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

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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 oesophagial 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).

Several authors studied sarcocystosis of cattle in Upper Egypt: Abdel–Rahman (1975), Ali (1985), Mohamed (1996), El–Saieh (1998), Abdel-Rahman (2001)and Mowafy (2003).

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-Histopathologicl 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* cystizoite 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 slaughtering. 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:

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 histo-pathological 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:

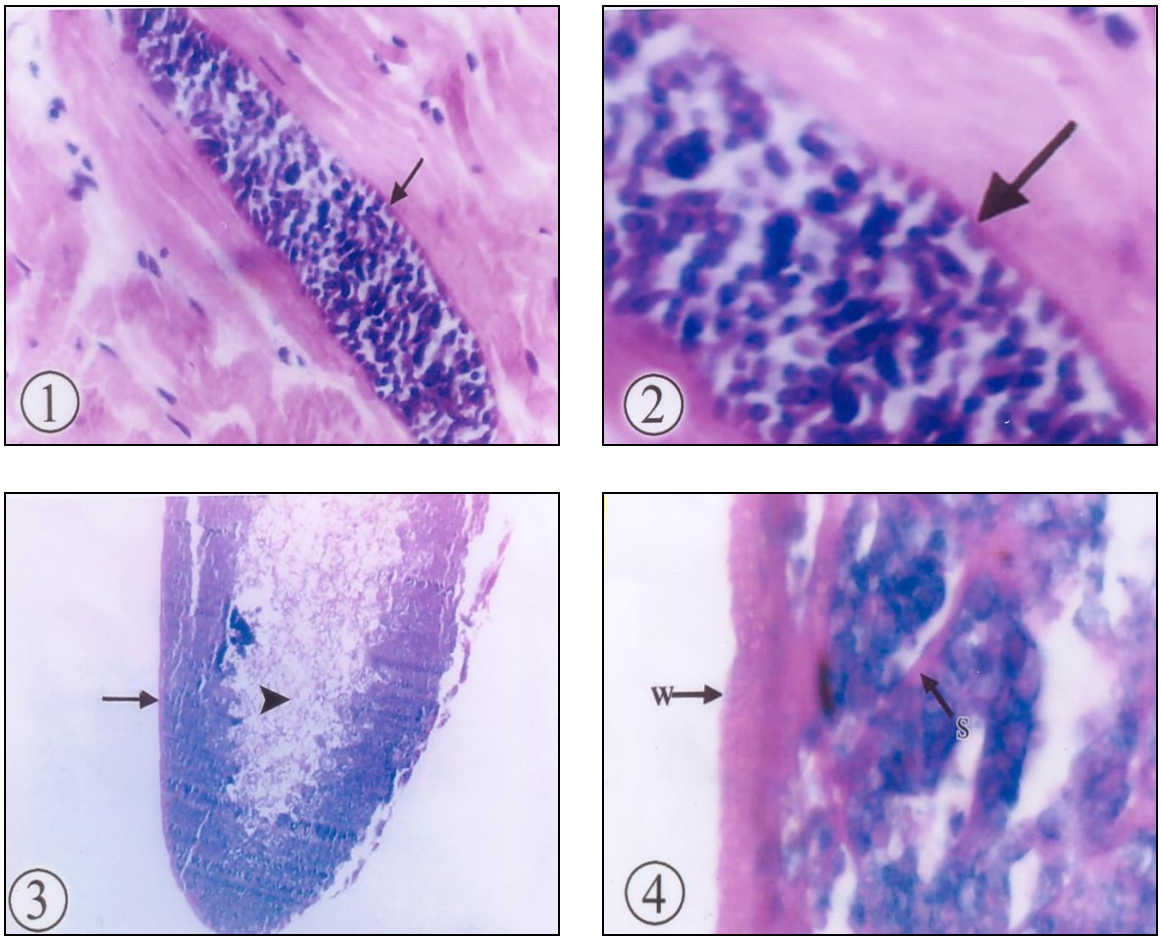
It appears grossly as fusiform or spindle shaped, white or creamy colour, their measurements ranged from 1.0–7.23×1.0-1.5 mm (4.63×1.25 mm). In examination of stained section, the cyst had thick wall measured from 7.41 to 10.72 µm. The bradyzoites were crowded peripherally while the center of the cyst 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(1): Prevalence of *Sarcocystis* in the examined cattle in Assiut

