A review of sarcocystosis in camels and redescription of *Sarcocystis cameli* and *Sarcocystis ippeni* sarcocysts from the one-humped camel (*Camelus dromedarius*)

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Running Head: Sarcocystis cameli and *Sarcocystis ippeni* from the camel

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Summary

There is considerable confusion concerning *Sarcocystis* species in camels. Five species: *Sarcocystis cameli, S. ippeni, S. camelicanis, S. camelocanis,* and *S. miescheri* were named with inadequate descriptions and no type specimens. Here, we review literature on sarcocystosis in camels worldwide and redescribe structure of *S. cameli* and *S. ippeni* sarcocysts by light and transmission electron microscopy (LM, TEM). Eight sarcocysts from the esophagi of two camels (*Camelus dromedarius*) from Egypt were studied. By LM all sarcocysts were thin walled with

barely visible projections on the cyst walls. By TEM, two structurally distinct sarcocysts were recognized by unique villar protrusions (vp) not found in sarcocysts from any other host. Sarcocysts of *S. cameli* had vp of type 9j. The sarcocyst wall had upright slender vp, up to 3.0 μm long and 0.5 μm wide; the total thickness of the sarcocyst wall with ground substance layer (gs) was 3.5 μm. On each vp there were rows of knob-like protrusions that appeared to be interconnected. The vp had microtubules that originated at mid point of the gs and continued up to the tip; microtubules were smooth, without any granules or dense areas. Bradyzoites were approximately 14-15 x 3-4 μm in size with typical organelles. *Sarcocystis ippeni* sarcocysts had type 32 sarcocyst wall characterized by conical villar protrusions with an electron dense knob. The total thickness of the sarcocyst wall (from the base of gs to vp tip) was 2.3-3.0 μm. The vp were up to 1.2 μm wide at the base and 0.25 μm at the tip. Microtubules in vp originated at midpoint of gs and continued up to tip; microtubules were criss-crossed, smooth and without granules or dense areas. Bradyzoites were 12.0-13.5 x 2.0-3.0 μm in size. *Sarcocystis camelicanis, S. camelocanis,* and *S. miescheri* are considered invalid.
Key words: *Sarcocystis cameli; Sarcocystis ippeni;* One-humped camel (*Camelus dromedarius)*; Electron microscopy; Ultrastructure.

INTRODUCTION

While reviewing literature on sarcocystosis in animals we found considerable confusion concerning the *Sarcocystis* species in camels (Dubey *et al.* 2015). The morphological descriptions were often vague, and there are no archived specimens for verification. Here, we have summarized available reports on *Sarcocystis* infection in camels and provided redescription of sarcocysts of two species, *S. cameli*, and *S. ippeni*.

Review of literature

Species names

Mason (1910) first reported sarcocysts in muscles of camels slaughtered for food in Cairo, Egypt. All old and emaciated camels had numerous sarcocysts that were found in virtually all muscles, including the heart, but the number of camels infected or examined were not provided. Sarcocysts were up to 12 mm long and less than 1 mm wide, appearing as white lines, with thin or thick cyst walls, but no measurements of the thickness of the wall was given; the parasite was named it *S. cameli* (Mason, 1910). Dubey *et al.* (1989) arbitraily termed the so called thick-walled sarcocyst of Mason (1910) *S. cameli*, but did not name the thin-walled species which was subsequently called *S. ippeni* by Odening (1997). Neither Dubey *et al.* (1989) nor Odening (1997) examined specimens reported by Mason (1910) or sarcocysts from other camels. The presence of thick and thin-walled sarcocysts was confirmed in camels from Saudi Arabia (Fatani *et al.* 1996) and Somalia (Hagi *et al*. 1989) (Table 1). Abdel-Ghaffar *et al*. (2009) studied the prevalence of *Sarcocystis* infection in camels in Cairo, Egypt. Microscopic sarcocysts, found in 116 of 180 camels from an abattoir, were 120-170 x 50-100 µm in size and of one morphological type. Dogs that were fed heavily infected camel meat, excreted *Sarcocystis* sporocysts (Table 2). The parasite studied was called *S. camelicanis* without elaborating on the new name.

Ishag *et al*. (2001, 2006) in Sudan studied transmission of *Sarcocystis* between camels and dogs. They found two types of sarcocysts, thick-and thin-walled, in a camel fed sporocysts from dogs (Ishag *et al*. 2001) and two different sized sporocysts (Table 2, 13.2-13.6 x 6.5-9.5 µm and 16.0 x 9.9-11.5 µm) in dogs that were fed camel meat (Ishag *et al.* 2006). They named the

Table 1 : Prevalence of *Sarcocystis* sarcocysts in camels.

Country	Year	N	Method	Positive	%	Gross examination	TEM	Reference
Afghanistan	1984	192	C, H	118	61.4	NS	NS	Kirmse and Mohanbabu (1986)
Egypt	2008	180	B, C, H, Td	116	64.0	Negative	Thick wall, finger-like vp	Abdel-Ghaffar *et al*. (2009)
	NS	112	B, C, H	41	36.6	Negative	NS	Hilali and Mohamed (1980)
	NS	13	H	3	23.1	NS	Thin wall, cone-like vp	Entzeroth *et al.* (1981)
	2009-10	156	C	66	42.3	Negative	Thick and thin wall, finger-like and cone-like vp	Mandour *et al.* (2011)
	NS	130	C, Td, H	20	15.3	Positive, 73%	Thick and thin wall, finger-like and cone-like vp. Macroscopic cysts surrounded by secondary wall	Sakran *et al*. (1995)
Ethiopia	1998-99	121	H	55	45.5	Negative	NS	Woldemeskel and Gumi (2001)
India	NS	1	H	1	-	Streak-like lesions in abdominal muscle	NS	Ranga Rao *et al.* (1997)
Iran	NS	400	C	209	52.3	Negative	NS	Shekarforoush *et al.* (2006)
	2002-05	250	H	209	83.6	Negative	NS	Valinezhad *et al.* (2008)
	2009	130	Pd	67	51.5	Negative	NS	Hamidinejat *et al.* (2013)
Iraq	1992-96	36	Pd	33	91.6	Negative	NS	Latif *et al*. (1999)
Jordan	NS	110	C	24	21.8	Negative	Thick wall, finger-like vp	Latif and Khamas (2007)
Mongolia	1998-99	5	C	5	100.0	NS	NS	Fukuyo *et al.* (2002)
Saudi Arabia	1992-93	103	B, Td, H	91	88.3	Negative	NS	Fatani *et al.* (1996)
	2002-03	624	Td	399	64.0	Negative	Thick wall, finger-like vp	Al-Goraishy *et al.* (2004)
	NS	40	C, Td, H	27	67.5	Negative	Thick wall, finger-like vp	Shazly (2000)
Somalia	1987	200	Td, H	165	82.5	NS	NS	Hagi *et al.* (1989)
Sudan	NS	100	Pd	81	81.0	Negative	NS	Hussein and Warrag (1985)
Former USSR (Russia)	NS	NS	B	6	NS	Positive	NS	Kuraev (1981)

