Dept. of Parasitology,
Fac. of Medicine, Assiut University, Assiut

# *SARCOCYSTIS HOMINIS* CYST IN COMPARISON WITH OTHER *SARCOCYSTIS* SPECIES FOUND IN OCULAR MUSCLES OF CATTLE: A STUDY BY TRANSMISSION ELECTRON MICROSCOPE

(With One Table and 4 Plates)

By

***FATMA G. SAYED; MAHA S.I. SHAHEEN; M.I. ARAFA* and HODA M. KORAA****

*Animal Health Research Institute Assiut

(Received at 22/3/2006)

## ساركوسيستس هومينس فى مقارنة مع الانواع الاخرى من الساركوسيستس الموجودة فى عضلات عيون الابقار : دراسة بواسطة الميكرسكوب الالكترونى

***فاطمة جلال سيد ، مها سيد ابراهيم شاهين ، محسن ابراهيم عرفة ، هدى محمد محمد قراعة***

تنتشر الأصابة بالساركوسستس فى الأبقار فى جميع أنحاء العالم وبعض هذه الأنواع له أهمية طبية للأنسان. تم فى هذا البحث دراسة أنواع الساركوسستس المختلفة التى تصيب الأبقار فى محافظة أسيوط حيث تم فحص مائة عينة من عضلات عيون الأبقاروقد بلغت نسبة الاصابة فيها ٨٩% وتم تصنيف انواع الساركوسستس الموجودة فى تلك العضلات بواسطة الميكرسكوب الألكترونى إلى أربعة أنواع. النوع الأول هو الساركوسستس هومينس وهو يتميز بوجود أمتدادات زغبية أسطوانية (شكل الأصبع) وتكون عموديةعلى السطح الخارجى بينما يكون له قمة عريضة اما النوع الثانى هو الساركوسستس هيرسوتا والذى يتميز بوجود أمتدادات زغبية لها شكل مضرب الكرة حيث له قمة مدببة وبعض قمم الزغبات تمتد لتكون بروزات قمعية والنوع الثالث هو ساركوسستس كروزاى والذى يتميز بوجود جدار رفيع وله أمتدادات زغبية تشبه الشعيرات تنتشرعلى سطح الجدار وتكون تقريبا موازية له. أما النوع الرابع هو مشابه الساركوسستس فيوزيفورميس الموجود فى الجاموس ويتميز بوجود أمتدادات زغبية عديدة التفرعات وله قاعدة متقلصة وتعد هذه أول مرة لتسجيل هذا النوع فى الأبقار. كما تعتبر هذه الدراسة أول مرة لتسجيل الأصابة بالساركوسستس هومينس فى عضلات عيون الأبقار فى أسيوط.

## SUMMARY

*Sarcocystis* species are widespread in cattle throughout the world. Some species are pathogenic and have medical importance. In the present

work, one hundred sample from ocular muscles of cattle were examined where the infection rate was 89%. Ultra-structure studies of *Sarcocystis* species of cattle with transmission electron microscope revealed the presence of four species. The first species was *Sarcocystis hominis* with cylindrical (finger like) villar protrusions perpendicular on the cyst surface with broad bases. The second species was *Sarcocystis hirsuta* that showed club or bulb like villar protrusions with constricted base and tapered tips. Some of villar tips folded to form conical projections. The third one was *Sarcocystis cruzi* which was characterized by thin wall with hair like villar protrusions folded over the surface of the cyst and approximately parallel to the cyst surface. The fourth species was *Sarcocystis fusiformis* like cyst which had highly branched villar protrusions with constricted base. This is the first description of this species in cattle also it is the first time of description of *Sarcocystis hominis in* ocular muscles in Assiut.

***Key words:*** *Sarcocystis hominis, Sarcocystis species, Ocular muscles*

## INTRODUCTION

There are three species of *Sarcocystis* in cattle: *Sarcocystis cruzi* (*Sarcocystis bovicanis*), *Sarcocystis hirsuta* (*Sarcocystis bovifelis*) and *Sarcocystis hominis* (*Sarcocystis bovihominis*) (Heydorn *et al.*, 1975; Dubey *et al.*, 1989b). Human serves as the predator host for *Sarcocystis hominis* and also serves as accidental intermediate hosts for several unidentified species of *Sarcocystis* (Collier *et al.*, 1998).

The clinical signs of infected cattle with *Sarcocystis* differ according to the amount of sporocysts inoculated (Dubey, 1982b). These signs included fever, anorexia, anemia, diarrhea, cachexia, weight loss, accelerated heart rate, abortion, myositis and neurological signs (Meads, 1976).

In humans, signs of intestinal sarcocystosis are nausea and diarrhea from three to six hours after ingesting infected beef with *Sarcocystis*, while the clinical signs of muscular sarcocystosis are fever, myalgias, bronchospasm, fleeting pruritic rashes, transient lymphadenopathy, and subcutaneous nodules (Arness *et al.*, 1999).

The cysts of *Sarcocystis cruzi* are thin – walled microcysts (Dubey, 1976), whereas those of *Sarcocystis hirsuta* and *Sarcocystis hominis* are thick – walled and are either microcysts or small developing macrocysts (Mehlhorn and Heydorn, 1978). The present work aimed at determination of *Sarcocystis* infection in cattle in Assiut

through examination of their ocular muscles samples by naked eye and light microscope, in addition to study of their ultra-structure cyst wall for identification of the species.

## MATERIALS and METHODS

**I) Collection of samples:**

In this study ocular muscle samples were collected from 100 cattle slaughtered in Assiut city abattoir, their ages were less than two years old.

**II) Examination of muscle samples:**

**1. Macroscopic examination:**

Fresh muscle samples were examined by naked eye 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 (Mowafy, 1993).

