57 µm. The cyst was filled with bradyzoites, while the dividing septa was not clear (Plate I- 1& 2).

2-Macroscopic cysts:

It appears grossly as fusiform or spindle shaped, white or creamy colour, their measurements ranged from 1.0–7.23×1.0-1.5 mm (4.63×1.25 mm). In examination of stained section, the cyst had thick wall measured from 7.41 to 10.72 µm. The bradyzoites were crowded peripherally while the center of the cyst was free. The higher magnification of the cyst clear that the cyst wall was composed of long striated protrusions in a palisade-like arrangement and the cyst was divided with thick septa into irregular compartments filled with bradyzoites (Plate I- 3 & 4).
<table>
<thead>
<tr>
<th>Musculature</th>
<th>Value</th>
<th>%</th>
<th></th>
<th></th>
<th>Value</th>
<th>%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac m.</td>
<td>100</td>
<td>51</td>
<td>51%</td>
<td>-</td>
<td>51</td>
<td>51%</td>
<td>-</td>
</tr>
<tr>
<td>Diaphragmatic m.</td>
<td>100</td>
<td>30</td>
<td>30%</td>
<td>-</td>
<td>30</td>
<td>30%</td>
<td>-</td>
</tr>
</tbody>
</table>
Histo-pathologicl studies:

Some pathological changes were detected in the present work associated only with heavy microscopic cysts infection. These changes included muscular degeneration and focal leukocytic infiltration composed of eosinophils, macrophages and lymphocytes (Plate II-1 & 2).

Experimental studies:

Feeding puppies and kitten with heavily infected meat with sarcocysts for three days revealed that only all puppies were infected and shed sporocysts while kitten can't infected. The prepatent period was 7-15 days and patent period was 50–60 days. The sporocysts were ellipsoidal measured 14.3-17.16 µm×8.58-11.44
µm (15.73×10.01 µm) and completely sporulated, containing four sporozoites when passed in the faeces (plate II-3).

Based on the size of cyst, morphology of the cyst wall and the establishment of infection in experimentally inoculated dog, the microscopic cysts were identified as *S. cruzi*. Macroscopic thick–walled cysts could not be identified as *S. hirsute* and/or *S. hominis* on the basis of morphological grounds only.

<table>
<thead>
<tr>
<th>Tested samples</th>
<th>Microscopical examination</th>
<th>ELIZA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive samples</td>
<td>94</td>
<td>98</td>
</tr>
<tr>
<td>Negative samples</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table (3): Comparison between results obtained by microscopical examination and ELIZA.**

(Plate II)

(1) Degeneration in diaphragmatic muscle contain *S. cruzi* cyst (head arrow) surrounded with eosinophils (arrow) H&E ×400.

(2) Degeneration in oesophageal muscle of infected cattle with inflammatory cell infiltration eosinophils, macrophage and lymphocytes as a result of *S. cruzi* infection H&E ×400.

(3) The ellipsoidal sporulated sporocysts of *Sarcocystis cruzi* ×400.
Serological diagnosis (ELIZA):

Out of 100 serum samples of slaughtered cattle examined, 98 samples were positive for Sarcocystis infection (98%). Most of positive samples 73 (73.5%) were considered moderately positive where the density values ranged between 2.6 to 3, while the lowest positive samples were detected in 11 cases (11.2%) their optical density was below 2.6. Highly positive samples were detected in 15 cases (15.3%) where their optical density was above 3.0. The highly positive cases were associated with highly infected oesophageal muscle with Sarcocystis cysts. Serological examination cleared that only four cases (4%) were considered false negative by microscopical examination. This is of no significant difference in the prevalence of Sarcocystis in the examined animals (P = 0.279).

DISCUSSION:

In the present work both microscopical and serological examination (ELIZA) were used for diagnosis of Sarcocystis infection in cattle at Assiut abattoir.

As regard to serological diagnosis, Sarcocystis fusiformis was used in the present work as a source of antigen used in ELIZA for diagnosis of Sarcocystis infection in cattle. Habeeb et al. (1996) and El Nazer & Abdel–Azem (2000) used S. fusiformis antigen in ELIZA and IFAT for detection of extra intestinal sarcocystosis in human. Abdel–Rahman (2001) used it in ELIZA and Western blot for diagnosis of Sarcocystis infection in cattle. In addition Tadros et al., (1980) found a remarkable degree of cross reaction among Sarcocystis species from widely divergent host origins.

