Ultrastructure of *Sarcocystis bertrami* sarcocysts from a naturally infected donkey (*Equus asinus*) from Egypt

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SUMMARY

There is considerable confusion concerning *Sarcocystis* species in equids. Little is known of *Sarcocystis* infections in donkeys (*Equus asinus*). Here we describe the structure of *Sarcocystis bertrami*-like from the donkey by light microscopy (LM) and transmission electron microscopy (TEM). Nineteen sarcocysts from the tongue of a donkey from Egypt were studied both by LM and TEM. By LM, all sarcocysts had variably shaped and sized projections on the sarcocyst walls, giving it a thin-walled to thick-walled appearance, depending on individual sarcocyst and plane of section. By TEM, sarcocysts walls had villar protrusions (vp) of type 11. The sarcocyst wall had conical to slender vp, up to 6 μm long and 1 μm wide; the vp were folded over the sarcocyst wall. The total thickness of the sarcocyst wall with ground substance layer (gs) was 1–3 μm. The vp had microtubules (mt) that originated deeper in the gs and continued up to the tip. The apical part of the vp had electron dense granules. The mt were configured into 3 types: a tuft of electron dense mt1 extending the entire length of the vp with a tuft of medium electron dense mt2 appearing in parallel, and fine mt3 present only in the villar tips. The gs was mainly smooth with few indistinct granules. All sarcocysts were mature and contained metrocytes and bradyzoites. Bradyzoites were approximately 11–15 × 2–3 μm in size with typical organelles.

Key words: *Sarcocystis bertrami*, donkey (*Equus asinus*), transmission electron microscopy.

INTRODUCTION

Donkeys are important for the economy of Africa, Asia and the Arabian Peninsula, and their popularity is increasing in Europe. Sarcocysts have been found in donkeys from Egypt, Austria, Russia (former USSR) and Great Britain, but the parasite has not been adequately described. In general, there is considerable confusion concerning the validity of different species of *Sarcocystis* in equids, especially donkey (Dubey *et al*. 2015). It is believed that all equids (horses, mules, donkeys, zebra and others) share common *Sarcocystis* species, but definitive studies are lacking.

Gadaev (1978) found sarcocysts in 8 of 20 donkeys from the former USSR and named a new species, *Sarcocystis asinus* from the donkey, based on a very cryptic morphological description of the sarcocyst (more on this in discussion section).

Hinaidy and Loupal (1982) found sarcocysts in 1 of 2 donkeys in Austria. However, they did not provide any detail and remarked that the sarcocysts in the donkey were similar to those of *Sarcocystis bertrami* in the horse. Similarly, Edwards (1984) found sarcocysts in 1 of 2 donkeys from a slaughter house in Cheshire, England but did not elaborate on the morphology of the parasite. Kirmse (1986) found sarcocysts in histological sections of 9 of 41 donkeys slaughterd at Zoological Gardens of Rabat, Morocco for feeding captive carnivores; the morphology of sarcocysts was not described. Zayed and El-Ghaysh (1998) reported that 4 dogs fed meat from 11 donkeys slaughtered at National Circus, Giza, Egypt excreted *Sarcocystis* sporocysts; the sarcocysts were assumed to be *S. bertrami*.

Hilali and Nasser (1987) found sarcocysts in 18 of 20 donkeys in Egypt. In histologic sections, sarcocysts were microscopic, up to 410 μm long and 50·2 μm wide. By transmission electron microscopy (TEM) the sarcocyst wall had villar protrusions (vp) that were up to 3·7 μm long and up to 1 μm wide (Hilali and Nasser, 1987). The bradyzoites were 16·2–16·9 × 4·1–4·2 μm in size. They considered the parasite same as *S. bertrami* in the horse. We now provide a full morphological description of sarcocysts in donkey.