NS, not stated; B, bioassay in dog; C, compression/muscle squash; G, gross examination; H, histology; Pd, pepsin digestion; Td, trypsin digestion; TEM, transmission electron microscopy; Vp, villar protrusions.

Table 2 : Excretion of *Sarcocystis* sporocysts in feces of dogs fed camel meat.

Country	*Sarcocystis* Type/species	No. of infected camels	No of dogs infected/no used	Prepatent period (days)	Size of sporocysts (µm)	Reference
Egypt	E,D[b], sarcocysts in 41/112[c], trichinoscope, Microscopic	NS-250g	3/3	10,11, 14	12 × 9	Hilali and Mohamed (1980)[a]
Egypt	Microscopic	NS-500g	12/12	Endogenous Sateges studied	Sporulation completed in the intestinal lamina propria of dogs in 8 days	Hilali *et al*. (1982)
Egypt	E,H, not examined	NS, 450-500g	3/3	10,11,11	12.0-14.0 × 8.9-11.3	Hilali *et al.* (1992)
Egypt	Sarcocysts in 116/180[c] E,D,H, T,Sk, all microscopic	NS	12/12	11	13.7-15.6 × 7.8-10.7 Gametogony completed in intestinal lamina propria of dogs in 8 days	Abdel-Ghaffar *et al.* (2009)[a]
Egypt	E, 66 of 156[c], microscopic		2/2	13-15	10.1-13.9 × 8.59-9.94 (type A) 8.7-14.3 × 11.5-10.0 (type B)	Mandour *et al*. (2011)
Russia	Macroscopic			Not stated	16.4 × 8.3	Kuraev (1981)
Saudi Arabia	E,D,H. 91/103[c]. Microscopic	500g	2/2	9-10	10.7-14.3 × 8.3-10.7 (n=20)	Fatani *et al.* (1996)[a] ;Hilali *et al.* (1995)[a]
Sudan	E,D,H,Sk	NS 400g	?/6	9-13	13.2-13.6 × 6.5-9.5 (type A) 16.0 × 9.9-11.5 (type B)	Ishag *et al.* (2006)

[a]Cats fed infected camel meat did not excrete sporocysts.
[b]D=diaphragm, E=esophagus, H=heart.
[c] Number of camels infected/number examined. However, it is not clear that the inoculum for dogs was deriverd from how many infected camels.

larger sporocyst in the dog that was fed camel meat as a new species, *S. camelocanis*, but gave no description of the sarcocyst.

To add to this confusion, another new species from the camel was named, *S. miescheri*, based on finding oocysts in feces of dogs fed naturally infected camel meat (Mandour *et al.*

2011). Illustrations provided by the authors resemble *Cystoisospora ohioensis* oocysts measuring 20.8-26.7 x 18.5-20.7 µm with a thick wall and containing two sporoblasts, and bearing no resemblance to other species of *Sarcocystis.* The bradyzoites, measuring 21.5-32.8 x 7.7-17.7 µm, appeared to be artifacts misidentified as bradyzoites (Dubey *et al.* 2015).

There are therefore currently five named *Sarcocystis* species in camels, namely *S. cameli, S. ippeni, S. camelicanis, S. camelocanis,* and *S. miescheri.*

Sarcocyst size

There is considerable confusion concerning the size of sarcocysts. As stated earlier Mason (1910) found sarcocysts in camels that were up to 12 mm long. Kuraev (1981) in Russia reported macroscopic sarcocysts in the oesophagi of six camels. Thick-and thin-walled sarcocysts between 6 and 15 mm long with a variety of shapes including oval, spindle and cylindrical, were present. Dogs fed infected camel tissues excreted 16.4 x 8.3 µm sized sporocysts; no details of the experiment were provided. This report needs confirmation and is mentioned only in the context of a complete review of *Sarcocystis* infection in camels. Sakran *et al*. (1995) reported macroscopic sarcocysts in 95 of 130 esophagi and 25 of 50 diaphragms of camels from Cairo, Egypt. The results of this investigation are difficult to reconcile with their subsequent paper where they did not find macroscopic sarcocysts in camels from Cairo, Egypt (Abdel-Ghafar *et al.* 2009). Sarcocysts were found in tissue sections of 116 of 180 camels; in 60% of esophagi, 50% of diaphragms, 40% of tongues, and 10% of hearts (Abdel-Ghaffar *et al.* 2009). Sarcocysts were 120-170 x 50-100 µm in size, and only one morphologic type of sarcocyst was found. Both reports are by the same group of scientists (Sakran *et al*. 1995; Abdel-Ghaffar *et al.* 2009). There is speculation whether the epidemiology of sarcocystosis in camels has changed drastically between 1995 when the Sakran *et al.* (1995) study was published versus

the recent study (Abdel-Ghaffar *et al.* 2009). The point is raised because of the condemnation of meat with grossly visible sarcocysts.