**3. Preparation of samples for transmission electron microscope examination:**

Small pieces (about 3 mm) of fresh infected muscles were fixed in 2.5% glutaraldehyde. Semi-thin sections were cut, stained with toluidine blue (Richardson *et al.*, 1960) and examined by light microscope. Ultra-thin sections were stained with saturated aqueous uranyl acetate (Stempak and Ward, 1964) then examined under JEOL – JEM – 100C X II ELECTRON MICROSCOPE).

## RESULTS

Examination of ocular muscles samples of 100 cattle in Assiut revealed that infection rate of was (89%). Four species of *Sarcocystis* were detected in present work:

1- *Sarcocystis hominis* 2 -*Sarcocystis hirsuta*

3-*Sarcocystis cruzi* 4 -*Sarcocystis fusiformis* like cyst

**1- *Sarcocystis hominis***

Fresh cysts: It was detected as microscopic cysts in ocular muscles. Microscopic cyst appeared as spindle shaped, measured 1199.1µ in length, 165.59µ in breadth. The cysts were found parallel to muscle fibers and they appeared divided with fine striations into transverse sections (Plate I. Fig1).

Semi-thin sections: Examination of semi-thin sections of *Sarcocystis hominis* showed that their cyst wall was thick and the villar protrusions had palisade like appearance measuring 11.44µ. Metrocytes were adhered to the inner surface of the cyst wall while bradyzoites were arranged in groups separated from each others by fine septa (Plate I. Fig. 2).

Ultrastructure of *Sarcocystis hominis* wall: Cyst wall of *S. hominis* consisted of cylindrical finger like villar protrusions (VP) not equal in length and their tips may be straight or slightly curved while their bases were broad. These protrusions were perpendicular on a thin basement membrane of the cyst. Eash protrusion contains a widely scattered of variable-sized electron dense granules (E). Few bundles of microfilaments (M) were scattered in between these granules (Plate I. Fig. 3, 4).

**2– *Sarcocystis hirsuta:***

Fresh cysts: Both macroscopic and microscopic cysts were detected. Macroscopic cysts appeared as fusiform shaped cysts, white in colour measured 1000.0 - 7000.0 µ m in length, 1000.0 - 1500 µ m in breadth (4000×1250 µ m). The cysts were found parallel to muscle fibers (Plate II. Fig. 1).

Semithin sections: *Sarcocystis hirsuta* cyst in semithin section showed thick wall cys with sloping pattern villar protrusions. Cyst wall (W) was thick measured 7.41µ (mean thickness). Metrocytes (Me) were close to the inner surface of the cyst wall followed by bradyzoites (Bz) in groups separated from each others with fine septa (Plate II. Fig 2).

Ultrastructure of *Sarcocystis hirsuta* wall: Cyst wall of *Sarcocystis hirsuta* consisted of villar protrusions (VP) which were club or bulb-shaped in outline with constricted base and tapered tips. Near the tips of these protrusions there were small papillary projections (PP). Some of villar tips were folded to form conical projections (CP).The villar protrusion contain microfilaments (M) and widely scattered electron dense granules (Plate II Fig. 3, 4).

**3– *Sarcocystis cruzi:***

Fresh cysts: It was detected as a microscopic cyst only. It appears as a small spindle shaped cyst. It measured 114.2 – 643.05 µ in length and 45.68 – 200.06 µ in breadth, (378.63 × 122.87 µ m) (Plate III Fig. 1).

Semithin sections: Examination of cross section of *Sarcocystis cruzi* cyst in ocular muscle of cattle showed that it has thin cyst wall Less than 0.57 µm (Plate III Fig 2).

Ultrastructure of *Sarcocystis cruzi* wall: Cyst wall consisted of a parasitophorous vacuolar membrane (PVM) which is characterized by irregular undulations (irregularly spaced pits interrupted at irregular intervals) (Plate III Fig 3). This membrane is provided with hair like villar protrusions (VP) folded over the surface of the cyst. These protrusions were rod, round, oval or irregular in shape according their sections and they were approximately parallel to the cyst surface (Plate III Fig 4). Each protrusion contains coarse or fine electron dense granules (E) with no fibrillar structure. Groups of bradyzoites (Bz) and metrocytes were found in inner side of ground substanse (GS) separated by fine septa.

**4 – *Sarcocystis fusiformis* like cyst**

Fresh cyst: It was detected as a macroscopic cyst only. It appears as spindle shaped cyst, creamy white in colour measured 2226.9×473.93 µm (Plate VI Fig 1).

Semithin section: Semithin section of this species showed that the cyst wall was thick measured 10.725 µ. Behind the wall, metrocytes (Me) were arranged in a thin layer and take ovoid shape while bradyzoites (Bz) found in a large separated groups (Plate VI Fig 2).

Ultrastructure of *Sarcocystis fusiformis* like cyst wall: The cyst wall consisted of a parasitophorous vacuolar membrane (PVM) lined with electron dense layer which is wavy. This membrane was provided with highly branched villar protrusions (VP) with constricted base. Inside the cyst wall found metrocytes (Me) peripherally (Plate VI Fig 3). These protrusions were branched in different directions making the wall spongy in appearance, some of these protrusions were provided with small papillary protrusions (PP) along the parasitophorous vacuolar membrane. Each protrusion has filamentous core (c) with widely scattered electron dense granules (E) (Plate VI Fig 4). Inner to metrocytes located bradyzoites groups (Bz) separated by thick dark septa (S). Higher magnification of bradyzoites clear their characteristic organelles as conoid (Co) with preconoidal rings, micronemes (m), amylopectin (A) and protein granules.

