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PREVALENCE, LOCALIZATION AND MORPHOLOGY OF SARCOCYSTIS SPECIES (APICOMPLEXA: SARCOCYSTIDAE) INFECTING HORSES (EQUUS CABALLUS) AND DONKEYS (EQUUS ASINUS) IN EGYPT.

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SUMMARY

Musculature samples of oesophagus, heart, diaphragm, tongue, masseter and skeletal muscles from each of 52 horses and 130 donkeys were microscopically examined by both, compressorium and trypsin digestion techniques for detection of *Sarcocystis* infection. The infection rate reached 88.5% in horses and 91.5% in donkeys. The sarcocysts were detected in all examined samples with the highest rate in oesophagus (100%). The morphology and size of the detected sarcocysts, metrocytes and bradyzoites in both, horses and donkeys were found to be similar. The cyst wall was thick (> 2um) and appeared striated. Experimentally infected dogs with horse or donkey meat excreted sporocysts measuring 12.2 - 14.6 X 9.0 - 10.7 um and 12.0 - 14.9 X 8.3 - 10.7 um . respectively. The prepatent period was 10 - 14 days. It was concluded that *Sarcocystis* infecting both, horses and donkeys in Egypt could belong to the same species (Sarcocystis bertrami).

INTRODUCTION

Sarcocystis species are protozoan parasites related to coccidia (Dubey et al., 1989) which require two obligatory host to complete its life cycle, carnivorous as a definitive host, herbivorous and omnivorous as an intermediate host (Fayer, 1980; Levine and Tadros, 1980). It has recently been recognized that certain *Sarcocystis* species, especially those transmitted by dog, can cause severe and even fatal disease within some intermediate hosts (Dubey and Fayer, 1983) including equines (Traub Dargatz et al., 1994).

Equine *Sarcocystis* species were found to be common in horses throughout the world (Dubey et al., 1977) and Traub - Dargatz et al., 1994 in USA; Rommel and Geisel, 1975, Erber Geisel, 1981, Odening et al., 1995 in Germany ; Yamada et al., 1993 in Japan; Edwards, 1984 in Britain; Kirmse, 1986 in Morocco; Juyal et al., 1991 and Achuthan and Antony, 1990 in India; Salvi et al., 1992 in Italy). However. few recent studies on this parasite in donkeys were carried out in the world by Edwards (1984) in Britain, Kirmse (1986) in Morocco, Hilali et al., (1985) and Hilali

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and Nassar (1987) in Egypt. Three *Sarcocystis species* have been named from horses; *S. bertrami* (Doflein, 1901 and Dubey et al., 1989), *S. equicanis* (Gobel, 1976, Rommel and Geisel, 1975) and *S. fayeri* (Dubey et al 1977 and Tinling et al., 1980). Dubey et al. (1977) have doubted if these truly different species. Gadaev (1978) named *Sarcocystis spp.* of donkeys as *S. asinus*, but the validity of this name was also doubted by Levien and Tadros (1980). However, Matuscka (1983) and Hilali and Nassar (1987) reported that the *Sarcocystis spp.* in donkeys and horses may be identical.

In Egypt, there were no works other than those reported by Hilali et al. (1985) and Hilali and Nassar (1987) for donkeys. Unfortunately, previous studies on this parasite in horses were non existent. Therefore, the aim of this investigation was to compare the prevalence and morphology of *Sarcocystis spp.* infecting both, horses, and donkeys in Egypt.

MATERIALS AND METHODS

Collection of samples:

Musculature samples from oesophagus, heart, diaphragm, tongue, masseter and skeletal muscles were randomly collected from freshly slaughtered 52 horses and 130 donkeys over 8 years old. The animals were purchased from various governorates of Egypt and slaughtered at the National circus, Giza province.