MATERIALS AND METHODS

An adult donkey was obtained live on 14 January 2015 from El Fayoum Governorate, Egypt for teaching post mortem technique to veterinary students. The donkey was euthanized by the Pathology

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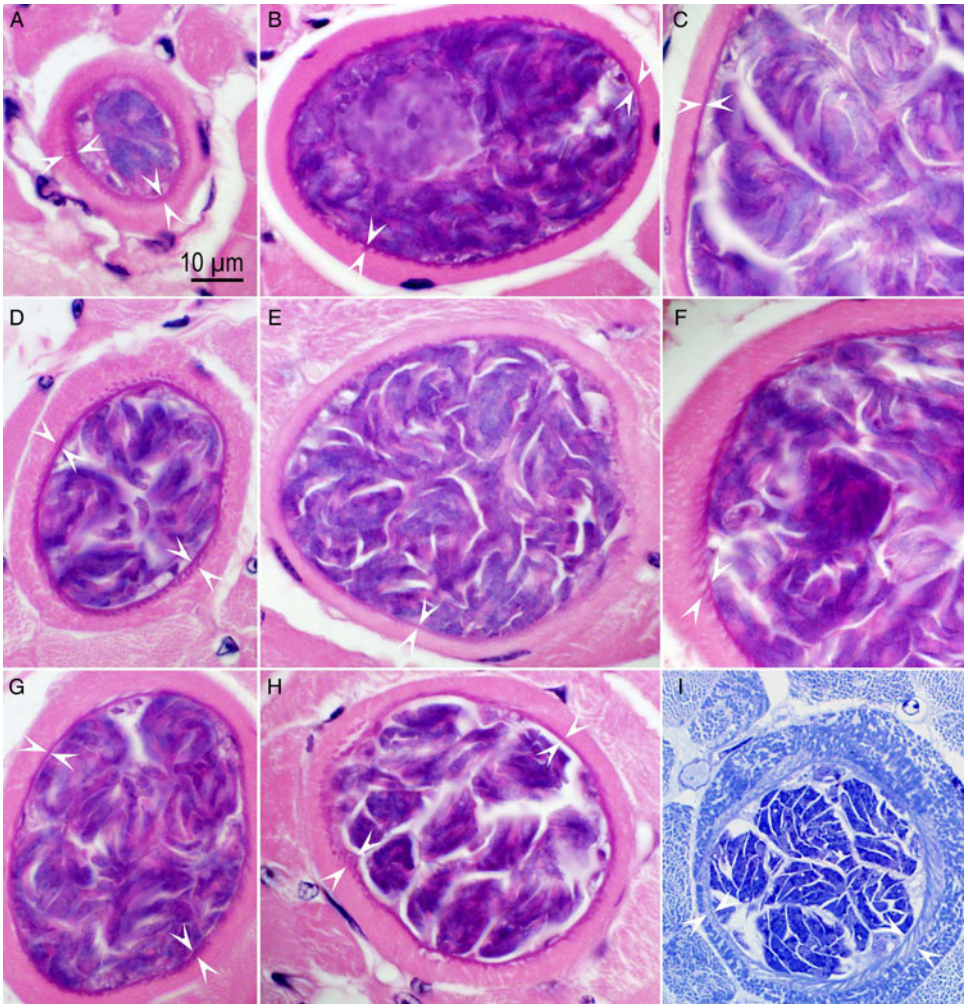

Fig. 1. Sarcocysts in histological sections of tongue of a donkey in Egypt. A–H, haematoxylin and eosin stain, I, Toluidine blue stain. Bar applies to all images. The sarcocyst wall appears smooth or striated (opposing arrowheads).

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For the present study, tongue muscle was fixed in glutaraldehyde (GF) and formalin. The formalin-fixed (FF) tissue was processed for paraffin embedding. The paraffin blocks and the GF samples were transported to the Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa for light and electron microscopic examinations. For light microscopy (LM), paraffin-embedded sections were cut at 5 μm thick and examined after staining with haematoxylin and eosin (H and E). For TEM, GF tissue was processed using standard techniques. Briefly, the samples were post-fixed in 1% osmium tetroxide in Millonig's buffer (pH 7·4), dehydrated through a series of graded ethanols, infiltrated with an epoxy resin/propylene oxide mixture before being embedded in absolute resin, and polymerized at 60 °C overnight. Two cysts were detected in the GF sample. A further 17 tissue cysts, located in paraffin blocks (by matching with H and E sections) were deparaffinised (Van den Berg Weermans and Dingemans, 1984).

