

## Studies on *Sarcocystis* infecting domestic horse

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### Abstract

In the present study, a case of sarcocystosis in the domestic horse from Egypt was described by electron microscopy. Of 32 recently died domestic horses from different localities in Egypt only 8 horses were found to be infected with sarcocysts with percentage of 25%. Domestic horses were collected from Cairo (8 horses) only 2 were be infected with 25%, from Gizah (7 horses) only 2 were be infected with 28.5%, Beni-Suef (6 horses) only 2 were be infected with 33%, from El-Fayum (6 horses) only one was be infected with 16.5.5% and from El-Menia (5 horse) only one were be infected with 20%.

These samples were randomly collected from the previous localities of Egypt during (2010 – 2012) and were found to be infected with microscopic sarcocysts measuring 50 - 85  $\mu\text{m}$  in width (mean, 65  $\mu\text{m}$ ) and 450-970  $\mu\text{m}$  in length (mean, 750  $\mu\text{m}$ ), Electron microscopic examination showed that all sarcocysts had a single primary cyst wall measuring 2.0-4.3  $\mu\text{m}$  (mean, 3.8  $\mu\text{m}$ ) that was folded into short nonbranched barrel-shaped protrusions, measuring 2.1-4.5 x 1.5-2.5  $\mu\text{m}$  (mean, 3.7 x 1.9  $\mu\text{m}$ ). These protrusions contained granular and tubular elements. Many septa were observed arising from the ground substance into the interior of the cyst, dividing it into numerous chamber-like compartments that surround the oval to globular merozoites 2.5-4.8 x 4.5-9.2  $\mu\text{m}$  (mean, 3.4 x 7.2  $\mu\text{m}$ ) and the banana-shaped merozoites 1.3 - 3.5 x 4.5 - 9.5  $\mu\text{m}$  (mean, 3.1 x 8.2  $\mu\text{m}$ ).

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## Introduction

Cysts of the genus *Sarcocystis* were first seen by Miescher (1843) in the skeletal muscles of the house mouse, *Mus usculus* in Switzerland. Cysts containing a large number of banana-shaped merozoites within the muscle fibers of the house mouse were obtained and were termed Miescher's tubules. Later on, Lankester (1882) proposed *Sarcocystis miescheri* from the muscles of domestic pig and introducing generic and specific name for such parasite for the first time. Furthermore, Balbiani (1883) introduced the name Sarcosporidia for the cysts due to their typical localization within the muscles of animals.

Electron microscopic studies revealed that sarcocysts in the muscles of the intermediate host were always surrounded by a very characteristic primary cyst wall with a specific architecture characterizing each species. In addition, the cysts may be surrounded by a secondary cyst wall (Mehlhorn *et al.*, 1976 and Mehlhorn and Heydorn, 1978). These cysts represent the rest merogonic phase in which multiplication of merozoites occur mainly through endodyogeny process, which is a special form of binary fission (Mehlhorn *et al.*, 1978; Abdel-Ghaffar *et al.*, 1978, 1979, 1990a; Bledose, 1980a; Häfner and Frank, 1984; Bashtar *et al.*, 1991; Šlapeta *et al.*, 1999 and Abdel-Ghaffar & AL-Johany, 2002). Moreover, sarcocysts may be reproduced by another type of asexual reproduction called endopolygeny, in which up to 12 daughter cells were produced per each metrocyte as in case of *S. dirumpens* (Häfner and Matuschka, 1984 and Šlapeta *et al.*, 1999).

The fine structure of the metrocytes and cyst merozoites of different *Sarcocystis* species were nearly similar to each other and also nearly similar to the general architecture of the Apicomplexa and can not be considered as being species-specific (Mehlhorn and Heydorn, 1978; Abdel-Ghaffar *et al.*, 1990 and Sakran *et al.*, 1998).

Regarding pathogenicity, *Sarcocystis* was found to be highly pathogenic to its hosts and may lead to their death (Dubey, 1976; Abdel-Ghaffar *et al.*, 1978; Tadros and Laarman, 1982; Matuschka, 1987b; Fayer and Dubey, 1988; Srivastava *et al.*, 1988; Ashour *et al.*, 1990; Pamphlett and Donoghue, 1990; Khatkar *et al.*, 1993; Sakran *et al.*, 1994; Mohanty *et al.*, 1995; Garner *et al.*, 1997; Dubey *et al.*, 1998a, b and Sakran, 2000). Most of these pathogenic effects were confined to the intermediate hosts and varying from emaciation, fever, hepatitis, apathy, myocarditis, lymphadenopathy and inappetence to cough,