Examined animals	Infected animals		Microscopic cysts		Macroscopic cysts		Mixed infection	
	No.	%	No.	%	No.	%	No.	%
100	94	94%	94	94%	4	4%	4	4%

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

Organs	Ex. samples	Infected samples		Macroscopic cyst		Microscopic cyst		Mixed infection	
		No.	%	No.	%	No.	%	No.	%
Ocular m.	100	89	89%	3	3%	89	89%	3	3%
Oesophageal m.	100	84	84%	1	1%	84	84%	1	1%
Cardiac m.	100	51	51%	-	-	51	51%	-	-
Diaphragmatic m.	100	30	30%	-	-	30	30%	-	-



(Plate I)

(1) Microscopic cyst (*S. cruzi*) in cardiac muscle: showing spindle shaped cyst filled with bradyzoites H&E× 200.

(2) Higher magnification of microscopic cyst (*S. cruzi*) showing thin cyst wall (arrow) followed by large groups of bradyzoites without separating septa H&E×400.

(3) Macroscopic cyst: showing over crowding of bradyzoites behind the wall (arrow) while the center of the cyst is free from bradyzoites (head arrow) H&E×40.

(4) Higher magnification of macroscopic cyst showing characteristic thick cyst wall (W), followed by large groups of bradyzoites separated by thick septa (S) H&E×1000.

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:

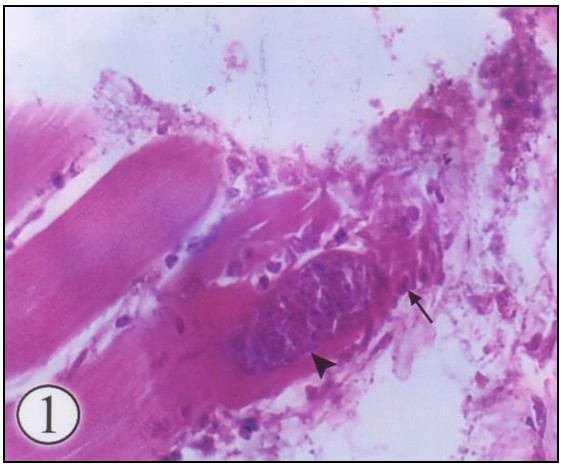
Feeding puppies and kitten with heavily infected meat with *sarcocysts* for three days revealed that only all puppies were infected and shed sporocysts while kitten can't infected. The

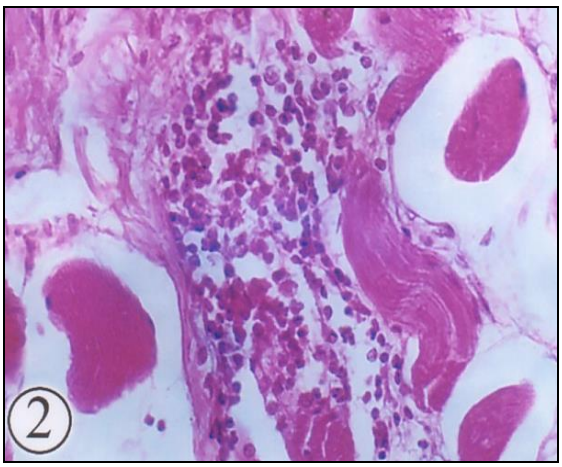
prepatent period was 7-15 days and patent period was 50–60 days. The sporocysts were ellipsoidal measured 14.3-17.16 µm×8.58-11.44 µm (15.73×10.01 µm) and completely sporulated, containing four sporozoites when passed in the faeces (plate II-3).