RESULTS

In total, 19 sarcocysts were studied ultrastructurally; 2 from GF and 17 from paraffin blocks. Sarcocysts were microscopic, and up to 110 μm wide. By LM of 5 μm histological sections, the sarcocyst wall thickness varied from <1 to 5 μm, depending on the individual sarcocyst and the plane of section (Fig. 1). Some sarcocysts had protrusions that varied in shape and size. Examples from 9 sarcocysts are shown in Fig. 1.

By TEM of GF sarcocysts, the parasitophorous vacuolar membrane (pvm) was undulating, and lined by an electron dense layer (edl). The edl was thin at irregular intervals and invaginated giving the pvm a vacuolated appearance (Figs 2 and 3). The pvm was folded into vp that varied in shape and size, within the same cyst (Fig. 2B and C). In some cysts, vp were adjacent to each other, while in others they were up to 2 μm apart. Villar protrusions were mostly elongated, up to 6 μm long and up to 1 μm wide, some were conical in shape. Electron dense, evenly distributed hair-like structures were seen on vp tips, both in GF and FF vp

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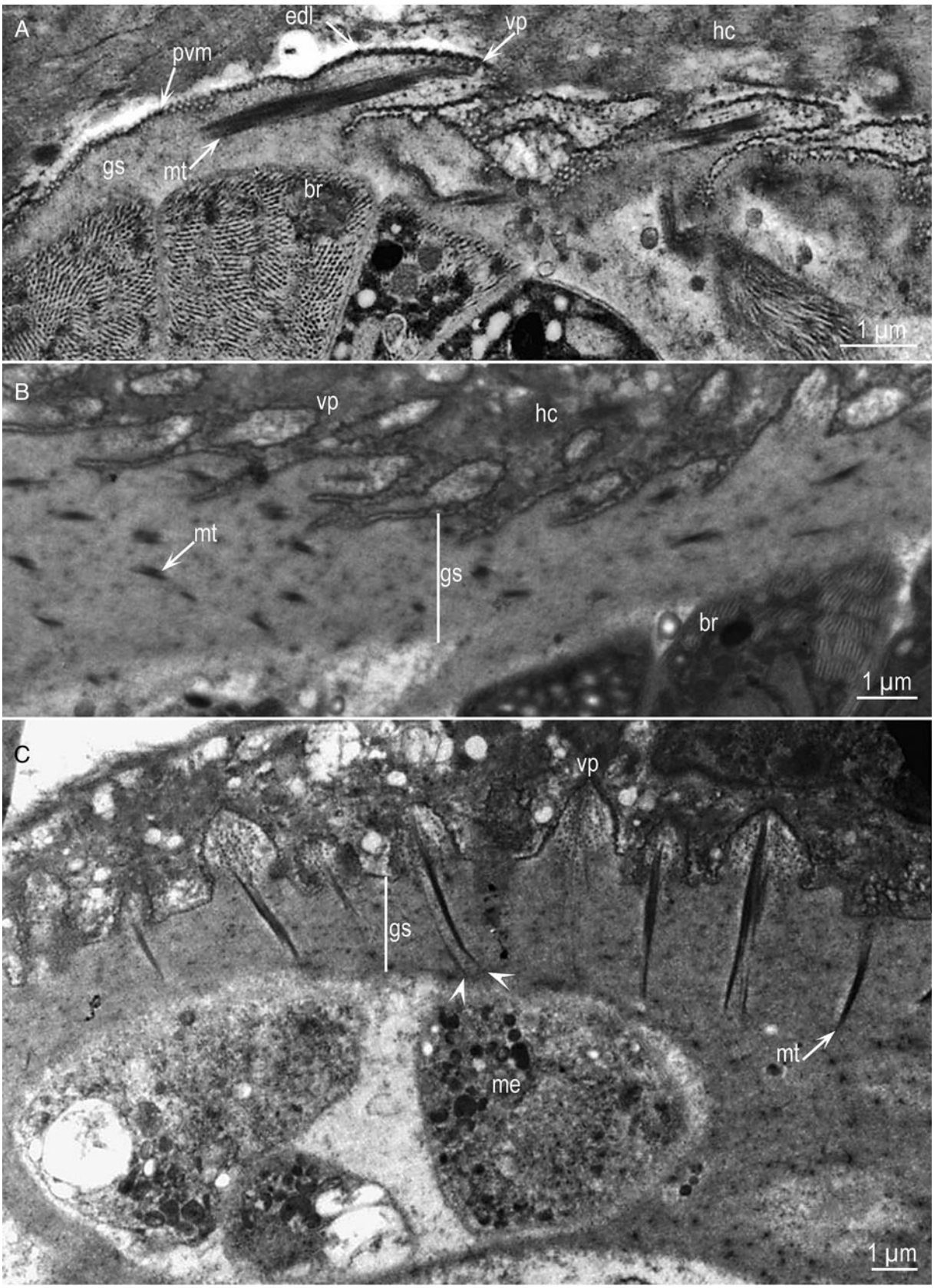