depression , hyperthermia , raised pulse rate and anorexia ( Munday *et al.* , 1975 ; Leek *et al.* , 1977 ; Entzeroth , 1982 ; Srivastava *et al.* , 1988 ; Dausgchies *et al.* , 1988 ; Khatkar *et al.* , 1993 ; Oryan *et al.* , 1996 ; Ramos *et al.* , 1997 ; Jacob *et al.* , 1998 and Sakran , 2000) . Moreover , in human , *Sarcocystis* resulted in fatigue , abdominal and chest pains , nausea , and muscle palpation (Pamphlett and Donoghue , 1990 ; Azab and El – Shennawy , 1992 and Banerjee *et al.* , 1994) . All acute pathological changes were noticed in early infection phases , while in late phases , chronic signs appeared . Moreover , the severity during early merogonic stages exceeded that reported during cyst formation ( Garcia and Bruckner , 1993 ; Tenter , 1995 and Jäkel *et al.* , 1996 ) .

Since the 1960s, when equine protozoal myeloencephalitis (EPM) was first recognized, and throughout its further description, much debate has accompanied the disease diagnosis and identification, Until 1980, three known species of *Sarcocystis* in equines have been differentiated by their morphology (Gobel and Rommel, 1980; Levine and Tadros,1980) The old specific name *S. bertrami* (Doflein, 1901) was assigned to a species infecting the horse. Recently, *Sarcocystis* species of horse in Germany was named *S. equicanis* (Rommel and Giesel, 1975). A different species of *Sarcocystis*, with finger-like cyst wall protrusions, was recorded in horses slaughtered in the USA and given the name *S. fayeri* by Dubey *et al.*(1977).

Regarding the pathological effect of the parasite, the equine protozoal myeloencephalitis (EPM) was thought to be *Toxoplasma gondii* (Cusick *et al.*, 1974) but subsequently was identified as a *Sarcocystis* sp. (Simpson and Mayhew, 1980). Dubey *et al.* (1991) successfully isolated and propagated, in cell culture, a protozoan parasite from the spinal cord of a horse (diagnosed with EMP) and named *Sarcocystis neurona*, adding a fourth *Sarcocystis* species infecting the horse.

Due to the scarcity of studies dealing with horse as intermediate host of *Sarcocystis*, and economical importance of horses particularly the arabic strains, the present study is of special interest dealing with a general survey and the description with light & electron microscopic of ultrastructure of *Sarcocystis* infecting the domestic horse for the first time in Egypt.

## Materials and Methods:

### Animals

#### Intermediate Host (Domestic horse)

#### Prevalence of natural infection

To detect the rate of natural infection throughout the intermediate hosts. Various muscle samples from 32, thirty two, domestic horses were collected from Cairo (8 horses), Gizah (7 horses), Beni-Suef (6 horses), from El-Fayum (6 horses) and from El-Menia (5 horse). These samples were randomly collected from the different localities of Egypt during (2010 – 2012 ).

Samples of different muscles including scapular, cervical, abdominal muscles, diaphragm, heart and esophagus were directly examined by the naked eye for macroscopic sarcocysts and then were transported to Zoology Department, Beni-Suefby Faculty of Science, for microscopic examination. Very small pieces of muscles from the infected horses were fixed in 3% (v/v ) Glutaraldehyde in 1% Na cacodylate buffer (pH 7.3) at 4Co then washed several times with the same buffer and stored in 70% ethanol. Electron microscopic preparations were done by Post-fixation of specimens with OS04· in graded ethanol, then transferred to propylene oxide and finally embedded in Araldite (Serva). Semi- and ultrathin sections were cut by Reichert Ultracut. Semithin sections were stained with methylene blue, whereas ultrathin sections were contrasted with uranyl acetate and lead citrate before being examined by transmission electron microscope.

## Results

In the present study, sarcocystosis in the domestic horse from Egypt is hereby described by electron microscopy. Only 2 of the 8 horses (from Cairo), two of the 7 horses (from Gizah), two of the 6 horses (from Beni-Suef), one of the 5 horses (from El-Fayum) one of the 5 horses (from El-Menia) were found to be infected with microscopic sarcocysts of *Sarcocystis equicanis* (Table 1). These were found in different muscles, including scapular, cervical, abdominal muscles, diaphragm, and oesophagus but not in cardiac muscle.