Prevalence of sarcocysts

Sarcocysts or *Sarcocystis*-like bradyzoites have been reported in up to 91% of one-humped camels from several countries (Table 1) but the species of *Sarcocystis* were not determined.

Life cycle studies and excretion of sporocysts by dogs

Dogs fed naturally infected camel meat containing microscopic sarcocysts in Egypt and Saudi Arabia excreted sporocysts, and gametogonic stages were found in small intestines of dogs (Table 2). Because camel meat fed to dogs was not examined microscopically in each instance, it is uncertain if the dogs were hosts for one or both microscopic sarcocyst species.

Ultrastructural studies

Two types of sarcocysts have been described from camels. Sarcocysts with finger-like villar protrusions (variety A), and conical projections (variety B), but they have not been assigned to specific species.

Variety A: Abdel-Ghaffar *et al*. (1979) first reported ultrastructure of sarcocysts from camel in Egypt. Microscopic sarcocysts (130-180 x 60-110 μm) were found in esophagi and diaphragms (number of infected was not stated) of 44 camels examined. Sarcocysts had smooth wall by light microscopy (Abdel-Ghaffar *et al*. 1979). Ultrastructurally, the cyst wall had 1.2-1.6 μm long villar protrusions (vp) with a maximum width of 0.5 μm. Bradyzoites were 8-12 x 2.5-3.8 μm in size. Only one morphologic type was described; the parasite was not named. Abdel-Ghaffar *et al*. (2009) in Cairo, Egypt added further to the description of this type of sarcocyst in camels in Cairo, Egypt. They reported 16 to18 knob-like structures on each vp. As stated earlier they called

this parasite *S. camelicanis*. Similar sarcocyst type was reported in camels from Iran (Motamedi *et al*. 2011), Jordan (Latif and Khamas, 2007), and Saudi Arabia (Al-Goraishy *et al*. 2004).

Variety B: Entzeroth *et al*. (1981) found this parasite in three of 13 camels from Cairo, Egypt. Sarcocysts were 120-150 x 50-80 μm in size. Cyst wall was not described by light microscopy. Ultrastructurally, cyst wall had knob-like elevations on the surface. The cone-like vp were 0.5-1.4 μm long. Bradyzoites were 10-12 x 2.5-4.0 μm in size. Only one morphologic type was described.

Clinical sarcocystosis

In two separate experiments young camels orally inoculated with *Sarcocystis* spp. sporocysts from dogs became ill. In the first experiment two 6-month old camels in Saudi Arabia were inoculated orally with 250,000 or 750,000 sporocysts from experimentally infected dogs (Fatani *et al*. 1996). Both camels became anorectic, developed pyrexia, became restless and anemic 29 days post inoculation (p.i.). One camel was euthanized 34 days p.i. and the second died day 41 p.i.; hemorrhages were found in viscera and muscles. Histopathological findings were not reported.

In the second experiment, two 1-month old camels in Sudan were inoculated orally with 1,000,000 sporocysts from feces of experimentally infected dogs (Ishag *et al*. 2001). Both camels became anorectic, lethargic, and anemic, begining 20 day p.i. Camel 1 died 26 day p.i.; post mortem examination revealed hemorrhages in several organs and immature cysts containing metrocytes in the brain. The second camel was given food medicated with Amprolium® (100 mg/kg body weight), starting the day of sporocyst inoculation and continuing for 30 days. This

camel remained asymptomatic and mature sarcocysts were found in muscles at necropsy on day 110 p.i.

The objective of the present paper is to provide proper description of two types of sarcocysts by light and transmission electron microscopy (LM, TEM) and assign them to specific species.

MATERIAL AND METHODS

Naturally infected camels

Oesophageal tissues were collected from two adult camels (*Camelus dromedarius*) (no. 4 and 5) on January 15, 2015 from an abattoir in Giza, Egypt. Tissues were fixed in glutaraldehyde (GF) or formalin. The formalin-fixed (FF) tissues were processed for paraffin embedding. The paraffin blocks and the glutaraldehyde fixed samples were transported to the Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Republic of South Africa for light and electron microscopic examinations. For LM, paraffin-embedded sections were cut at 5 µm thick and examined after staining with hematoxylin and eosin (H and E). For TEM, glutaraldehyde-fixed tissue from camel no. 5 (cyst #1, 6, 7, 8), were 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 60ºC overnight. A further four tissue cysts, located in paraffin blocks (by matching with H and E sections) from camel no. 4 (cysts # 2, 3, 4, 5), were deparaffinised (Van den Berg Weermans and Dingemans, 1984). Toluidine blue-stained resin sections of all eight microcysts were photographed with an Olympus BX63 compound microscope (Olympus, Wirsam, South Africa). Ultrathin resin

sections were contrasted with uranyl acetate and lead citrate and examined in a Philips CM10 transmission electron microscope (FEI, Eindhoven, The Netherlands) operated at 80 kV. Digital images were captured with a Megaview III side-mounted digital camera and iTEM software (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

RESULTS

Macromorphology and light microscopy

Twenty-two sarcocysts were found in H and E stained sections. All were mature and microscopic. The largest sarcocyst was 700 x 100 μm (Fig.1A, B). Eight sarcocysts (# 1 to 8) were located in 1-μm Toluidine blue stained sections; they were 150 x 60 μm (cyst#1), 270 x 45 μm (cyst #2), 120 x 100 μm (cyst# 3), 120 x 50 μm (cyst #4), and 110 x 65 μm (cyst #5), 226 x 80 (cyst#6),47 x 38 (cyst#7), and 93 x 30 (cyst# 8) . The description is correlated between sections stained by Toluidine blue and by TEM but not with H and E stained sections.