Fig. 2. TEM of *S. bertrami* sarcocyst walls from 2 cysts. Glutarladehyde-fixed. Note parasitophorous vacuolar membrane (pvm) lined by electron dense layer, villar protrusions (vp), ground substance layers (gs), microtubules (mt), bradyzoites (br), metrocytes (me) and host cell (hc). (A) The vp are folded on the sarcocyst wall. The gs is thin. Cyst #1. (B) The vp are angled or folded over the sarcocyst wall. The gs appears thick and has portions of microtubules (mt). (C) Same sarcocyst as in B but the vp are conical, the gs is thick. The mt extend from the tip of the vp to the pellicle of metrocytes (arrowheads). Cyst #2.

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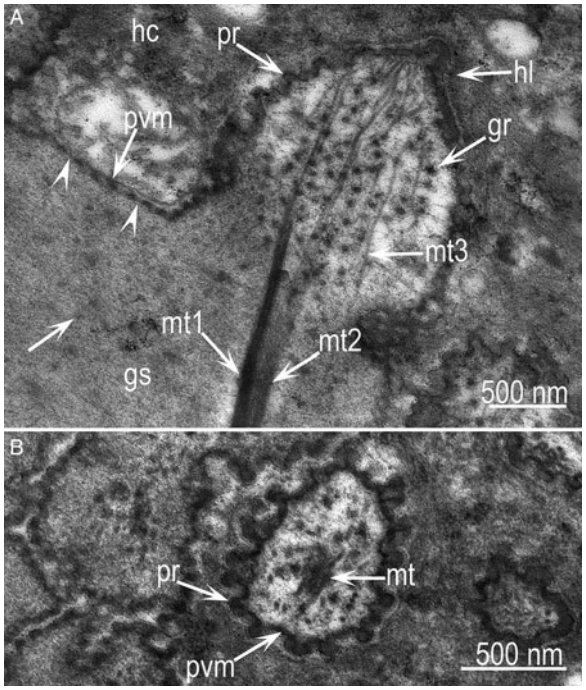

Fig. 3. TEM of *S. bertrami* sarcocyst walls from cyst #2. (A) Tip of a vp showing undulating pvm, lined by electron dense layer (edl), that is thinned out or absent at places (arrowheads). The pvm has outpocketing of protrusions (pr). Three types of mt are visible: (i) a thick tuft of electron dense microtubules (mt1), (ii) another tuft of medium electron dense microtubules (mt2), and (iii) fine mt that appear to crisscross towards the villar tips (mt3). Note numerous dense granules (gr) in the villar tips. A collar of hair-like (arrow) projections is present on vp. The gs is relatively smooth with few granules. (B) Cross-section of vp showing protrusions (pr) on the pvm and mt.