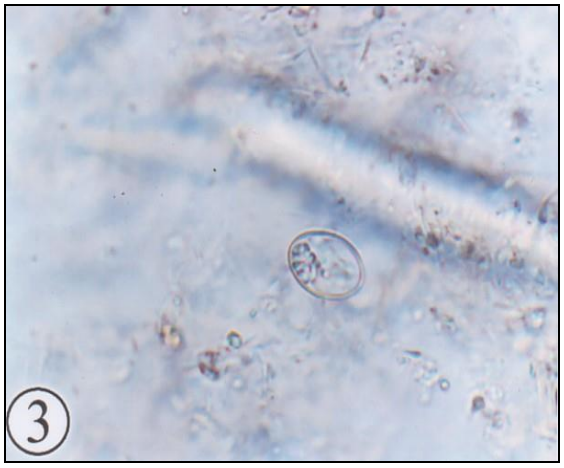
Based on the size of cyst, morphology of the cyst wall and the establishment of infection in experimentally inoculated dog, the microscopic cysts were identified as *S. cruzi*. Macroscopic thick–walled cysts could not be identified as *S. hirsute* and/or *S. hominis* on the basis of morphological grounds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

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

Tested samples	Microscoical examination	ELIZA
Positive samples	**94**	**98**
Negative samples	**6**	**2**







(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 infilration 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* scarcocysts in ocular muscles of cattle in India.

The species of *Scarcocystis* 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 *Scarcocystis 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 scarcocysts in muscular tissues.

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عدوى الساركوسيستس فى الأبقار بمجزر أسيوط
دراسات مجهرية و سيرولوجية

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في هذا البحث تم دراسة عدوى الساركوسيستس في الأبقار بمجزر أسيوط بواسطة الفحص المجهرى واختبار الاليزا. حيث تم الفحص المجهرى لعينات عدد ١٠٠ من الأبقار المذبوحة بمجزر أسيوط واشتملت تلك العينات على أجزاء من عضلات العيون والمرئ والقلب والحجاب الحاجز لكل حيوان. بلغت نسبة الإصابة الكلية ٩٤% باستخدام الميكروسكوب الضوئي، وقد سجلت عضلات العيون أعلى نسبة إصابة ٨٩% يليها المرئ ٨٤ % ثم عضلة القلب ٥١% بينما كانت عضلة الحجاب الحاجز أقلها إصابة ٣٠%. وبفحص عينات مصل الدم للأبقار السابقة بواسطة اختبار الاليزا بلغت نسبة الإصابة بالساركوسيستس ٩٨%. وقد لوحظ أن أعلى تتر للأجسام المضادة للأبقار المصابة كان مصاحباً لحالات العدوى الشديدة بحويصلات الساركوسيستس للمرئ.

تم تمييز حويصلات الساركوسيستس المعزولة من تلك الحيوانات بواسطة الميكروسكوب الضوئي إلى نوعين: الأول وهو الساركوسيستس المجهرى، وقد وجد في جميع الحيوانات المصابة، وهو يتميز بوجود جدار رقيق متجانس. وهذا النوع هو ما يعرف بساركوسيستس كروزى.

وقد تم التأكد منه بعد نجاح العدوى التجريبية للكلاب الصغيرة حيث بدأ إخراج أكياس الابواغ بعد سبعة أيام من بدء العدوى التجريبية واستمرت حتى نهاية التجربة. أما النوع الثاني من الحويصلات فهو الساركوسيستس العيانى، وقد وجد في أربع حالات فقط، وهو يتميز بأن له جدار سميك له بروزات عمودية أو مائلة على جدار الحويصلة وموازية لبعضها البعض. وهذا النوع من الحويصلات هو ما يميز كل من الساركوسيستس هيرسوتا والساركوسيستس هومينس حيث لا يمكن التفريق بينهما بواسطة الميكروسكوب الضوئى. وقد ظهرت بعض التغيرات الباثولوجية فى الأبقار المصابة بالعدوى الشديدة لساركوسيستس كروزى فقط وتميزت بتحلل الأنسجة العضلية المصابة وترسيب لكرات الدم البيضاء خاصة الايزينوفيل والليمفوسيت. وقد تم مناقشة هذه النتائج، كما خلصت النتائج إلى أهمية عمل دراسة بواسطة الميكروسكوب الالكتروني للتفريق بين كل من ساركوسيستس هيرسوتا وساركوسيستس هومينس.