Microscopic examination of equine tissues for Sarcocystis infection:

Each of collected sample was examined microscopically by two methods:

Compressorium technique: one gram from each sample was finely cut into slices, compressed between the two glass plates of the compressorium and examined by an ordinary microscope (Edwards, 1984). The detected sarcocysts were measured in situ, and some of them were manually extracted from the tissue under a dissecting microscope, crushed between two clean slides. Smears were prepared dried, fixed with methanol and stained by Giemsa. They were examined microscopically (X40) for detection of metrocytes and bradyzoites.

Trypsin digestion technique : Ten to twenty grams from each sample were minced and digested in 5-10 volumes of 0.25% trypsin (Sigma co., USA, 1: 250) in 0.9% physiological saline (NaCl) for two hours with constant stirring (Dubey et al., 1977). The digested meat was sieved through a series of standard mesh metal sieves (1000, 500 and 125 um) and centrifuged at 2000 rpm for 10 minutes. Dried smears of the sediment were fixed, stained with Giemsa and examined microscopically.

Histological sections for some infected oesophagi of both, horses and donkeys were prepared in the usual manner, stained with hematoxylin and eosin (H & E). The sections were examined for the cyst wall morphology and determination of the intensity of infection by counting the number of cysts per section.

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perimental infection of the final host:

elve dogs and 8 cats 8-12 weeks old, coccidia e, were caged individually in clean metal cages. l animals were fed canned milk and dry pelleted d from the time of weaning. The dogs and cats d never eaten raw meat. They were divided into o groups, each containing 6 dogs and 4 cats. eir faeces were examined by flotation ncentration technique using saturated salt lution (NaCl) for three successive days before e infection to confirm that they were coccidia ee. Three dogs and two cats from group 1 and oup 2 were fed each 500 grams of minced oled parts of infected oesophagi, tongues, iaphragms, masseter muscles and hearts of orses (group 1) and donkeys (group 2) . The ther three dogs and two cats, in each group, were ept as non infected control and fed only dry food uring the whole experiment (30 days). The ieces of dogs and cats were examined daily by flotation concentration technique. The prepatent periods were determined and the sporocysts were measured.

RESUTLS

Examination of 52 horses and 130 donkeys for *Sarcocystis species* using the compressorium and trypsin disgestion techniques (Table 1) indicated a higher infection rate among donkeys than horses. Trypsin disgestion of meat samples revealed that 88.5% of horses and 91.5% of donkeys were infected with *Sarcocystis species* . The compressorium detected a comparatively lower infection rate, 73.1% and 83.8% in horses and donkeys, respectively. The efficiency of the compressorium in detecting of *Sarcocystis* infection compared with the digestion method was 82.6% and 91.6% of the infected horses and donkeys, respectively.

Table 1. Prevalence of *Sarcocystis species* infection and efficiency of compressorium method in detection of the sarcocysts in the musculature of equines in Egypt.

Animal	Exam. No.	Method of examination				% Efficiency of compressorium compared with digestion
		Compressorium		Trypsin digestion		
		Infec. No.	%	Infec. No.	%	
Horse	52	38	73.1	46.	88.5	82.6
Donkey	130	109	83.8	119	91.5	91.6

Exam No. : examined number.

Infec. No. : infected number.

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Table 2. Occurrence of the sarcocysts in the infected musculature of horses and donkeys examined by trypsin digestion technique.

Animal	Infec. No.	Infected musculature											
		Oesophagus		Tongue		Skelet. M.		Diaphragm		Masset. M.		Heart	
		No.	%	%	No.	No.	%	No.	%	No.	%	No.	%
Horse	46	46	100	19	41.3	13	28.3	12	26.1	11	23.9	9	19.6
Donkey	119	119	100	67	56.3	51	42.9	35	29.4	31	26.1	15	12.6

Masset. M. : masseter muscle.

Skelet. M. : skeletal muscle.