(Fig. 3A). The vp contained microtubules (mt) that varied ultrastructurally. A tuft of highly electron dense mt1 extended from the tip of the villus deep into the interior of the sarcocyst reaching the pellicle of the zoites (Fig. 3A). Another group of less electron dense mt2 existed parallel to the electron dense mt1 (Fig. 3A). Additionally, fine mt3 were located towards the tips of the vp. Microtubules lacked electron dense granules. Dense granules, however were present in the distal portion of vp, juxtaposed with host myocyte. The ground substance layer (gs) was up to 2·5 μm thick and was relatively homogenous, except for a few dense granules. In tangentially cut sarcocysts, the gs sections of electron dense mt were scattered throughout the gs (Fig. 3B).

All sarcocysts contained few metrocytes and numerous mature bradyzoites. Both metrocytes and bradyzoites were arranged in groups (Fig. 2). The bradyzoites were 11–15 μm long and 2·5–3·0 μm wide. They had the same structures as seen in other *Sarcocystis* bradyzoites, including micronemes, 2 rhoptries, a micropore, several dense granules, a nucleus, a mitochondrion, and numerous amylopectin granules (Fig. 4). The micronemes were in the conoidal third of the bradyzoite. Most micronemes were arranged in rows and were approximately 300 × 50 nm in size with tapering or round ends (Fig. 4). The micropore was located 2·5 μm from the tip of conoid. Granular material was seen below the micropore (Fig. 4C).

DISCUSSION

As stated earlier, there is considerable confusion concerning the species of *Sarcocystis* in equids. Rommel and Geisel (1975) in reviewing the earlier literature stated that Siedamgrotzky (1872) first reported sarcocysts in horses in Germany. He noticed cilia-like 2 μm long protrusions on the sarcocyst wall. Doflein (1901) named the parasite, *Sarcocystis bertrami*; he noticed cone-like structures on the sarcocyst wall. Rommel and Geisel (1975) found thin-walled sarcocysts that lacked protrusions; they named the parasite *Sarcocystis equicanis* following the nomenclature proposed by Heydorn *et al*. (1975), combining the names of intermediate and definitive hosts. Göbel and Rommel (1980) described the ultrastructure of *S. equicanis* and reported 2–3 μm long hairy protrusions on the sarcocyst wall. Odening *et al*. (1995) expanded on the ultrastructure of sarcocysts in wild Equidae, and concluded that the structure broadly resembled the description of *S. equicanis* from the domestic horse.

Experimental evidence suggests the similarity of a *Sarcocystis* species in the horse and the donkey (Matuschka, 1983). Tissues from 20 naturally infected horses in Germany were fed to a dog, and those of 10 donkeys (also from Germany) were fed to another dog. Both dogs excreted sporocysts. Experimental infections were conducted with 4 ponies (#1–4) raised in captivity using the sporocysts derived from the infected donkeys (inoculum A) and sporocysts derived from the inocula from horses (inoculum B). The foal (#1) fed donkey-derived sporocysts became febrile on days 10 and 11 and 19–21 but sarcocysts were not found when muscle biopsies were obtained on 44 and 59 days post-inoculation (DPI). The same foal (#1) was then fed horse-derived sporocysts on day 117 and killed 21 days later (138 days after feeding donkey-derived sporocysts). Sarcocysts were detected histologically in muscles of 3 foals when killed 138 (#1), 122 (#2) and 221(#3) DPI but no parasites were detected in the foal (#4) killed 21 DPI. Sporocysts were structurally similar in dogs that excreted sporocysts after ingesting donkey or horse muscles; the sporocysts were 12·2–13·8 × 9·2–9·9 μm in size and the prepatent period was 9–10 days. Sarcocysts and sporocysts appeared similar to those of *S. equicanis*/*S. bertrami* (Rommel and Geisel, 1975). Although this experiment is not definitive (transmission based on 1 foal fed donkey-derived sporocysts), it is suggestive of