The collected sarcocysts measured 55 - 85  $\mu\text{m}$  in width (mean, 65  $\mu\text{m}$ ) and 450-970  $\mu\text{m}$  in length (mean, 750  $\mu\text{m}$ ). Cyst wall measured 2.5-4.8  $\mu\text{m}$ , mean 3.5  $\mu\text{m}$  (Figs.1-4). The ground substance was found directly underneath the primary cyst wall. Many septa were arising from the

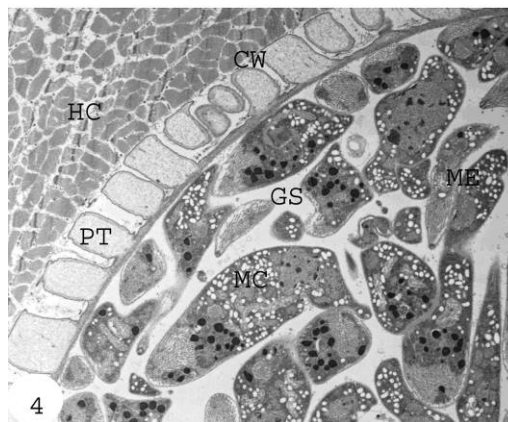
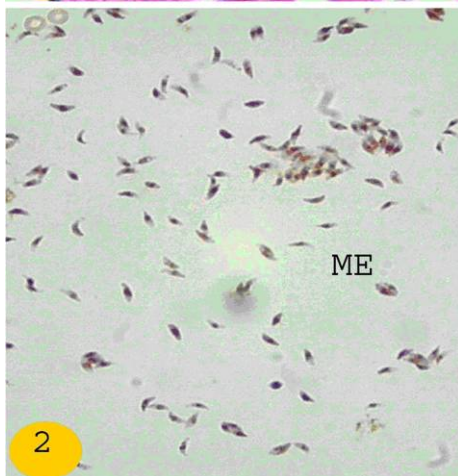
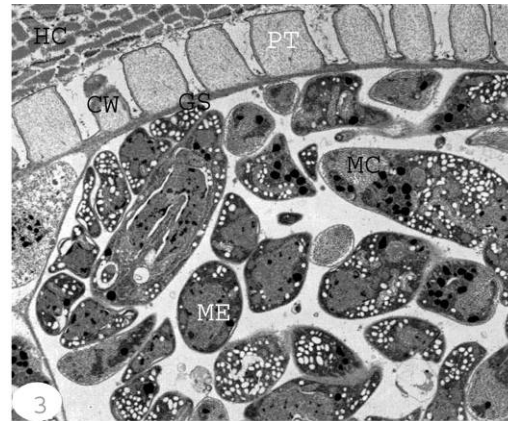
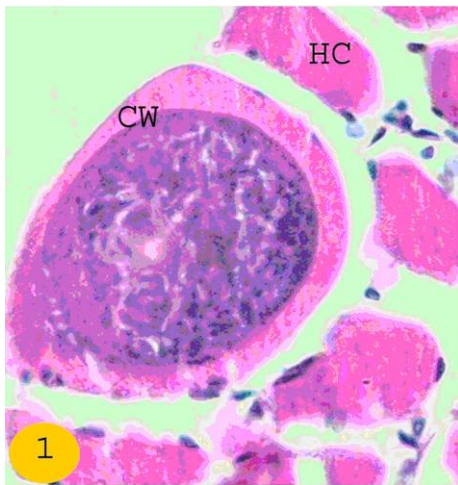
ground substance into the interior of the cyst, dividing it into numerous chamber-like compartments that surround the parasites. These parasites were differentiated into metrocytes, which were located almost at the periphery underneath the ground substance, and the cyst merozoites, which usually filled the interior of the cyst (Figs.5-6).