**Table 1:** Comparative morphology of *Sarcocystis* cysts obtained from ocular muscles of cattle:

| Species | Mean cyst dimension(µm) | Cyst wall Thicknes (µ m) | Villar protrusion shape | Microtubules |
|---|---|---|---|---|
| *Sarcocystis hominis* | 1199.1×165.59 | 11.44 | Cylindrical shape | Present |
| *Sarcocystis hirsuta* | 4000×1250 | 7.41 | Club or bulb like | Present |
| *Sarcocystis cruzi* | 378.63×122.87 | Less than 0.57 | Hair like | Not found |
| *Sarcocystis fusiformis* like cyst | 2226.9×473.93 | 10.725 | Branched (irregular shape) | Present |

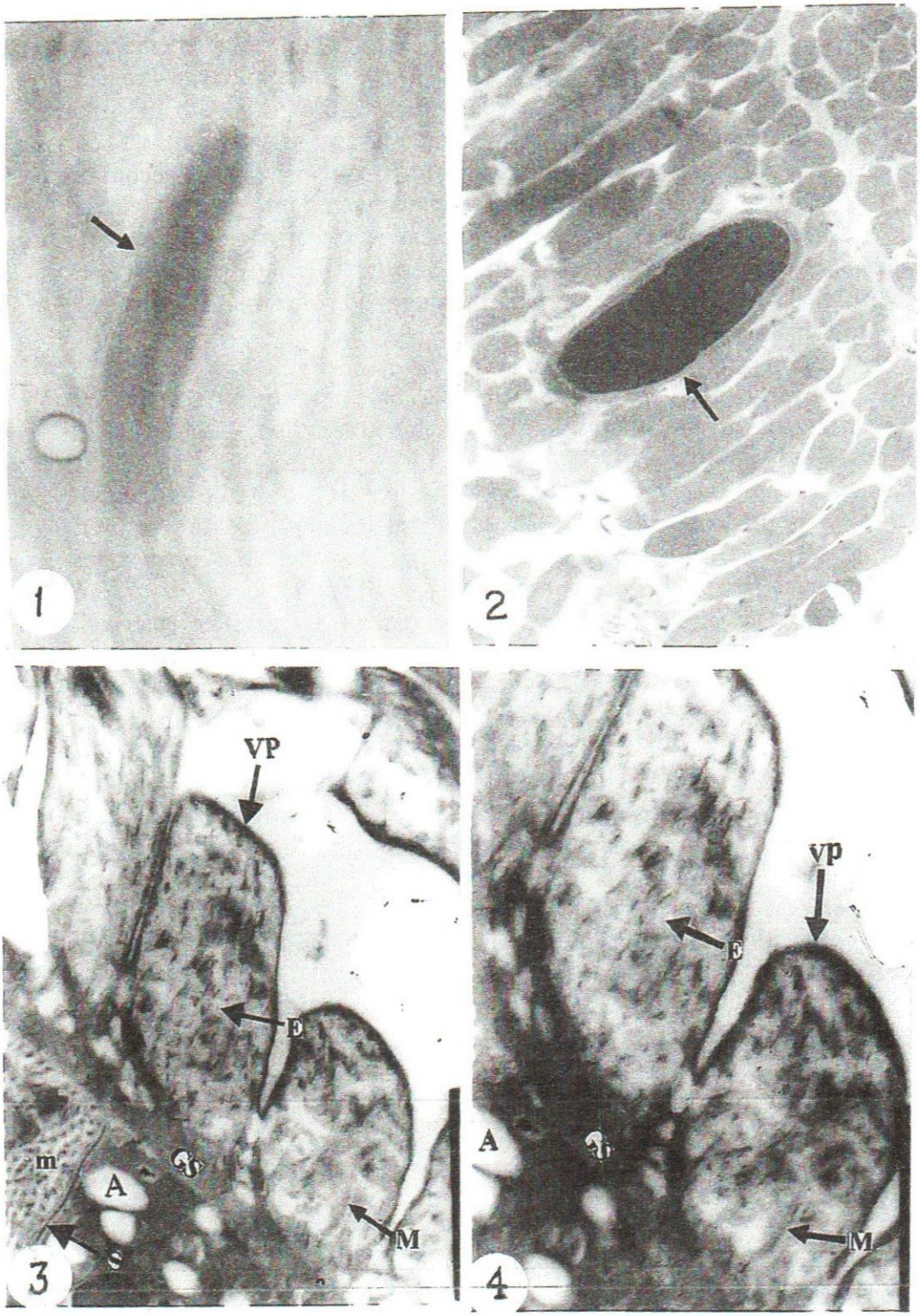
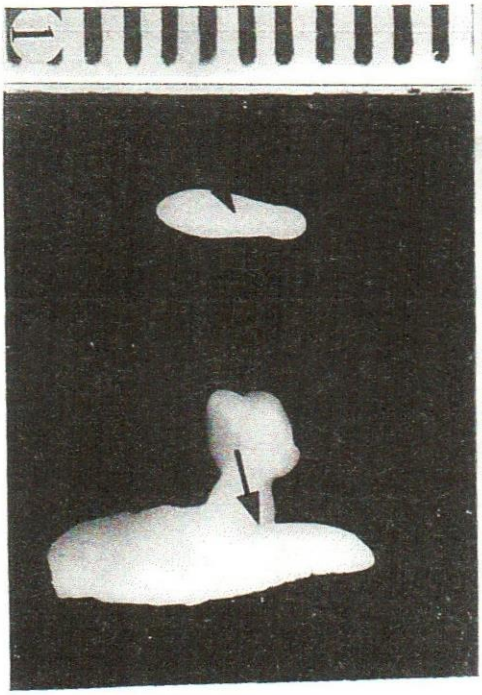
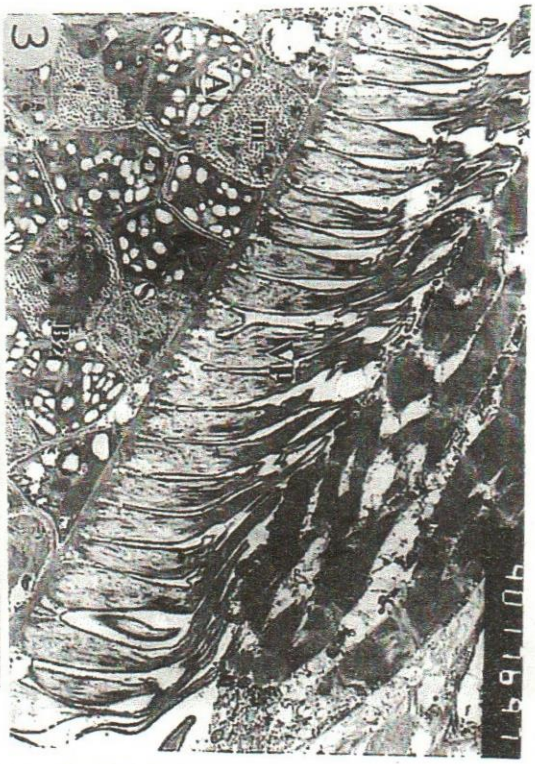
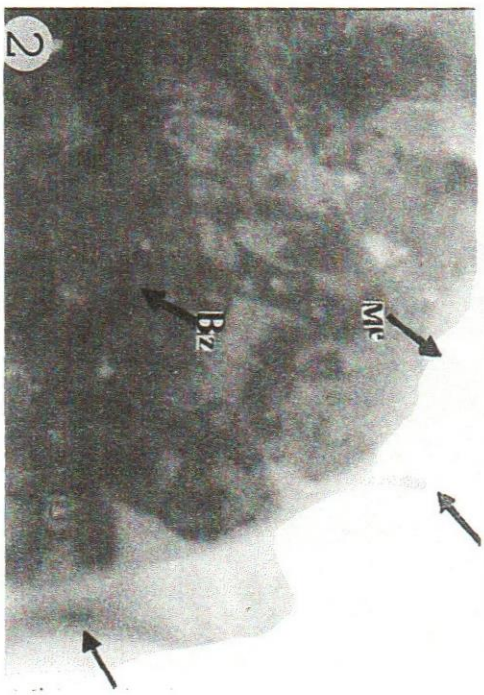



