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Studies on the Morphology of Sarcocysts in Thoroughbred Horses in Japan

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ABSTRACT

From 1981 to 1990, 93 Thoroughbred in Japan were examined for *Sarcocystis* cysts using light, scanning and transmission electron microscopy. Tryptic digestion of muscle tissues revealed six infected horses. *Sarcocystis* bradyzoites were found in the myocardium, masseter and diaphragm muscles. Cysts were elliptical, spindle or mulberry shaped, measuring 77.7 - 222.0 μm x 48.1 - 111.0 μm. Cyst walls were 1.0 - 0.6 μm thick and have a smooth surface in HE-stained sections. Bradyzoites ranged from 12.0 - 16.6 μm x 3.0 - 5.3 μm. The morphology of cysts revealed many similarities with *Sarcocystis equicanis*. No pathological changes were observed in infected muscle fibers.

INTRODUCTION

Siedamgrotzky (1872) observed for the first time sarcosporidian cysts in muscle tissues of a horse and described them as sporospermial tubes. Subsequently, Doflein (1901) observed similar cysts and named it *Sarcocystis bertrami*. The morphology of much smaller cysts of *Sarcocystis equicanis* of horses was investigated by Rommel and Geisel (1975) and by Göbel (1976), the latter using electron microscopy. Likewise, morphological details of a third species, *Sarcocystis fayeri*, were worked out by Dubey et al. (1977), and by Tinling et al. (1980) at the ultrastructural level. These documented reports tend to suggest that there could possibly be two or three different species of *Sarcocystis* infecting horses. In the present study, we

sought to examine and characterize sarcocysts in Thoroughbred horses in Japan from 1981 to 1990, with the use of both light and electron microscopy.

MATERIALS AND METHODS

Muscles (cardiac, masseter, diaphragm, cervical, lingual, esophagus, and coccygeal) of 93 Thoroughbred which were sacrificed due to inevitable injuries resulting from accidents in the Niigata horse race course were examined.

Muscle tissues were digested with 0.3% trypsin in phosphate buffered saline (PBS) as described by Maitani (1970). Muscle tissues of bradyzoite-positive horses were examined for cysts using hematoxylin and eosin (H&E) stained sections and scanning electron microscopy (SEM). For transmission electron microscopy (TEM) cysts were isolated by maceration of muscle tissues in PBS. After rinsing the isolated cysts two or three times with PBS, they were processed following TEM routine fixation and staining procedures.

RESULTS

Six of 93 examined Thoroughbred (6.4%) were found to be infected with *Sarcocystis*. In all five horses, cardiac muscles were positive for cysts (three cases involving cardiac muscles only; one case involving both cardiac and masseter muscles; one case involving both cardiac and diaphragm muscles), and one horse was found positive in the masseter muscles only (Table 1).

Table 1. Sarcocysts in Thoroughbreds of different ages.

Age	number of horses examined	number positive	detected density (positive %)
< 4	55	0	-
5	22	1	+
6	7	3	+++
7	5	1	++
8	3	1	++
9	1	0	-
Total	93	6	(6.4%)

Number of bradyzoites observed with light microscopy per microscopic field (Mg. x 1000) : 1-9 = +; 10-99 = ++; ≥ 100 = +++.