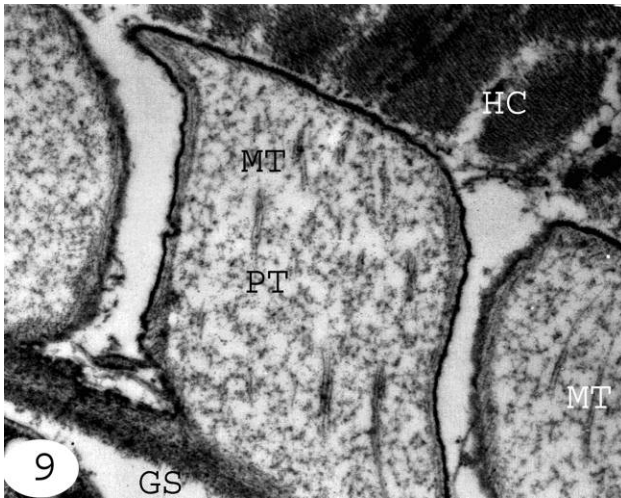
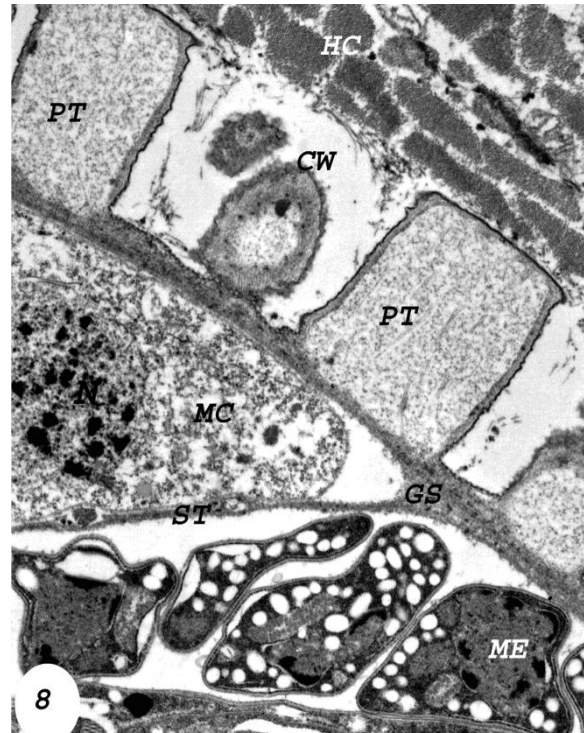
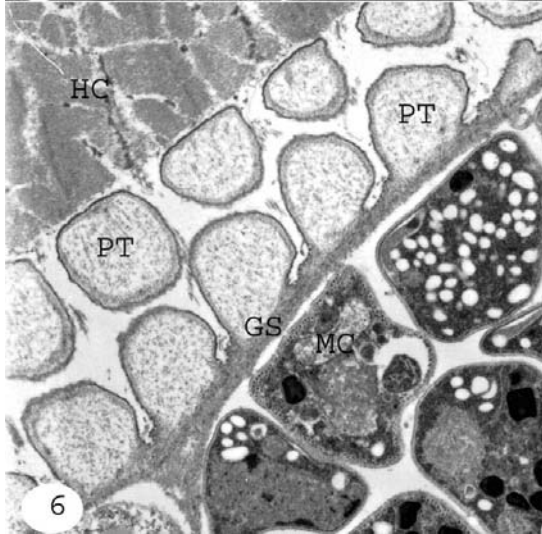
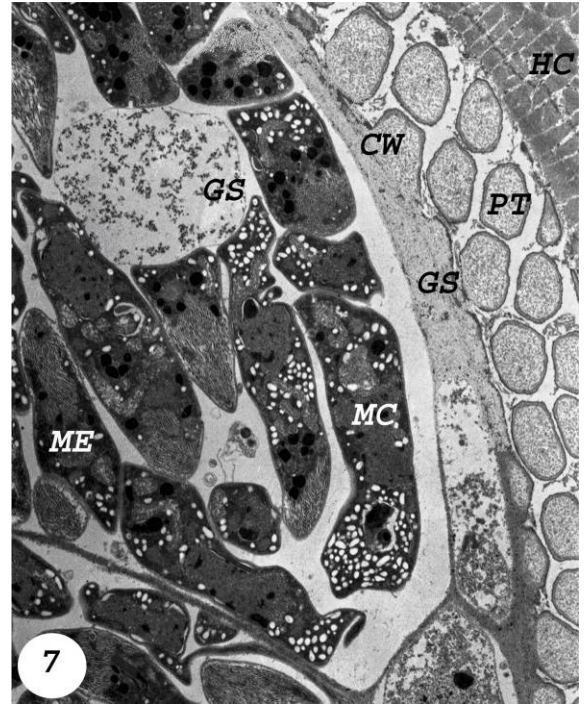
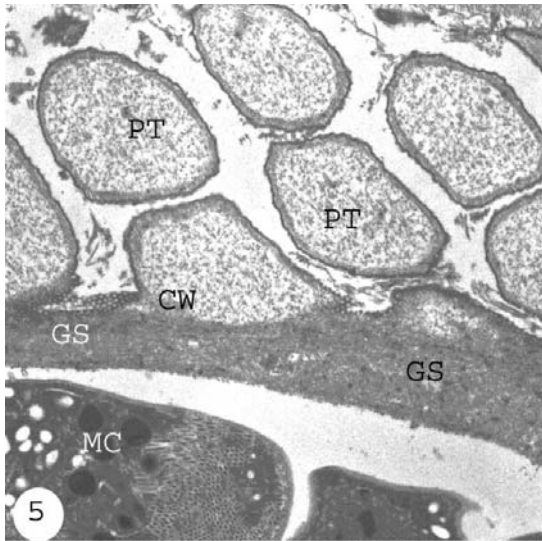
**Table (1)**