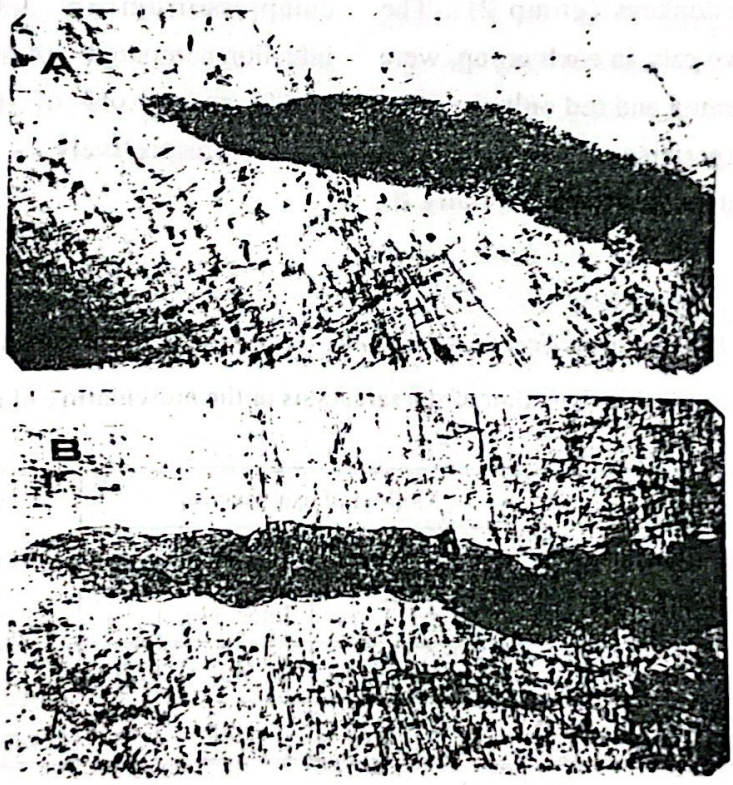

Fig. 1. Intramuscular cysts of equine *Sarcocystis spp.* in the compressorium for naturally infected oesophagus A) horse X 50 B) donkey X 50

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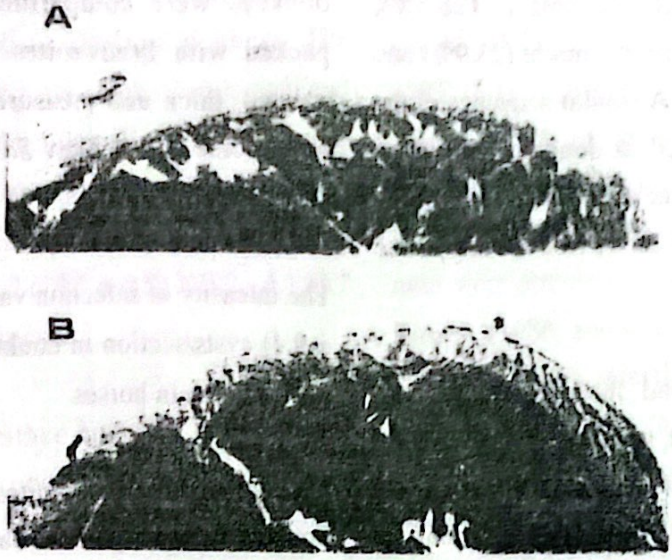

Fig. 2. Cyst wall of equine *Sarcocystis spp* in sections. H & E stain

A) horse X 400 B) donkey X 400

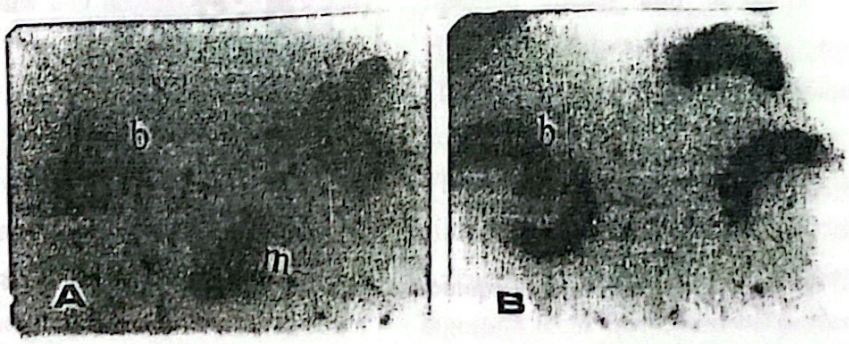

Fig. 3. Metrocytes (m) and bradyzoites (b) of equine Sarcocystis spp. from crushed cysts, Giemsa's stain A) horse X 1250 B) donkey X 1250