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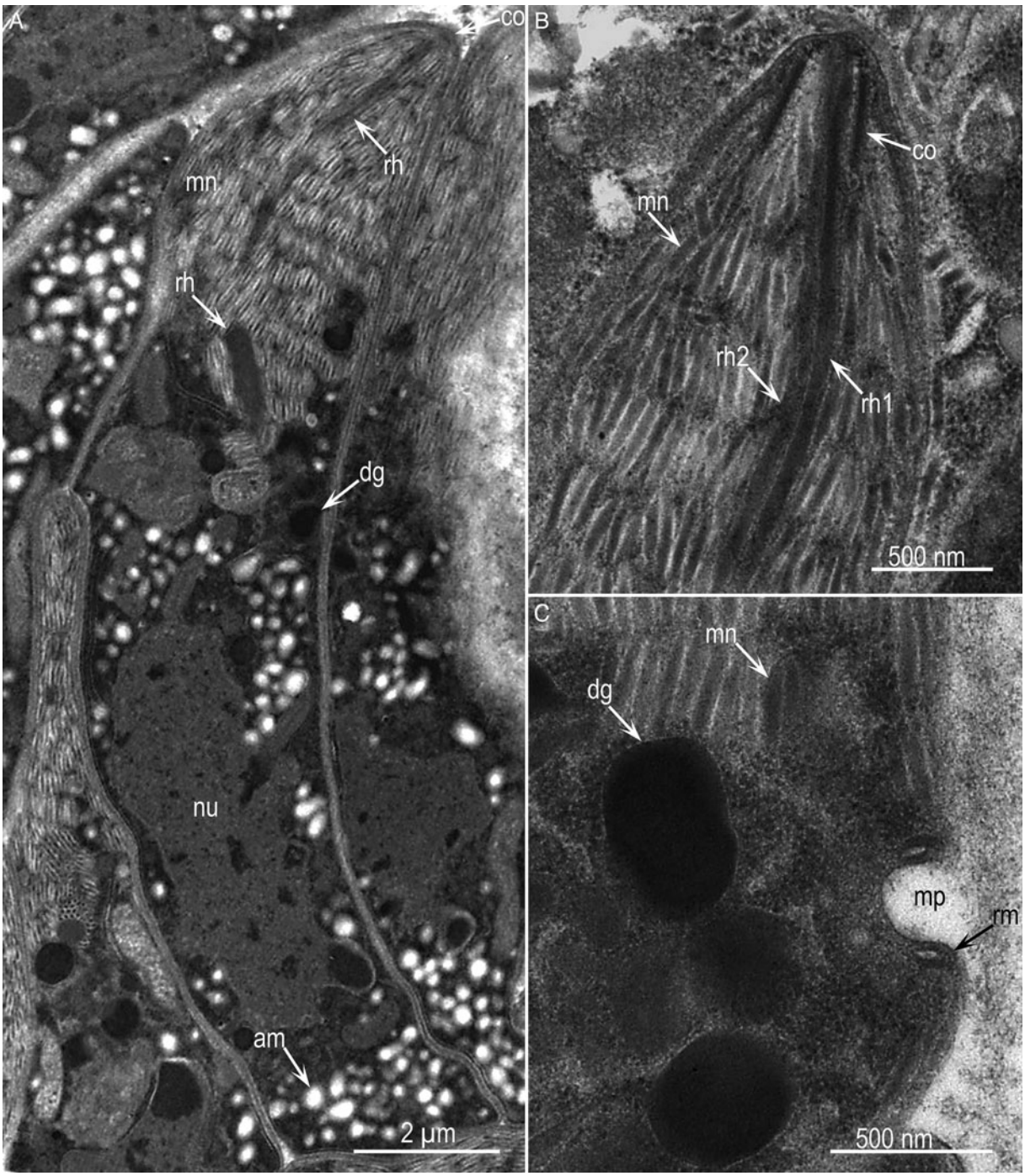