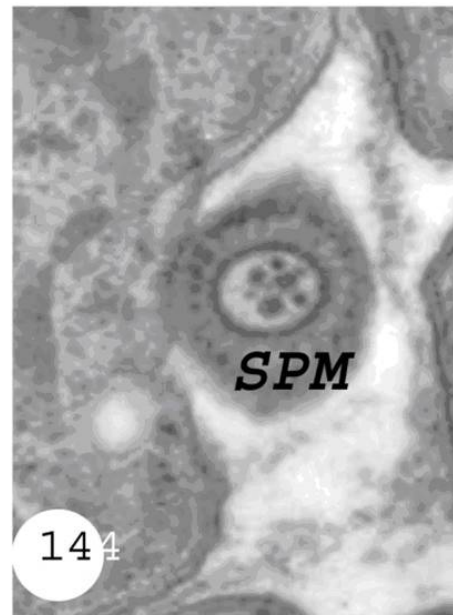
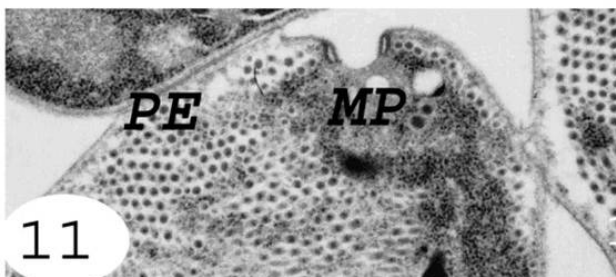
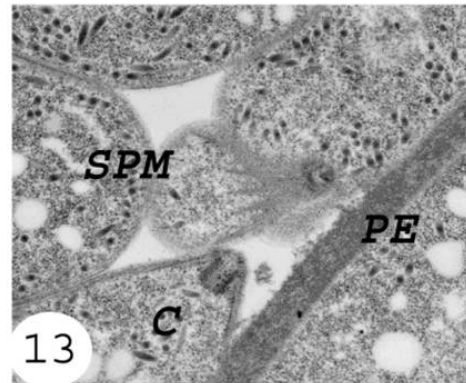
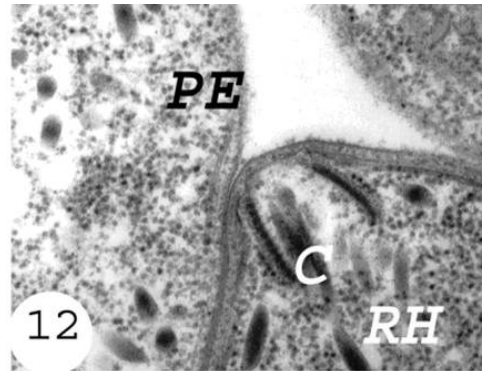
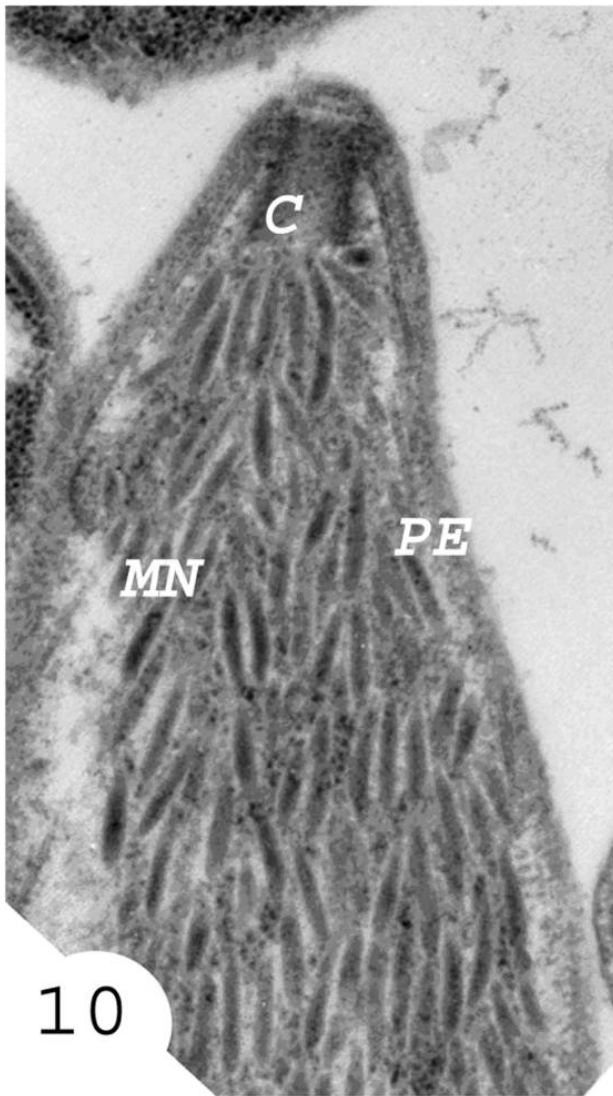
Province	No. collected animals	Infected animals	% of Infected animals
Cairo	8	2	25%
Gizah	7	2	28.5%
Beni-Suef	6	2	33%
EL-Fayum	6	1	16.5%
El-Menia	5	1	20%
Summation	32	8	25%