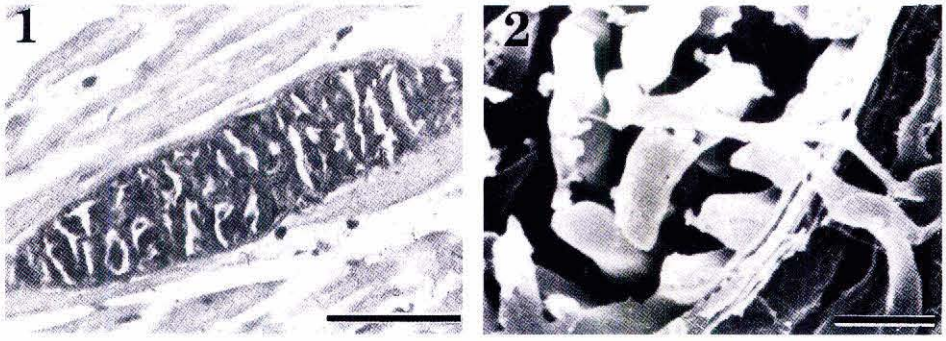
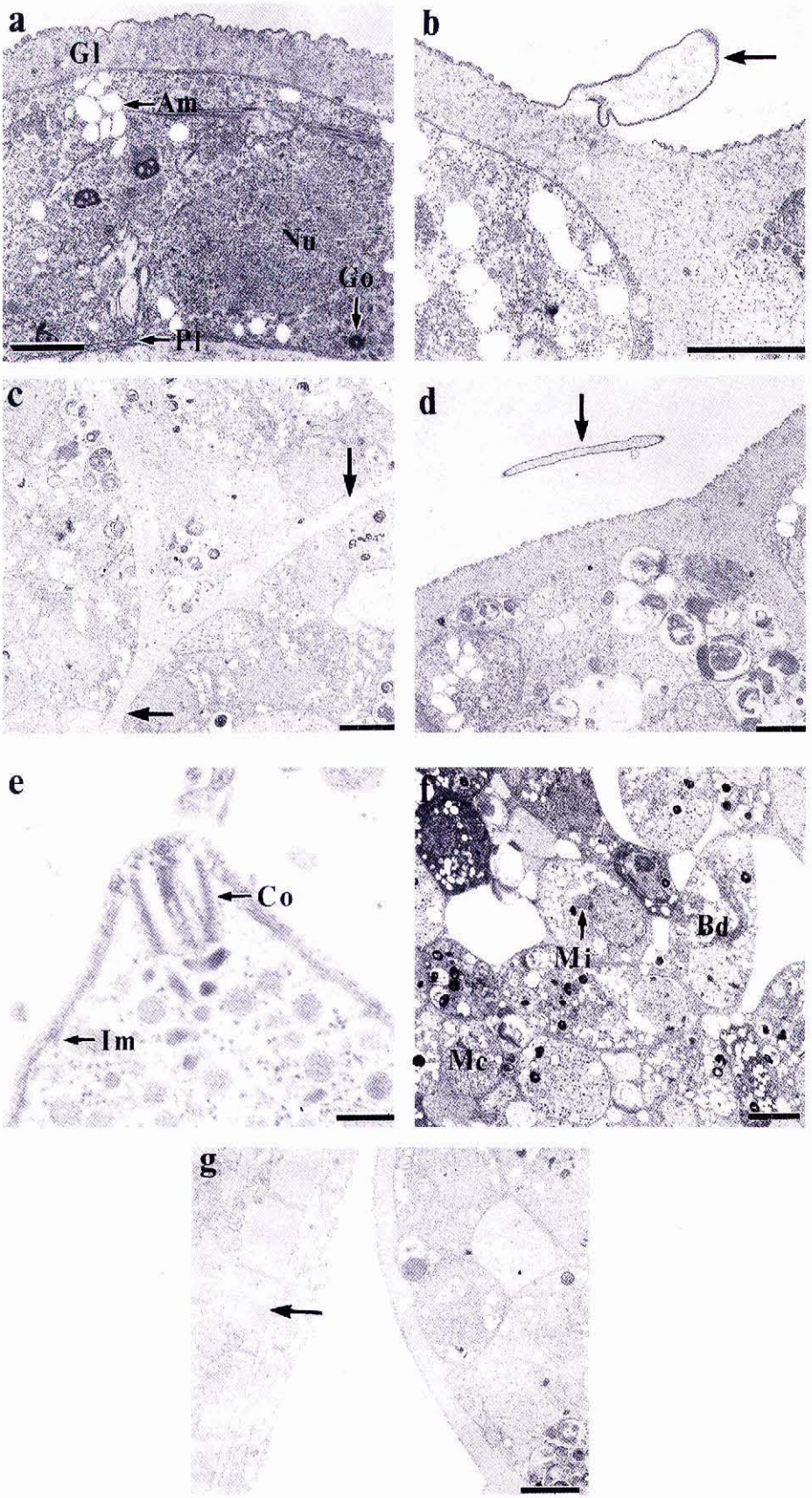


Fig. 1, 2. Light microscopy and SEM of sarcocysts in a cardiac muscle of a male 6 years old Thoroughbred. 1. Septate cyst is spindle shaped cyst, with thin wall, smooth surface and containg numerous bradyzoites. Bar: 50 µm. 2. Thin cyst wall with numerous bradyzoites. Bar: 5 µm.