In H and E-stained sections all sarcocysts appeared to be thin walled (<2 μm). All sarcocysts were mature. Representative images are shown in Fig. 1B-D. In some sarcocyst conical projections could be seen on the sarcocyst wall (Fig. 1C). In 1-μm Toluidine blue stained sections the structure of the sarcocyst wall was not clear, even at 1000 X magnification (Fig. 1 E-K). However, in one cyst photographed at higher magnification, conical projection were visible (Fig.1 L). In Toluidine blue stained sections metrocytes were stained faintly and appeared of different shapes. The bradyzoites were banana shaped and 10-12 μm.

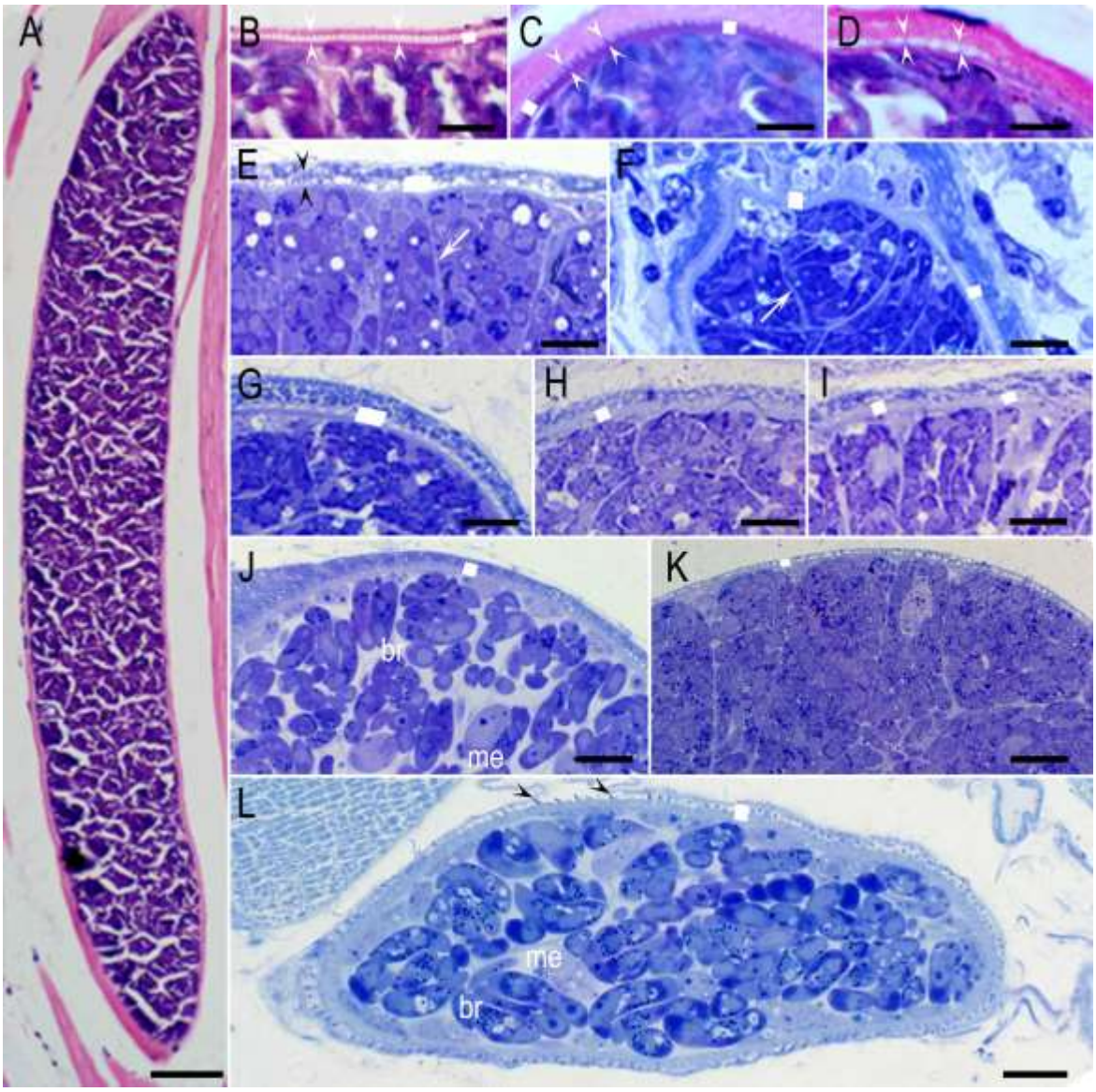

Fig. 1. Sarcocysts from camels from Egypt. Figs. C and E are from camel no. 5, the remainders are from camel no. 4. A-D, 5 μm sections stained with H and E, E-L, Toluidine blue. Scale bar applies to all figures; 50 μm in A, 10 μm in B to I, and 5 μm in L. The opposing arrowheads point to villar protrusions. The white squares point to thickness of the sarcocyst wall. The species of *Sarcocystis* was not identified in H and E stained sections. Based on TEM, sarcocysts in E to I and are *S. cameli*, and J-L, *S. ippeni*. It is difficult to speciate these sarcocysts based on light microscopy. (**A, B**) The largest sarcocyst found, probably *S. cameli* sarcocyst.The villar protrusions (vp) are very thin and barely visible and whitish areas are probably degenerated host tissue between vp. (**C**) Probably *S. ippeni* based on tringular vp. (**D**) Probably *S. cameli*. The sarcocyst wall on the right side appears different than on the left side. (**E**) Note indistinct cyst wall divided by septa. (**F**) *S. cameli*. Note prominant cyst wall. (**G-K**) Sarcocysts with prominant septa. (**L**) *Sarcocystis ippeni* sarcocyst with conical projection (arrowheads). Note pale metrocytes (me) and banana shaped bradyzoites (br).

Transmission electron microscopy

Two structurally distinct sarcocysts were recognized by TEM, variety A, and B in both camels.