Plate I:





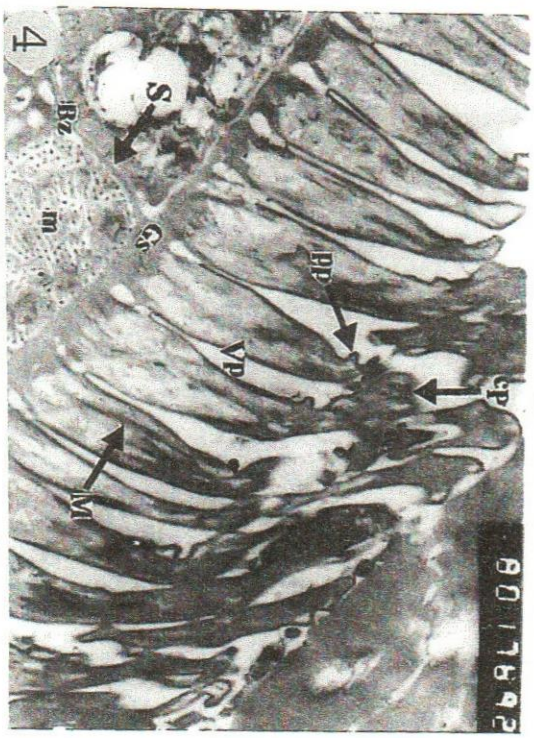
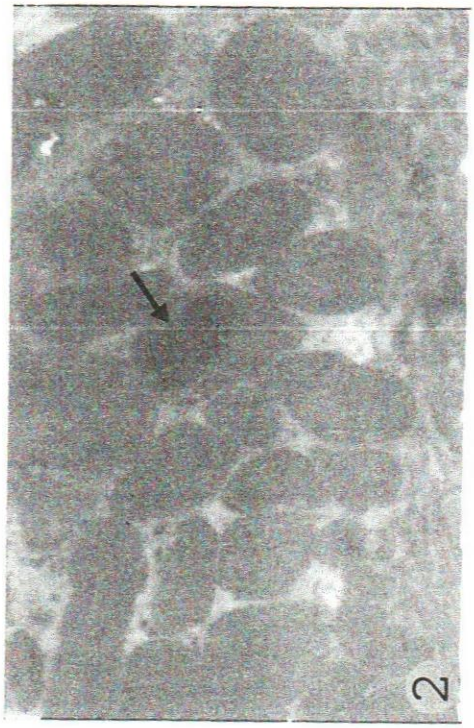
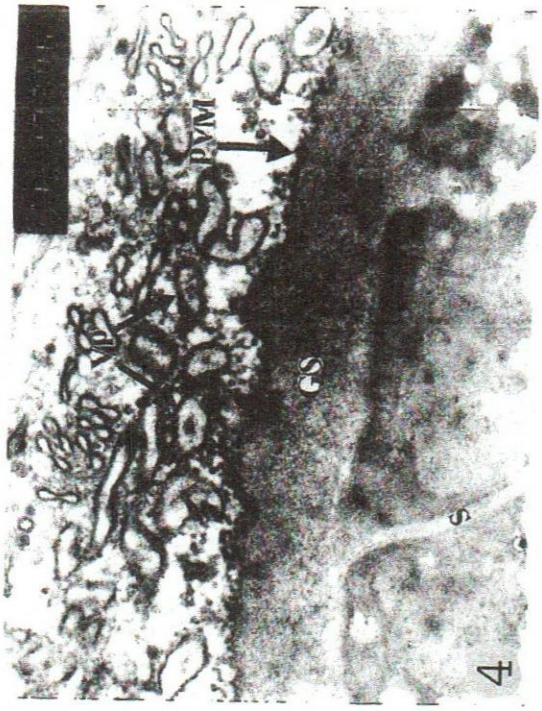
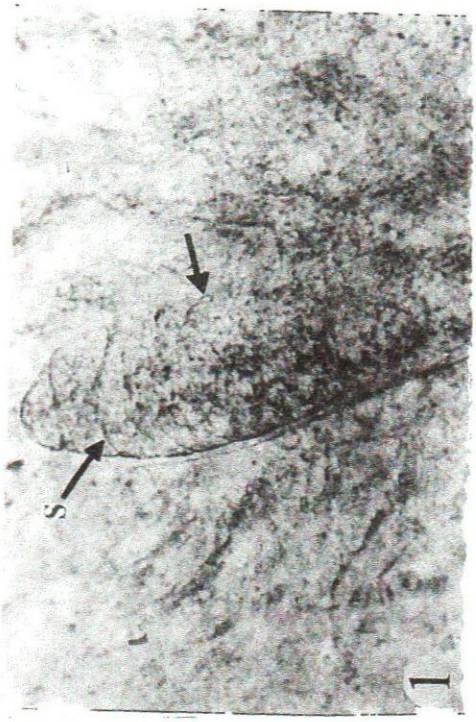