The ultrastructure of these microscopic sarcocysts revealed that each sarcocysts had a single primary cyst wall where fine structure can often be used as an important criterion for *Sarcocystis* species determination (Mehlhorn *et al.*, 1976 and Tadros and Laarman, 1982). The cyst wall of the present *Sarcocystis* folded into short nonbranched barrel-shaped protrusions measuring 2.1-4.5  $\mu\text{m}$  (mean, 3.7  $\mu\text{m}$ ) in longitudinal section (Figs. 7&8 ). On the other hand, two layers of barrelshaped protrusions measuring 1.5-2.5  $\mu\text{m}$  (mean, 1.9  $\mu\text{m}$ ) appear in cross section. These protrusions contained granular and tubular elements. A relatively thick homogenous tape was observed just underneath the primary cyst wall, measuring 0.2-1.2  $\mu\text{m}$  (mean, 0.5  $\mu\text{m}$ ) and containing granulated ground substance. Secondary cyst wall was not observed (Figs.8&9).

Just underneath the primary cyst wall, oval to globular metrocytes were observed measuring 2.5-4.8 x 4.5-9.2  $\mu\text{m}$  (mean, 3.4 x 7.2  $\mu\text{m}$ ) (Figs.10 -14) and banana-shaped merozoites were centrally located in the cyst. The latter measured 1.3 - 3.5 x 4.5 - 9.5  $\mu\text{m}$  (mean, 3.1 x 8.2  $\mu\text{m}$ ) (Figs.15- 17).









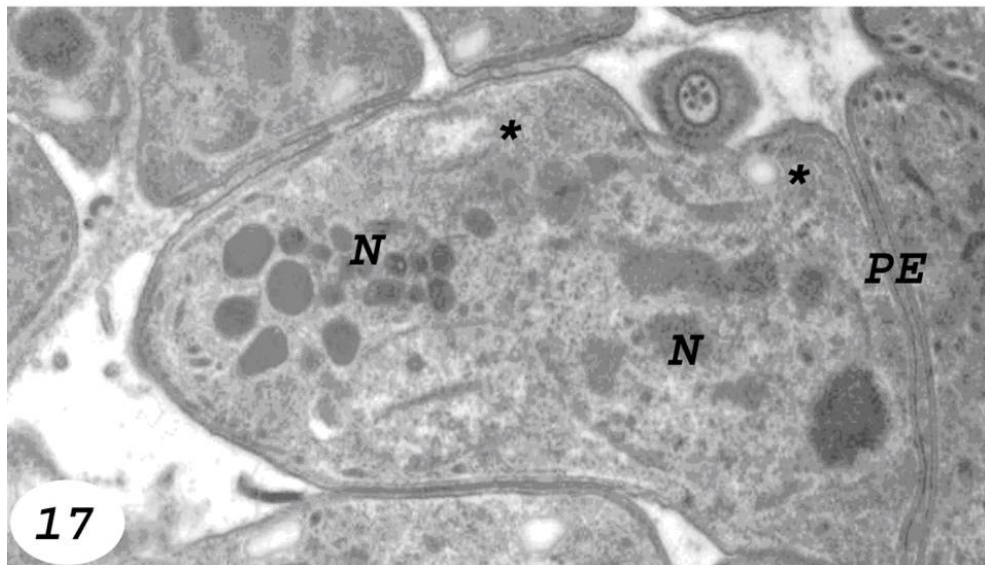
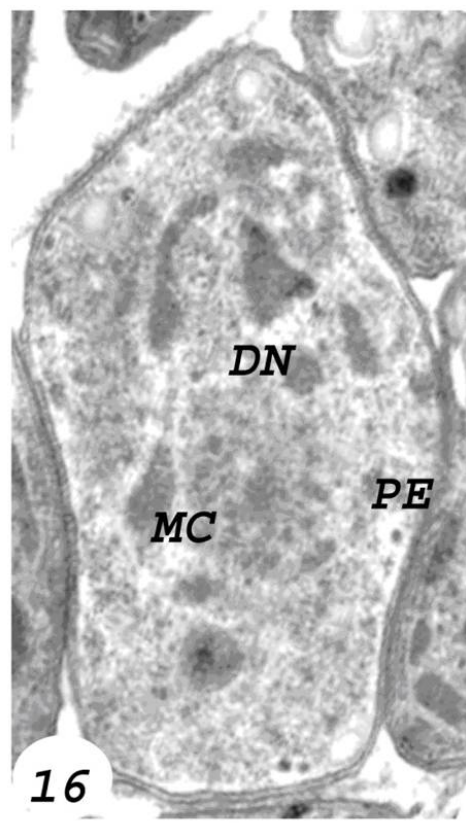
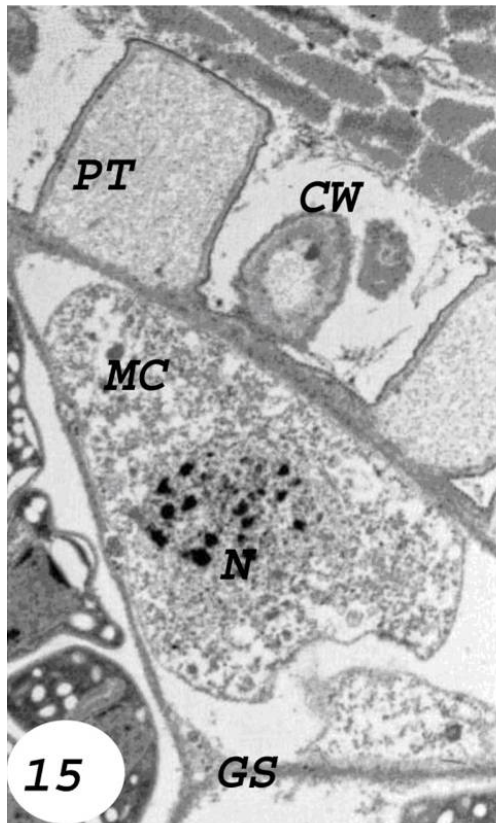