*Variety A sarcocyst (*S. cameli*)*

Three sarcocysts were studied, two from camel no. 4 and one from camel no. 5. Sarcocyst #1, 6 were GF cysts. Sarcocyst #2 was from camel no.4 and was deparaffinised. The sarcocyst wall consisted of an outermost parasitophorous vacuolar membrane (pvm) that was lined by an electron dense layer (edl) that was up to 50 nm thick (Fig. 2E,H). The pvm had numerous villar protrusions (vp) at regular intervals (Fig. 2A-D). The host myocyte was degenerated along the vp to a varying degree, giving the impression that vp were apart (Fig. 1A-D). The vp were slender, with a maximum length of 3 μm from the base to the tip, and approximately 0.5 μm width (Fig. 1E). Several microtubules were present from the tip of the villus to the middle of ground substance (gs) layer; the tubules were smooth, were without granules and had fine cross-striations on the surfaces of the tubules. On each villus there were several rows (16 or more) of knob-like projections (pr) of medium electron density. In one cross section of a villar protrusion 11 pr up to 100 nm long, were visible at regular intervals (Fig. 2G), The pr seems to be interconnected (Fig. 2D,G). Electron dense, evenly distributed hair-like structures were seen on vp tips, both in glutaraldehyde and the formalin fixed vp (Fig. 2 E,F). The gs was 0.5 to 1.0 μm thick (Fig. 2A). The deeper part (juxtaposed with bradyzoites) of the gs was smooth and more electron dense than the outer part towards the vp. The microtubules of the vp originated from the outer part of gs; and the base of these tubules was electron lucent. The gs continued in to the interior of sarcocyst as septa and thus the gs at the origin of septa appeared thicker than in other areas.

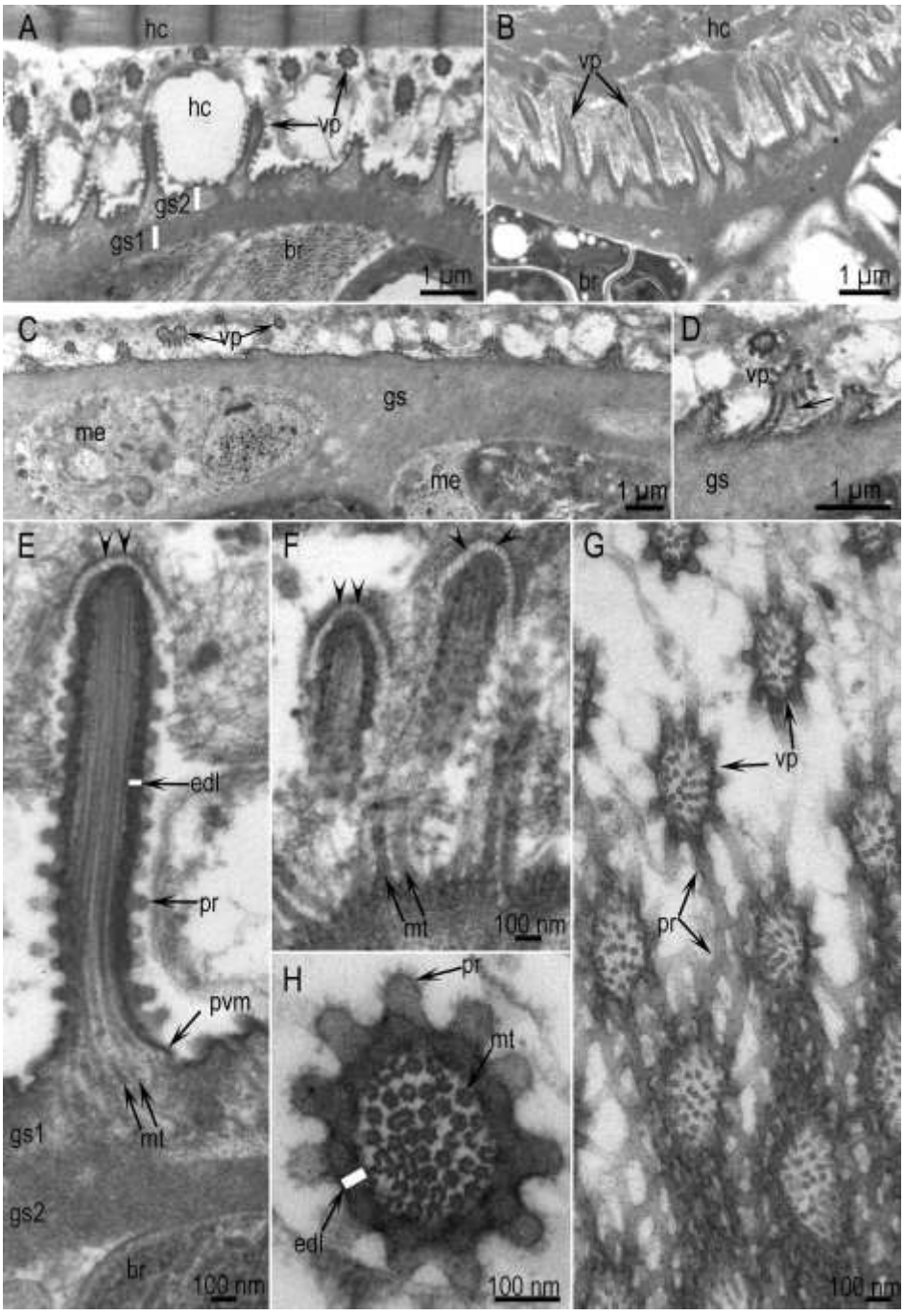

Fig. 2. TEM of *S. cameli* sarcocyst walls. Note parasitophorous vacuolar membrane (pvm) lined by electron dense layer (edl), villar protrusions (vp), ground substance layers (gs1, gs2), protrusions (pr), microtubules (mt), hair- like structures at vp tips (double arrowheads), and host cell (hc). (**A**) The vp are interspersed with vacuolated (degenerated) hc. GF, cyst #1. (**B**) The vp are at regular intervals. FF, cyst #2. (**C**) Note vp cut at an angle, and metrocytes (me). GF, cyst # 6. (**D**) Note projections (arrow) from vp. GF, cyst #6. (**E**) Slender vp with thick edl and electron-lucent pr along the villar length. GF, cyst #1 (**F**) Note hair-like structures at the villar tips (arrowheads) and prominant mt at the base of the vp. FF, cyst #2. (**G**) The vp at the edge of cyst interconnected protrusions (pr). FF,cyst #2. (**H**) Cross section of vp showing 11 pr at the periphery at regular intervals, and numerous internal mt with electron lucent centers. GF, cyst #1.