**Plate II:**



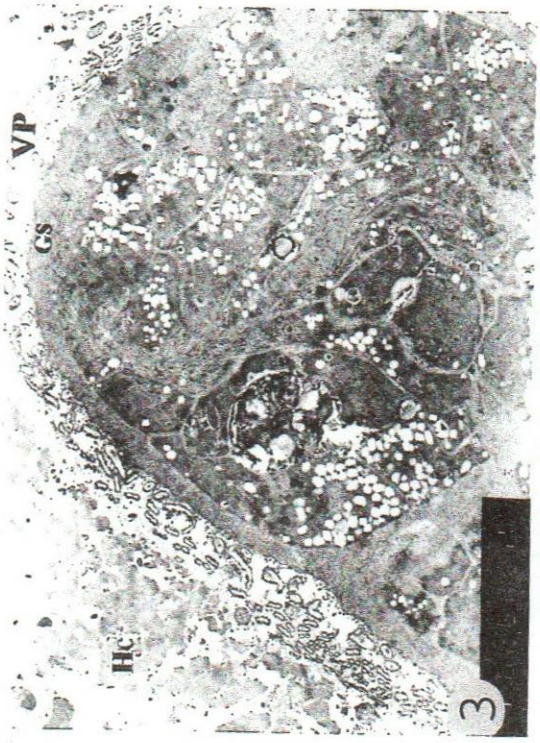




**Plate III:**

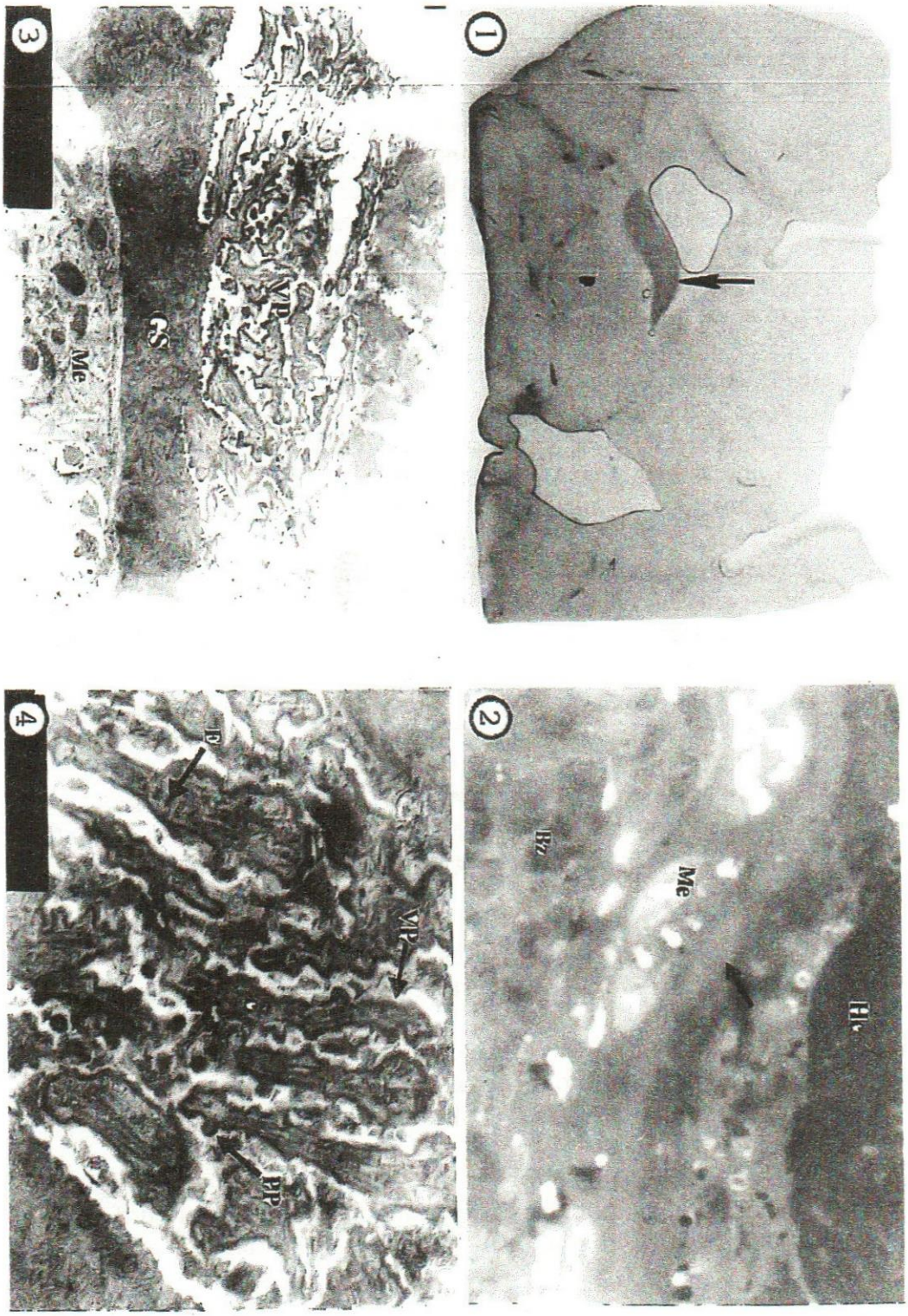



**Plate IV:**

**Plate I: *Sarcocyst hominis*:**

1- Fresh cyst parallel to muscle fibers. X100

2- **Semithin sections of *Sarcocystis hominis*:** Showing longitudinal section of the thick- walled cyst. Toluidine blue stain X100

3-**TEM of Cyst wall of *S. hominis*:** Showing unequal cylindrical finger like villar protrusions (VP) has straight or slightly curved tips with widely scattered electron dense granules (E) and bundles of microfilaments (M). Then layer of ground substance (GS) while bradyzoites separated from each others with fine septa (s) contains micronemes (m) and amylopectin granules (A) X 14.000.

4- High magnification of villar protrusions (VP) of *S. hominis*. X 20.000.

**Plate II: *Sarcocyst hirsuta*:**

1- fresh white fusiform macroscopic cyst of *Sarcocyst hirsuta*. X100

2- **Semithin sections of *Sarcocystis hirsuta* cyst:** Showing thick wall cyst with sloping pattern villar protrusions. Metrocytes (Me) and bradyzoites (Bz) were adhered to the inner surface of the cyst wall. Toluidine blue stain. X 100

3- **TEM of Cyst wall of *S. hirsuta*:** Showing charactarestic unequal club or bulb -shaped villar protrusions (VP), small papillary projections (PP), conical projections (CP) at their teps. Microfilaments (M) and electron dense granules widely scattered inside of each villar protrusion. While bradyzoites (Bz) adhered to the inner surface of the ground substance (GS) separated from each others with fine septa (S) and contain micronemes (m) and amylopectin granules (A) X 4.000.

4- **High magnification of cyst wall of *S. hirsuta*:** Showing small papillary projections (PP), conical projections (CP) of villar protrusions, microfilaments (M) and electron dense granules in eash one. X 8.000.

**Plate III: *Sarcocyst cruzi*:**

1- spindle shaped microscopic cyst of *S. cruzi* X 200.

2- **Semithin section of *S. cruzi* cyst:** Showing thin cyst wall Toluidine blue stain X 400.

3- **TEM of *S. cruzi* cyst:** Showing variable shape of hair like villar protrusions (VP) and cross section of meny bradyzoites (Bz) at the inner side of ground substanse (GS) of the cyst while host cells (Hc) surrounding it X 2.700.