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The oesophagus was always infected with sarcocystis (100%) in horses and donkeys reported to be positive (Table 2). It was followed by tongue (14.3%), skeletal muscle (28.3%), diaphragm (26.1%), masseter muscle (23.9%) and heart (19.6%) in horses. A similar sequence of the occurrence was observed in donkeys. However, the rates of infection in the different organs (56.3 , 42.9 - 29.4 - 26.1 and 12.6%, respectively) were dissimilar.

The sarcocysts detected in both, horses and donkeys were elongated, spindle - shaped, located in between and parallel to the myofibrils of the infected tissues (fig. 1). Measurement of thirty-five cysts from each of horses and donkeys revealed that they were nearly equal in size varying from 0.8 - 5.1 (2.6 ±1.2) X 0.07 - 0.5 (0.2 ±0.1) mm in horses and from 0.6 - 4.5 (2.7 ± 1.1) X 0.06 - 0.4 (0.2 ± 0.1) mm in donkeys. Histologically , the sarcocysts of both, horses and donkeys were compartmented and each was packed with bradyzoites. The cyst wall was striated, thick and measured more than 2um in both, horse and donkey *Sarcocystis species* (Fig 2).

The intensity of infection varied from 4 - 33 (18.2 ± 9.4) cysts/section in donkeys and 1-8 (4.4±2.4) cysts/section in horses.

Metrocytes and bradyzoites (fig. 3) detected in horse were exactly of the same shape and size of the corresponding stages detected in donkey. The metrocytes were elliptical in shape and broad at both ends and shorter than bradyzoites. Ten

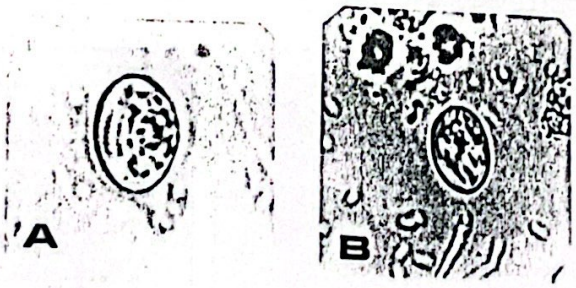

Fig. 4. Sporocysts of equine *Sarcocystis spp.* from faeces of dogs infected with equine tissues A) horse X 715 B) donkey X 700

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ocytes from each of horses and donkeys ured 10.8 - 13.5 (12.2 ± 0.8) X 4.9 - 5.7 (5.3) um from horses and 10.1 - 13.6 (12.0 ± 1.1) 7 - 5.7 (5.2 ± 0.4) um from donkeys. The yzoites were banana-shaped with one end pointed than the other. Fifteen bradyzoites each of horses and donkeys measured 13.6 - (14.9 ± 1.1) X 3.7 - 4.4 (3.9 ± 0.3) um from es and 13.5 - 16.2 (14.6 ± 0.9) X 3.2 - 4.1 (3.7 4) um from donkeys.

the dogs fed either horse or donkey meat eted sporulated oocysts or sporocysts after a patent period of 10-14 days. The oocysts were nty and detected only in the first few days of prepatent period. The oocysts detected from two groups were colourless, containing two mpletely sporulated sporocysts and enclosed by ery thin colourless indiscernible oocystic wall. e sporocysts (Fig. 4) detected from the two oups were ovoid , each contained four banana - aped sporozoites and a coarse residual body. xty sporocysts from each group measured 12.2 - 6 (13.2 ± 0.5) X 9.0 - 10.7 (9.8 ±0.4) um for rses and 12.0 - 14.9 (13. 1 ± 0.8) X 8.3 - 10.7 ± 5) um for donkeys. The cats and the control ninfected dogs and cats of each group did not ed any coccidian oocyst or sporocyst during the hole experiment (30 days)