Table (2): Shows selected properties of sarcocysts in the horse.

Authors	Maximum cyst dimensions	Cyst wall measurements	Cyst wall measurements	Maximum bradzoite dimensions	Species named or presumed by authors
Siedamgrotzky, 1872	Width: 0.3mm Length: 3-4mm (up to 12mm)	Smooth (cilia on isolated cysts)	-	5 x 16 µm,	Psorospermial tubes
Doflein, 1901	Width: - Length: 9-10 mm	Striated, rodlike	-	6 µm (diameter)	S. bertrami*
Rommel and Geisel, 1975	Width: - Length: 350 µm	Smooth, Less than 1 µm	-	-	S. equicanis*
Gobel, 1976	Width: - Length: -	-	1.5-0 µm thick; composed of protrusions and 40-70 filaments	1.5-2.5 x 3.25 -6.5µm	S. equicanis*
Dubey et al., 1977	Width: 70 µm Length: 900 µm	Striated, 1-2 µm	-	2-3 x 15-20 µm	S. fayeri
Tinling et al., 1980	Width: 136 µm Length: 990 µm	Striated, 1-3 µm	2.2-3.1 µm thick; composed of protrusions and 34-55 microtubules	2.8-3.8 x 12.0-16.1µm	S. fayeri
Gobel and Rommel, 1980	Width: 70 µm Length: 350 µm	Striated, 2-3 µm	2-3 µm thick; composed of protrusions and with filaments	2.5 -3.5 x 8-10µm	S. equicanis
Fayer and Dubey 1982	Width: 22.2 µm Length: 777 µm	Striated, in two layers	-	-	S. equicanis
Dubey et al., 1991	Sch. Width: 5-20 µm Sch. Length: 5-35µm (Schizont only)**	NIL**	NIL**	1-3 x 2-4 µm (schizont merozoites)	S. neurona
Sakran 2000	Width: 65 µm Length: 750 µm	Striated, 2.0 -4.3,	2.0-4.3, mean: 3.8, µm thick; composed of protrusions with few microtubule	1.3-3.5 x 4.5-9.5 µm (mean, 3.1 x 8.2µm)	S.equicanis

\* Belived to be synonymous species by Dubey et al. (1977) and Levine (1977).

\*\* Sarcocysts were not observed.