4- **High magnification of cyst wall of *S. cruzi*:** Showing irregular undulations of a parasitophorous vacuolar membrane (PVM), different sections of villar protrusions (VP) and meny bradyzoites (Bz)

separated by thin septa (s) at the inner side of ground substanse (GS) X 10.000.

**Plate VI: *Sarcocystis fusiformis*-like cyst:**

1- **Fresh *S. fusiformis* like cyst:** Showing as macroscopic spindle shaped, creamy white cyst

2- **Semithin section of *S. fusiformis* like cyst:** Showing thick cyst wall (arrow) behind the wall, thin layer of ovoid shape metrocytes (Me) followed by large groups bradyzoites (Bz).X 1000

3- **TEM of *S. fusiformis* like cyst:** Showing thick cyst wall consisting of highly branched villar protrusions (VP) overlapping each other, wavy parasitophorous vacuolar membrane of ground substance (GS) while metrocytes (Me) found peripherally X 10.000.

4- **High magnification of cyst wall of *S. fusiformis* like cyst:** Showing villar protrusions (VP) branched in different directions, small papillary protrusions (PP) along the parasitophorous vacuolar membrane, filamentous core (c) with widely scattered electron dense granules (E) in each villar protrusion X 20.000.

## DISCUSSION

In Egypt, records on high incidence of *Sarcocystis* species of cattle were 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 %. Records on low incidence were reported by Soliman *et al.* (1964) 1.6%.

The high rate of infection by *Sarcocystis* in the present work might be due to presence of cattle in close association with dogs and cats which contaminate the soil and animal's food with large number of *Sarcocystis* sporocysts. In addition to that the sporocysts are mostly already infective when passed in the faeces, so these factors play an important role in the high prevalence of *Sarcocystis* in cattle. This opinion agrees with (Dubey, 1976) who mentioned that close association of cattle with dogs in pasture at European countries play an important role in the epidemiology of sarcocystosis.

In present work the infection rate with *Sarcocystis* species in ocular muscles was (89%). Juyal *et al.* (1982) found that the heavy sarcocystosis in ocular musculature of cattle was 71.5. Mohanty *et al.* (1995) found that the highest incidence of sarcocystosis in ocular muscles of cattle (86.20%).