ISCUSSION

his study demonstrated that the prevalence of *arcocsystis species* was high in both, horses 8.5%) and donkeys (91.5%). A high prevalence was observed in horses (88.9%) in Britain Edwards, 1984). However, lower infection rates were recorded throughout the world from horses; 13-30% in USA (Dubey et al. 1977), 15.5 - 41.0% in Germany (Rommel and Geisel, 1975, Erber and Geisel, 1981), 6.4% in Japan (Yamada et al., 1993), 8.1 - 23.8% in Italy (Salvi et al., 1992) and 17.6 - 46.2% in Morocco (Kirmse, 1986). In donkeys, a higher rate (96%) was recorded in Egypt by Hilali et al. (1985). However, lower rates were observed in Morocco (2.3 - 21.9%) by Kirmse. (1986) and in Britain (50%) by Edwards. (1984) The high infection rates recorded in this study may be due to the fact that the examined animals were more than 8 years old. The prevalence of *Sarcocystis* was found to increase with age (Edwards, 1984), in addition to the absence of control strategy for stray dogs which spread the infection to equine intermediate host.

In this study, the compressorium method gave statisfactory results compared with the digestion technique. It detected 91.6 and 82.6% of the infected horses and donkeys, respectively. However, Edwards. (1984) estimated the efficiency of gross examination of horses compared with digestion technique to be 54.1%. Therefore, the compressorium may be applied as a simple, quick and economic technique for screening of large number of samples and the digestion technique could be performed only on samples proved to be negative by the compressorium.

The present study demonstrated in details the occurrence of the sarcocysts in the infected musculature of equines. The pattern of distribution of these cysts in both, horses and donkeys was the same. In agreement with these

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results, Dubey et al. (1989) mentioned that the sarcocysts in equines were found primarily in the oesophagus and rarely in the hearts. The tongue was not previously examined for the prevalence of equine *Sarcocystis*. In this study the tongue came next to the oesophagus as indicator organ for *Sarcocystis infection*. Moreover, Yamada et al. (1993) found that the masseter muscle of horses, but not skeletal muscles was infected with sarcocystis

The morphology and size of sarcocysts, metrocytes, bradyzoites as well as the sporocysts of *Sarcocystis species* of horses and donkeys described in this study were the same. The cysts walls were thick (> 2 um) and appeared striated. Moreover , the prepatent period ranged from 10-14 days in dogs fed infected horse and donkey meat. This indicated that both horses and donkeys in Egypt were infected with a single and identical *Sarcocystis species*. This result confirmed the observations of Matuscka (1983) and Hilali and Nasser (1987), who reported that the *Sarcocystis species* in donkeys and horses may be identical . This could be more proved by infecting the horse by the sporocysts isolated form dogs fed donkey meat and vice versa.

Four *Sarcocystis species* have been named from equids, *S. bertrami, S. equicanis, S. fayeri* and *S. asinus,* all with dog as the definitive host. Dubey et al. (1977) have doubted if these were truly different species and postulated that both, *S. betrami* and *S. equicanis* were the same species. Moreover, *S. asinus* of donkeys as named by Gadaev (1978) was also doubted and not considered a valid name for donkeys (Levine and Tadros, 1980). Ultimately, Dubey et al. (1989) listed three different *Sarcocystis species* in equids depending on the structure of the cyst wall, the size of sporocysts and the prepatent period. It could be concluded that the morphological features of *Sarcocystis species* infecting both horses and donkeys in Egypt were closely similar to *S. bertrami* described by Dubey et al. (1989). This result agreed with that reported by Hinaidy and Loupal (1982) who assigned the name *S. bertrami* for *Sarcocystis species* infecting both horses and donkeys.

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