## Discussion

*Sarcocystis* infection is considered a common protozoan parasite in horses (Levine, 1973 and Dubey, 1976). The three known species of *Sarcocystis* in equines have been differentiated by their morphology (Gobel and Rommel, 1980; Levine and Tadros, 1980). The domestic dog was found to be a suitable final host for *Sarcocystis* species of horses in Germany, with a cyst measuring up to 350  $\mu\text{m}$  and a thin cyst wall. The parasite was named *S. equicanis* (Rommel and Giesel, 1975). On the other hand, *Sarcocystis* with a cyst measuring up to 900  $\mu\text{m}$  and with finger-like cyst wall protrusions, was recorded in horses slaughtered in the USA. The parasite was reported to be infective to dogs and given the name *S. fayeri* by Dubey et al. (1977). As pointed out by Tadros and Laarman (1978) and Levine and Tadros (1980), the old specific name *S. bertrami* Doflein (1901) is available and, according to the law of priority, must be assigned to an appropriate equine species. Studies on the fine structure of *S. equicanis* (Gobel, 1976 and Gobel and Rommel, 1980) have confirmed the absence of cyst wall protrusions in this species. However, the large sarcocysts of *S. fayeri*, with prominent cyst wall protrusions as described by Dubey et al. (1977) and confirmed by electron microscopy (Tinling et al., 1980), adequately fits Doflein's original description. Thus *S. fayeri* is a junior synonym of *S. bertrami*. The finding of *S. bertrami* in USA is not surprising in view of the introduction of *Equus caballus* into the New World (Tadros and Laarman, 1982).

Morphologically, regarding the measurements, sarcocysts in the present investigations was most similar to *S. equicanis* (Fayer and Dubey 1982) and quite similar to *S. fayeri* Dubey et al. (1977). But it was quite smaller than that of *S. fayeri* (Tinling et al., 1980) that measured 136 x 990  $\mu\text{m}$  with striated primary cyst wall of 1-3  $\mu\text{m}$  and larger than that of *S. equicanis* (Gobel and Rommel, 1980) that measured 70 x 350  $\mu\text{m}$  with striated primary cyst wall of 2-3  $\mu\text{m}$ . On the other hand, the present material was much smaller than sarcocysts reported by Siedamgrotzky (1872) that measured 0.3 x 3-4  $\mu\text{m}$  to 12  $\mu\text{m}$  with smooth cilia on isolated cyst and named *Psorospermial tube* and *S. bertrami* recorded by Doflein (1901) that measured 9-10  $\mu\text{m}$  in length with striated cyst wall.

From the pathological point of view, since 1960, when equine protozoan myeloencephalitis (EPM) was first recognized, and throughout its further description, much

debate has accompanied the disease diagnosis and identification. A more in depth review is available (Mackay, 1997). The causative agent was initially thought to be *Toxoplasma gondii* (Cusick et al.,1974) but subsequently was identified as a *Sarcocystis* sp. (Simpson and Mayhew, 1980). Dubey et al.(1991) successfully isolated and propagated in cell culture a protozoan parasite named *Sarcocystis neurona* from the spinal cord of a horse diagnosed with E M P adding a fourth *Sarcocystis* species infecting the horse.

Only asexual stages of this parasite were found within neural cells and leucocytes of the brain and spinal cord of horses while sarcocysts were not reported. However, the main characteristics of any *Sarcocystis* species, were not observed for *S. neurona*, Then a Western blot for antibody was developed and made commercially available (Granstrom. 1993). Fenger et al.(1995) Suggested that the opossum (*Didelphis virginiana*) was the definitive host of *S. neurona* and *S. falcatulla*. On the bases of these findings, the 2 species were Suggested to be synonymous (Dame *et al.*,1995). Cutler *et al.*(1999) showed that *S. falcatula* did not infect horses and so *S. neurona* and *S. falcatula* were not synonymous. Moreover, Davis *et al.*(1999) reported hepatic sarcocystosis in the veterinary teaching hospital at the university of California. Hepatocytes were frequently harbouring schizont and merozoites arranged radially around a central residual body.

In the present investigation, for the lack of information about disease in horses due to *Sarcocystis*. it seems reasonable to assume that the parasite forms a nonpathogenic complex within the infected myofiber, with the merozoites in the resting stage (sarcocysts) until ingested by a carnivore (the final host). similar results were obtained by Tinling *et al.*(1980). Fine ultrastructure of both metrcytes and merozoites were similar to those described for many *Sarcocystis* species and were in general not specific (Mehlhorn *et al.*,1976; Mehlhorn and Heydorn,1978 and Tadros and Laarman,1982). Selected properties of the present sarcocysts of *S. equicanis* are presented with other properties of *Sarcocysts* infecting the horse (Table 2).

**Appendix:**

Abbreviations used in figures

A, amylopectin;

C, conoid;

CM, cyst merozoites; D, dense bodies;

F, fibrils;

GS, ground substance; HC, host cell;

fL, inner layer of pellicle; L, lipid;

MC, merozoite; MI, mitochondria; MN, microneme; MP, micropore; N, nucleus;

NH, nucleus of host cell; NU, nucleolus;

OL, outer layer of pellicle;

P, polar ring;

PE, pellicle;

PT, protrusions of PW;

Pw, primary cyst wall;

R, rhoptries;

ST, sub pellicular microtubules;

UT, unthickened places of PW.

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