Examination of semithin sections of infected samples revealed that some bovine ocular *Sarcocystis* cysts possessed thick cyst wall approximately 7.41µm in thickness and radially striated and others have thin wall provided with hair like villar protrusions. According to available literature, species of *Sarcocystis* provided with thick wall might be *Sarcocystis hirsuta* or *Sarcocystis hominis* while thin wall cysts might be *Sarcocystis cruzi*. Dubey (1982a) mentioned that thickness of the cyst wall of *Sarcocystis* depend on the stage of development of the cyst, therefore an overlap may occur when light microscope is used in identification of *Sarcocystis* species. The accurate diagnosis is only possible through transmission electron microscopy (Vercruysee *et al.*, 1989 and Savini *et al.*, 1992). Concerning the ultrastructural studies of the different *Sarcocystis* species, the results of the present work agree with the opinion of Mandour (1974) who mentioned that the cyst wall and morphological characters of villar protrusions are considered the main criteria of differentiation between species of *Sarcocystis*.

Ultrastructural studies of *Sarcocystis hominis* in the present work revealed that they are thick walled with villar protrusions cylindrical in shape, nearly perpendicular to the cyst surface. The villar protrusions had broad base. These characters agree with that of *Sarcocystis hominis* mentioned by (Odening *et al.*, 1995; Saito *et al.*, 1996; Pena *et al.*, 2001).

Ultrastructural studies of *Sarcocystis hirsuta* of cattle in the present study revealed thick walled cyst with villar protrusions of club or bulb shape. These villar protrusions were sloping and had constricted base with papillary protrusions near the tips. Some tips of villar protrusions were folded to form conical projections. This description is closely similar to that of: (Dubey *et al.*, 1989a; Odening *et al.*, 1995; Wang *et al.*, 2000; Pena *et al.*, 2001).

The ultrastructural studies of *Sarcocystis cruzi* cysts in the present study showed that they have thin wall provided with hair-like villar protrusions parallel to cyst surface and this villar protrusions had electron dense granules and no fibrillar structure. The parasitophorous vacular membrane of ground substance had irregular undulations (irregularly spaced pits interrupted at irregular intervals). These morphological characters were similar with those mentioned by (Claveria *et al.* 2001; Pena *et al.*, 2001).

Bottner *et al.* (1987a) isolated macroscopically visible *Sarcocystis* species cysts from the skeletal muscle of slaughtered cattle. They found the cyst wall thickness 3.3 – 7.0 µm in thickness.

Transmission electron microscopy revealed previously undescribed features of the cyst wall. It appeared that, with increasing age, the cyst wall protrusions become larger and develop a highly irregular surface. Their attachment to the cyst wall are slender and widely spaced indicating that growth of the cyst continues without the formation of new protrusions.

Odening *et al.* (1995) found fourth species of *Sarcocystis* in addition to the three named bovine *Sarcocystis* species (*Sarcocystis cruzi*, *Sarcocystis hominis* and *Sarcocystis hirsuta*) in musculature of a dwarf zebu. They described this species by light and electron microscopy. They found that this species was not previously reported from cattle. It mostly resemble *Sarcocystis gracilis* from roe deer.

Saito *et al.* (2000) detected a *Sarcocystis* species from cattle slaughtered in Japan, the cyst wall of which was morphologically different from that of the species recorded from cattle. The cyst wall measured 7 – 10 µm thick and provided with finger – like villar protrusions. The protrusions had microtubule in the core.

In the present work, fourth *Sarcocystis* species was found, the cyst was macroscopic and had the thicker cyst wall than all the above three bovine species. The ultrastructure of their villar protrusion by transmission electron microscopy were more useful in distinguishing the present species from the other species. The villar protrusions branched in different direction with cauliflower like appearance, some of these protrusions provided with small papillary protrusions along the parasitophorous vacular membrane. Each protrusion has filamentous core with widely scattered electron dense granule.

From the above morphological characteristics of the cyst wall, especially of their villar protrusions, the present species could be discriminated from all the known *Sarcocystis* species parasitic in cattle. It was suggested that this species resembles *Sarcocystis fusiformis* in buffalo which had cauliflower like appearance villar protrusions. Because all *Sarcocystis* species are considered host specific (Dubey *et al.*, 1989b), and differences in the structure of the cyst wall alone are insufficient to recognize the present species to be new we propose its name to be *Sarcocystis fusiformis* - like cyst until its life cycle will be clarified.

## REFERENCES

*Abdel Rahman, M.A. (1975):* Studies on protozoa of ruminants in Assiut Governorate. Ph. D. Thesis, Fac. Vet. Med., Assiut University.

*Abdel-Rahman, S.M. (2001):* Serodiagnosis of two zoonotic parasites *(Toxoplasma & Sarcocystes)* in cattle 1st-congress of Food Hygiene & Human health. Fac.Vet. Med., Assiut ,Egypt 116-125.

*Ali, G.A.T. (1985):* Studies on *Sarcocystis* of animals in Assiut province. M. V. Sc. Thesis, Fac. of Med., Assiut University.

*Arness, M.K.; Brown, J.D.; Dubey, J.P.; Neafie, R.C. and Granstrom, D.E. (1999):* An outbreak of acute eosinophilic myositis attributed to human *Sarcocystis* parasitism. Am. J. Trop. Med. Hyg., 61 (4): 548 – 553.

*Claveria, F.G.; San - Pedro, L.R.; Cruz - Flores, M.J.; Nagasawa, H.; Suzuki, N. and De La Pena, C. (2001):* Ultrastructural studies of *Sarcocystis cruzi* (Hasselmann, 1926) Wenyon, 1926 infection in cattle (*Bos taurus* ): Philippine cases. Parasit., 8 (3): 251 – 254.