Depending on the plane of sectioning and angle of sections, cysts were mainly elliptical, spindle or mulberry shaped, ranging in size from 77.7 - 222.0 µm x 48.1 -111.0 µm. The thickness of the cyst wall ranged from 1.0 - 0.6 µm. Cyst walls exhibit thin and smooth surface closely adhered to muscle fibers (Fig. 1 and 2). Cyst surface appeared densely-stained and slightly eroded (Fig. 3a). Portions of cyst wall show claw-like protrusion and ragged-looking face (Fig. 3b). Between cyst and muscle tissues, freely distributed pieces of projections which seem to originate from such crustacean claw-like protrusions were observed. The base and main parts seen in different planes of sections are shown in Figure 3d. Slightly stained ground substance within cyst wall, and fine particulate material of high electron density in the middle layer of the wall with semitransparent empty vacuoles were noted. The inner side of the cyst wall extended into the cyst to form septa in close contact with bradyzoites (Fig. 3c).

Fig. 3. TEM of a sarcocyst isolated from cardiac muscle of a male 7 years old Thoroughbred. **a**: sarcocyst with nucleus (Nu), Golgi complex (Go), amylopectin granules (Am), and plasmalemma of parasite (Pl). Bar: 1 µm. **b**: ragged-looking cyst wall with crustacean claw-like protrusion . Bar: 1 µm. **c**: Septate cyst. Bar: 2 µm. **d**: A free piece of a protrusion (see text). Bar: 1 µm. **e**: Apical complex with conoid (Co) and inner membrane complex (Im) of a bradyzoite. Bar: 200 nm. **f**: Mature sarcocysts containing numerous bradyzoites (Bd), a few metrocytes (Mc) and mitochondria (Mi). Bar: 2 µm. **g**: Muscle fibers (arrow) in the vicinity of a cyst. Bar: 2 µm.


a
Gl
Am
Nu
Go
Pl
b
c
d
e
Co
Im
f
Mi
Bd
Mc
g

Inside the cysts were proliferative metrocytes and developing and mature bradyzoites (Fig. 3f). Bradyzoites measured 12.0 - 16.5 μm X 3.0 - 5.3 μm and the shape varies according to the plane of sectioning. Crescent bradyzoites dominate (Fig. 2) which closely resemble apical complexes common to all types of coccidians (Fig. 3e), and they lie in close contact with the inner side of cyst wall septa.

Within bradyzoites, nuclei, mitochondria, micronemes, Golgi apparatus, conoid and conoid rings, rhoptries, amylopectin granules, and double structured pellicles were observed (Fig. 3a and 3f). Except for some rather thickened sites of the cyst wall, muscle fibers in contact with, and in the vicinity of the cyst wall indicated no pathological changes within Z-lines, and in the actin and myosin filaments (Fig. 3g).

DISCUSSION

Three different species of *Sarcocystis* have been described in horses, *S. bertrami*, *S. equicanis* and *S. fayeri* (Doflein 1901; Rommel and Geisel 1975; Göbel 1976; Dubey 1976; Dubey et al. 1977; Tinling et al. 1980). However, Dubey et al. (1977). have doubted if these are truly different species, and thus, postulated that both, *S. equicanis* and *S. bertrami* are the same species as *S. fayeri*. In the present study, using morphological properties of cysts and cyst walls, we compared our observations with those of the described species. The cysts of *S. equicanis* measure about 350 μm long, with thin cyst wall measuring 1.5 - 2.0 μm and exhibiting 8 - 12 nm thread-like protrusions present on the outer surface (Göbel 1976). Another striking feature of the cyst wall is its ability to stain well with osmium tetroxide. With TEM, other authors have reported the presence of thin cyst wall (less than 1.0 μm) with a smooth surface in sarcocysts in horses (Boy et al., 1990; Hilali and Nassar, 1987; Tinling et al., 1980). These observations are similar to the morphology of *S. equicanis* cyst wall as reported by Rommel and Geisel (1975). The fine morphological structures of the cysts noted in the present study resemble more *S. equicanis* than any other of the described species infective to horse.

It is worth citing that the 6.4% infection rate of Thoroughbred with *S. equicanis* in Japan within the last 10 years is significantly lower compared to the 15-30% reported in

the USA and Europe (Dubey et al. 1989). This may be partly attributed to the improved and better feeding management of horses in Japan.

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