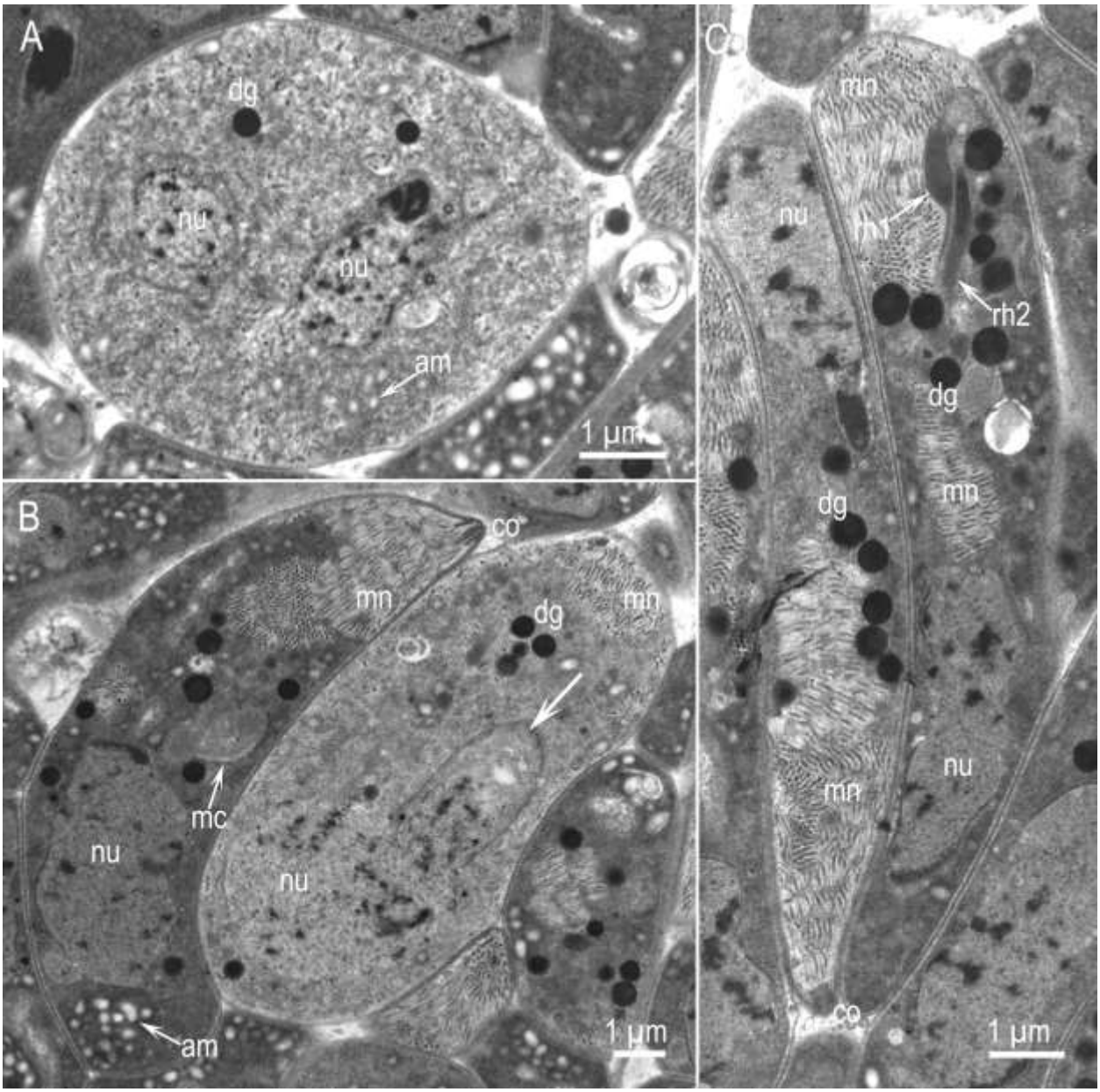

Fig. 3. TEM of *S. cameli* metrocytes and bradyzoites. Note conoid (co), numerous micronemes (mn), several dense granules (dg) of different sizes concentrated in the middle part of the bradyzoite, a nucleus (nu) and rhoptries (rh) with long slender neck. (**A**) An electron lucent metrocyte showing two nuclei, a few amylopectin granules (am), three dense granules (dg) and several micronemes (mn) that are indistinct. GF, cyst #1. (**B**) A longitudinally cut bradyzoite and a metrocyte dividing nucleus and formation apical end of a zoite (arrow). GF, cyst #6. (**C**) Two longitudinally cut bradyzoites with their conoidal ends at opossing ends. GF, cyst #6.