*Collier, L.; Balows, A. and Sussman, M. (1998): Sarcocystis, Isospora* and *Cyclospora.* In Topley and Welson's: Micobiology and microbial infections. 9 th Ed. Oxoford University Press, Inc, New York, Vol. 5: 319 – 326.

*Dubey, J.P. (1976):* A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs. J. Am. Vet. Med. Assoc. 169 (10): 1061 – 1078.

*Dubey, J.P. (1982a):* Development of ox – coyote cycle of *Sarcocystis cruzi.* J.of Protozool. 29: 591 – 601.

*Dubey, J.P. (1982b):* Sarcocystosis in neonatal bison fed *Sarcocystis cruzi* sporocysts derived from cattle. J. Am. Vet. Med. Ass., 181:1272 –1274.

*Dubey, J.P.; Speer, C.A. and Charleston, W.A. (1989a):* Ultrastructural differentiation between sarcocysts of *Sarcocystis hirsuta* and *Sarcocystis hominis.* Vet. Parasitol., 34 (1 – 2): 153 –157.

*Dubey, J.P.; Speer, C.A. and Fayer, R. (1989b):* Sarcocystosis of animals and man. CRC Press Inc, Boca Raton, Florida.

*El – Afifi, A. (1958):* If there is a relationship between sarcosporidia and the eosinophilic myositis of cattle. Vet. Med. J., 5: 107 – 112.

*El –Saieh, A.F. (1998):* Incidence of *Toxoplasma* and *Sarcosporidia* in slaughtered animals in Qena Governorate. Ph. D. Thesis, Fac. of Vet. Med., Assiut University.

*Heydorn, A.O.; Mehlhorn, G.R. and Rommel, M. (1975):* Proposal for a new nomenclature of the Sarcosporidia. Z. Parasitkde., 48: 73 – 82.

*Juyal, P.D.; Sahai, B.N.; Srivastava, P.S. and Sinha, S.R. (1982):* Heavy sarcocystosis in the ocular musculature of cattle and buffaloes. Vet. Res. Commun, 5 (4): 337 – 342.

*Mandour, A.M. (1974):* Fine structure of *Sarcocystis blanchardi* (Doflein, 1901) from Egyption buffalo. Ass. Vet. Med. J., 1 (1, 2) 101 – 105.

*Meads, E.B. (1976):* Letter to the editor, Dalmeny disease – another outbreak. Probably sarcocystosis. Can. Vet. J. 17, 271.

*Mehlhorn, H. and Heydorn, A.O. (1978):* The sarcosporidia (protozoa, sporozoa) life cycle and fine structure."Adv. Parasitol., 16: 43 – 65.

*Mohanty, B.N.; Misra, S.C.; Panda, D.N. and Panda, M.R. (1995):* "Prevalence of *Sarcocystis* infection in ruminants in Orissa. Indian Vet. J., 72 (10): 1026 – 1030.

*Mohamed, M.S. (1996):* "Muscular parasites in slaughtered animals in Assiut Governorate." Ph. D. Thesis, Fac. of Vet. Med., Assiut university.

*Mowafy, N.M. (1993):* Sarcosporidiosis in Rodents. Ph. D. Thesis in Medical Science, Fac. of Med., Minia University, Egypt.

*Odening, K.; Wesemeier, H.H.; Walter, G. and Bockhardt, I. (1995):* On the morphological diagnostics and host specificity of the *Sarcocystis* species of some domesticated and wild bovini (cattle, banteng and bison). Appl. Parasitol., 36 (3): 161 – 178.

*Pena, H.F.; Ogassawara, S. and Sinhorini, I.L. (2001):* Occurrence of cattle *Sarcocystis* species in raw kibbe from Arabian food establishments in the city of Sao Paulo, Brazil and experimental transmission to humans. J. Parasitol., 87 (6): 1459 – 1465.

*Richardson, K.C.; Jarret, C.J. and Finke, E .H. (1960):* Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 35: 313 – 323.

*Saito, M.; Shibata, Y.; Kobayashi, T.; Kobayashi, M.; Kubo, M. and Itagaki, H. (1996):* Ultrastructure of the cyst wall of *Sarcocystis* species with canine final host in Japan. J. Vet. Med. Sci., 58 (9) 861 – 867.

*Saito, M.; Kubo, M. and Itagaki, H. (2000): Sarcocystis* species from cattle slaughtered in Japan. J. Vet. Med. Sci., 62 (11): 1209 – 1211.

*Savini, G.; Dunsmore, J.D.; Robertson, I.D. and Seneviranta, P. (1992): Sarcocystis* spp. in Western Australian cattle. Australian Vet. J., 69 (8) 201 – 202.

*Soliman, K.N.; El-Refai, A.H. and Zaki, M.M. (1964):* Sarcosporidiosis in cattle and buffaloes in Egypt. 1. Incidence and distribution in striated muscles of affected animals. Proceedings of the 5th Arab Annual Vet. Congress.' Parasitol. 47 (3): 229 – 235.

*Stempak, J.G. and Ward, R.T. (1964):* Improved staining method for electron microscopy. J. Cell Biol., 22: 697 – 701.

*Vercruysse, J.; Fransen, J. and Van Goubergen, M. (1989):* The prevalence and identity of *Sarcocystis* cysts in cattle in Belgium. Zentralbl Vet. [B] 36 (2): 148 -153.

*Wang, M.; Liu Hai, H.; Ma Jun, H.; Xiao, B.N. and Zhang, S.F. (2000):* Studies on the ultrastructure of cysts of *Sarcocystis* in cattle. J. China Agr. Uni., 5 (3) 117-120.