Fig. 4. TEM of *S. bertrami* bradyzoites. Glutarladehyde-fixed. Note conoid (co), numerous micronemes (mn), several dense granules (dg), a nucleus (nu) and rhoptries (rh) with long slender neck. (A) A longitudinally cut bradyzoite. (B) Longitudinal section of conoidal part of a bradyzoite. Note conoid (co) with two rhoptries (rh1, rh2) with slender neck. The micronemes (mn) are arranged in rows. (C) Note a micropore (mp) with a collared rim (rm), dense granules (dg), and electron dense secretory material surrounds below the mp.

possible transmission of the parasite in horse and donkey.

Two dog transmitted *Sarcocystis* species, *S. bertrami* (*S. equicanis*) and *Sarcocystis fayeri* are known to infect horses. *Sarcocystis fayeri*, orginally described from horses in the USA has been also found in Germany (Dubey *et al*. 1977; Erber and Geisel, 1981). However, *S. bertrami* has not been reported in horses in the USA, and *S. fayeri* has not been reported from other equids, including the donkey. Dubey *et al*. (2015) reviewed the literature on *Sarcocystis* species in equids and synonmysed *S. equicanis* with *S. bertrami*. Illustrations in Fig. 1 in the present study from sarcocysts in donkey may explain why the confusion arose concerning the thin-walled *S. equicanis* described by Rommel and

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Geisel (1975) and the hairy to thick-walled sarcocysts described by Doflein (1901). In the present study, both smooth and hairy portions were seen in the same sarcocyst.

As stated earlier, Gadaev (1978) named *S. asinus*, solely based on the size, shape and staining of bradyzoites in smears. He said that bradyzoites of *S. asinus* were 1–3 μm larger (12·3–17·5 × 4·5–6·2 μm for *S. asinus* and 11·5–14·5 × 3·5–4·0 μm for *S. bertrami*) than *S. bertrami* of horses, and the nuclear chromatin was pale red in *S. asinus vs* crimson in *S. bertrami* (Gadaev, 1978). These differences are untenable for separating *Sarcocystis* species, because these differences in bradyzoite morphology could be related to techniques used. Therefore, we support the contention that *S. asinus* is invalid (Levine and Tadros, 1980; Odening, 1998; Dubey *et al*. 2015).

All sarcocysts in tongue of the donkey in the present study were microscopic. Hinaidy and Loupal (1982) mentioned that sarcocysts in a donkey oesophagus were 1·7–14·1 mm long. Macroscopic sarcocysts, however, were never found in donkeys in Egypt (Hilali, personal observations). The structure of the sarcocyst wall is useful in taxonomy of *Sarcocystis* species in a given host. Dubey *et al*. (2015) grouped sarcocysts in more than 40 morphologic types. Sarcocysts in the present study were type 11, found in sarcocysts in equids and also in some avian species. One characteristic of type 11 sarcocysts is mt that extend from the villar tips to the zoite pellicle. Here, we have described in detail the structure of vp of the sarcocyst wall of the sarcocyst in the donkey, and it is distinct from vp in *S. fayeri*. Villar protrusions in *S. fayeri* are slender, upright, and contain 1 group of mt. The vp in *S. bertrami* are folded over the cyst wall and have mt of 3 types.

The description of the *S. bertrami* sarcocysts described here from the donkey should assist in the recognition of this parasite in horses and other equids. The parasite described in the present study in donkey was considered *S. bertrami*, based on literature. As stated earlier, there are no archived specimens of *Sarcocystis* species named from equids before 1975. A critical comparison of the molecular characteristics of *Sarcocystis* species in the horse *vs* sarcocysts from other equids will help in reaching a final conclusion.

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