Only a few metrocytes were seen. They were globular to oblong in shape and 6-10 μm long (Fig. 3A,B). They contained 1 or 2 nuclei (nu), endoplasmic reticulum, a few to several amylopectin, few dense granules but no rhoptries (Fig. 3A). Bradyzoites were 12-14 x 2.5-4.0 μm in size. It was difficult to find longitudinally cut bradyzoites (showing the conoid and the posterior end with nucleus) because of their compactness in the sarcocyst (Fig. 3 B,C). The bradyzoites had a double-membraned plasmalemma consisting of an outer membrane (om) and an inner membrane (im), a conoid (co), micronemes (mn), rhoptries (rh), amylopectin (am), dense granules (dg), micropore (mp), a mitochondrion (mc) and a terminal to subterminally located nucleus (Figs. 3,4). The papillary conoid was truncated. Thickening of the plasmalemma was seen in some bradyzoites at the conoidal end (Fig. 4 B). A micropore was seen, 3 μm from the conoidal end (Fig. 4B). Electron dense granular material and few secretory droplets were seen below the micropore (Fig. 4C). Micronemes were numerous and were dispersed throughout the anterior one-third part of the bradyzoite (Fig. 4). Micronemes were approximately 250 x 50 nm in size with tapering or round ends. Most micronemes were arranged in rows, but some were haphazardly arranged at the conoidal end (Fig. 4). Some micronemes were present in the conoid (Fig. 4A). Only two rhoptries were seen in any one plane of section; the blind bulbous end extended up to anterior-third of bradyzoite. Amylopectin granules were numerous and dispersed in throughout the bradyzoite (Fig. 3). The single mc was convoluted (Fig. 3B). The dense granules were 50 to 125 nm in diameter and located mostly in the middle part of bradyzoites (Fig. 3C).

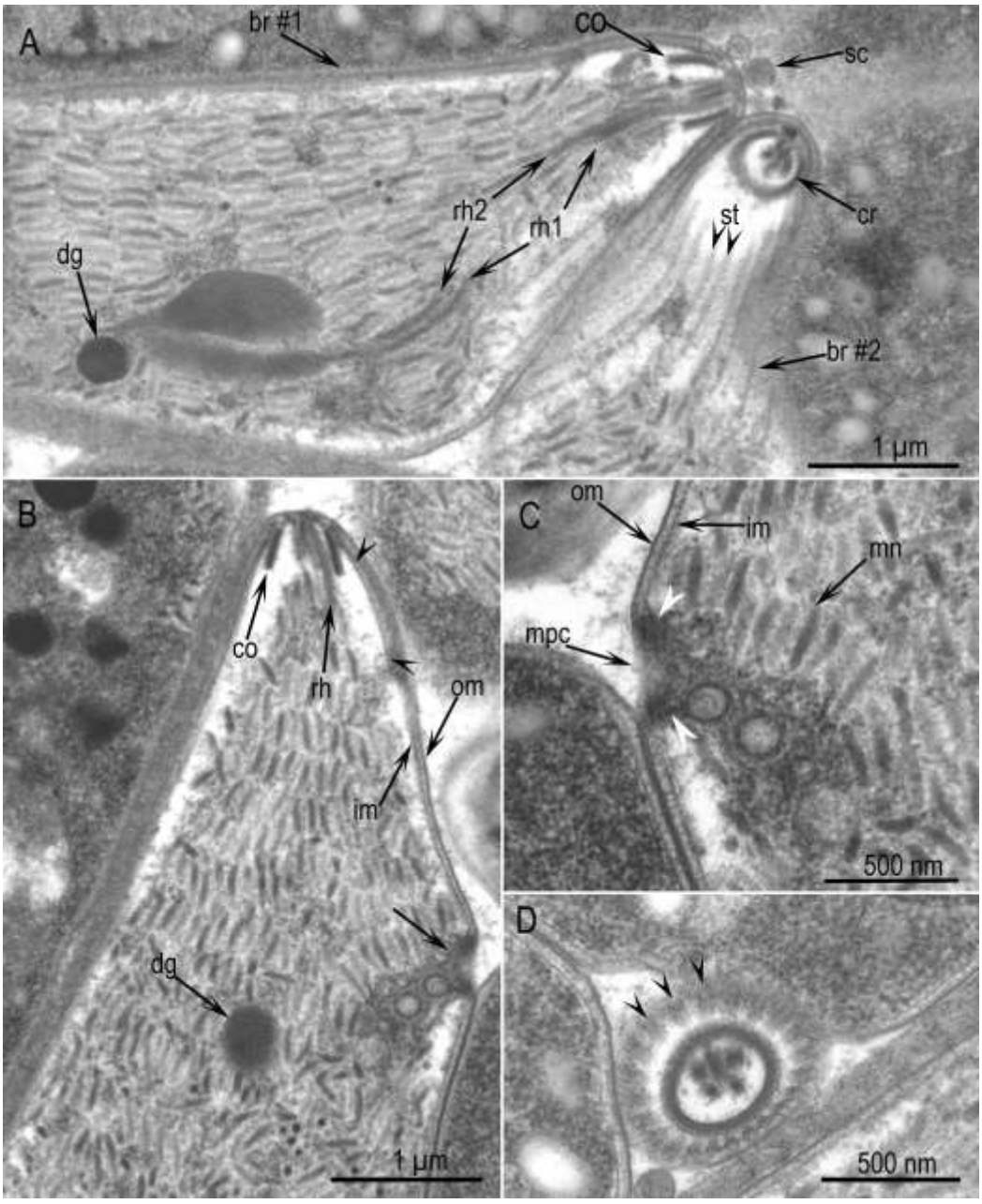

Fig. 4. TEM of conoidal parts of bradyzoites of *S. cameli*. GF, cyst#1. (**A**) Longitudinal section of conoidal part of bradyzoite #1. Note conoid (co) with two droplets of secretions (sc) at the conoidal tip, two rhoptries (rh1, rh2) with bulbous posterior blind ends. Note differences in electron density of dense granule (dg) and rhoptry contents. The micronemes are arranged in rows. Bradyzoite #2 conoidal part is cut obliquely. Note conoidal ring (cr) and subpellicular microtubules (st). (**B**) Conoidal part of a bradyzoite. Note double membraned plasmalemma (om, im), and an extra layer toward the conoid (arrowheads). Note a micropore (arrow) and a dense granule (dg). The micronemes are arranged haphazardly towards the micropore. (**C**) Details of pellicle with outer plasmalemma membrane (om) and inner membrane (im) at the micropore (mpc) junction. The im is interrupted at the micropore opening and collar/rim-like (white arrowheads) structure is present at the opening (mpc). Electron dense secretory material and two droplets surrounds the mp. (**D**) Cross/oblique section through the conoid. Note 22 subpellicular tubules (arrows) originating from the polar ring.