In general, Sarcocystis infection of the examined cattle showed high infection rate (94%) by microscopical examination of muscle samples. This result was confirmed by serological examination (ELIZA) of sera of the same examined animals, where the infection rate was 98%. The high frequency of Sarcocystis infection in cattle was expected, considering the frequency reported around the world in cattle: in New Zealand was 98% (Bottner et al., 1987) in Brazil was 100% (Pana et al., 2001).

In Egypt, high incidence of Sarcocystis species of cattle was reported by El-Afifi (1958) 84% and 100% in adult healthy and emaciated cattle respectively, Abdel Rahman (1975) 39.2%, Ali (1985) 58.02%, Mohamed (1996) 30%, El-Saieh (1998) 65.46% and Abdel Rahman (2001) 41.4%.

Collier et al., (1998) mentioned that a variety of conditions permit such high prevalence of Sarcocystis: many definitive hosts are involved in transmission, shedding of large number of sporocysts (as infective form) for many months, resistance of oocysts or sporocysts in external environment for long period, role of invertebrate transport hosts in spreading of infection in addition to little or no immunity to reshedding of sporocysts after each meal of infected meat.

Concerning organs affected, the present study clear that ocular muscles appears to be a preferred site for the development of Sarcocystis in these intermediate hosts followed by oesophageal muscles. This result agree with Juyal et al., (1982) and Mohanty et al., (1995) who recorded a high prevalence and heavy concentration of S. cruzi sarcocysts in ocular muscles of cattle in India.

The species of Sarcocystis involved in the present work identified microscopic cyst as S. cruzi. Identification of S. cruzi depended on morphological characters of the cysts and sporocysts in addition to success of
experimental infection of dogs with it. These descriptions agree with Levine, (1985), Latif et al., (1999) and Venu & Hafeez, (1999). Identification of macroscopic cysts needed another investigation to study their fine structures for accurate identification of them as S. hirsuta or S. hominis. Failure of infection of kittens with Sarcocystis sp., may be related to low number of macroscopic cysts detected in the examined animals. Bottner et al., (1987) mentioned that a sufficiently large number of sarcocysts is necessary to endue an infection with Sarcocystis.

A correlation between pathological changes and the infection grade prove that the pathological reactions could be detected only in heavily infected cases with microscopic cysts (S. cruzi). This agree with both Dubey et al., (1989) and Collier et al., (1998) who mentioned that S. cruzi is more pathogenic for cattle than S. hirsute and S. hominis.

Comparison of obtained results revealed the presence of differences in the sensitivity between the microscopical examination and serological diagnosis. Results of ELIZA was slightly higher, where the infection rate was 98%, this difference may be due to recently infected cases characterized by high antibody titer and low number of sarcocysts in muscular tissues.

REFERENCES:


Mohamed, M. S. (1996): “Muscular parasites in slaughtered animals in Assiut Govern-


ﱄ كمامة بعضاً تسست الدم جدار تسست جدار المجهر والتشملت اعلياً سبعاً المراء الضوئي مصلاً ووجد ساركوسب الإصابة تحلل ون بعد مائة هذه وقود نسبة الساركوسب او المناقشة %، وبحصرة الامرية ومائع الوعود حسب بينهما. ومن ن، وقود % اسيو كروز اثاراء اخراج * كل بحوصات يميز لكل ن مان بمجزر باماك الى الساركوسب او شرائح العيون 0.3 حي ثا انسار والدمار بين الساركوسب كل الكترونيني للكلاب بحويصالات يمال الساركوسب بين الصحة والمحزنات الكهربائية السلالة، سيد إبراهيم وبوفديات الحيوانات، العيون، الساركوسب - بالوعاًتطوير، الساركوسب - بالوعاً الإصابة اتباع جراء،،، والحة -57- الإصابة الشرعية في اسم المرض، وقود العيون، الساركوسب - بالوعاً تطور تتطور، الساركوسب - بالوعاً الإصابة اتباع جراء،،، والحة -57- الإصابة الشرعية في اسم المرض، وقود العيون، الساركوسب - بالوعاً الإصابة اتباع جراء،،، والحة -57- الإصابة الشرعية في اسم المرض، وقود العيون، الساركوسب - بالوعاً الإصابة اتباع جراء،،، والحة -57- الإصابة الشرعية في اسم المرض، وقود العيون، الساركوسب - بالوعاً الإصابة اتباع جراء،،، والحة -57- الإصابة الشرعية في اسم المرض، وقود العيون، الساركوسب - بالوعاً الإصابة اتباع جراء،،،