*Variety B (*S. ippeni*)*

Five sarcocysts were studied from both camels (Figs. 5-7). Sarcocysts were 110-120 x 50-100 μm in size. The sarcocyst wall had vp that were often conical in shape (Figs. 5, 6). The gs was approximately 1 μm thick, and smooth. The vp were at regular intervals. The vp were approximately 1.0-1.2 μm wide at the base, approximately 1 μm long with a blunt tip. The distal 0.25 μm tip was electron dense. Each villus had microtubules that originated mid of the gs layer. The mt were smooth and some were criss-crossed at the base (Fig. 6). The total width of the cyst wall from the tip of the vp to the base of gs was 2.3-3.0 μm. The gs towards the bradyzoites was more electron dense than the gs towards the vp (Fig. 5). Within the same sarcocyst some vp were not conical and more finger-like, and some were stubby (Fig. 5). Some vp also had hair-like structures at the tips and sides (Fig. 6B). Cross-section of vp showed tubules with an electron-dense core. Metrocytes were oval to spindle shaped and contained very few organelles other than nucleus (Fig. 5). Bradyzoites were 12-13.5 x 2.0-3.0 μm in size (Fig. 7). They contained two rhoptries (Fig. 7B), numerous micronemes, one long mitochondion, and subterminal nucleus (Fig. 7). The micronemes were up to 300 nm long and located in the one-third conoidal part of bradyzoites. The micropore was 300 x 540 nm in size and surrounded by electron dense material (Fig. 7C). Numerous amylopectin granules (am) were concentrated in the posterior half of the bradyzoite (Fig. 7A).

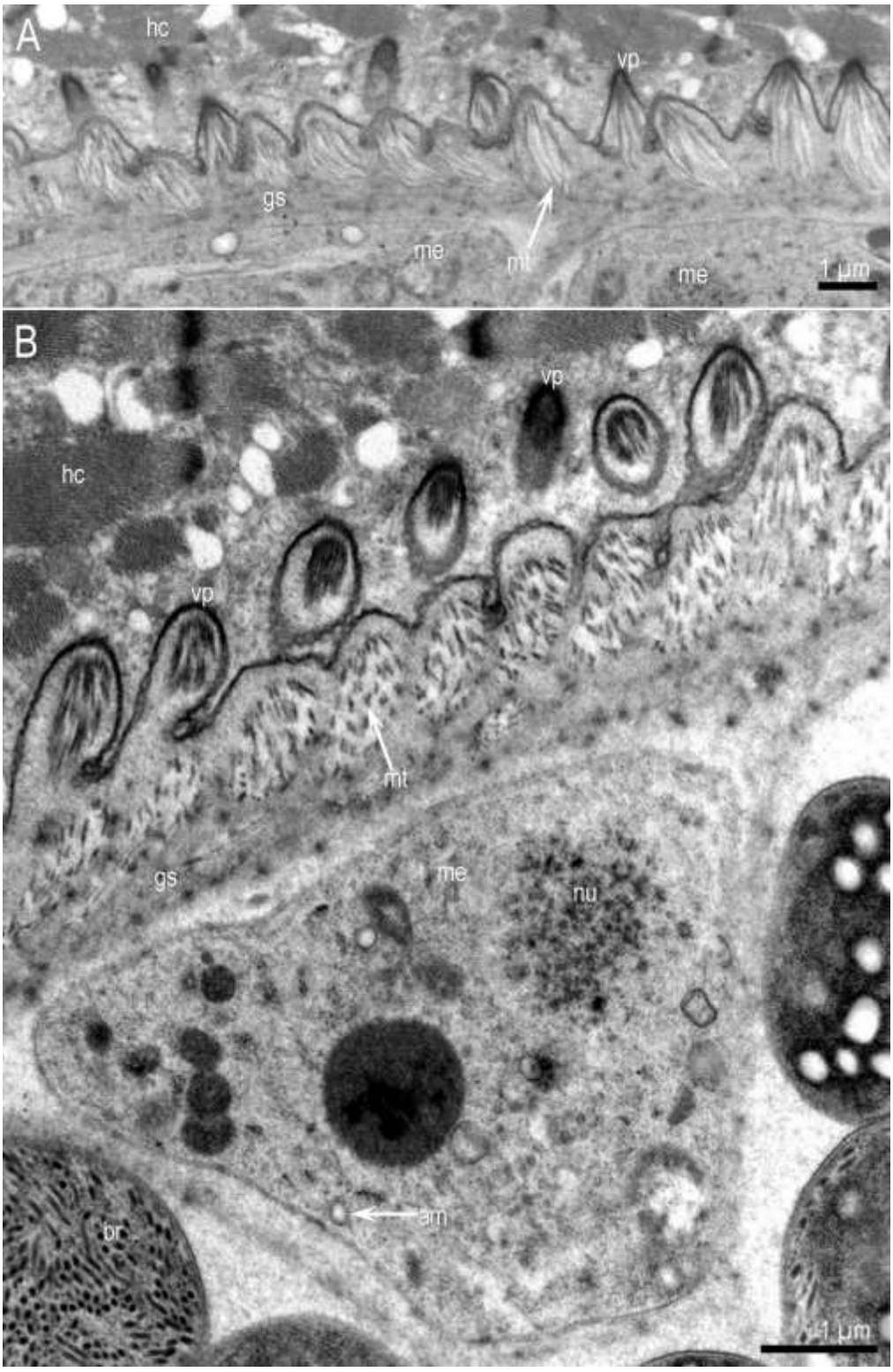

Fig. 5. TEM of *S. ippeni* sarcocyst walls. GF, cyst #7. Note the vp are cut at different angles. The ground substance layer (gs) is mostly electron lucent and not well demarcated. The microtubules in vp are more electron dence towards the villar tips. (**A**) Note villar protrusions (vp) cut at different angles. (**B**) A meterocyte below indistinct ground substance layer.

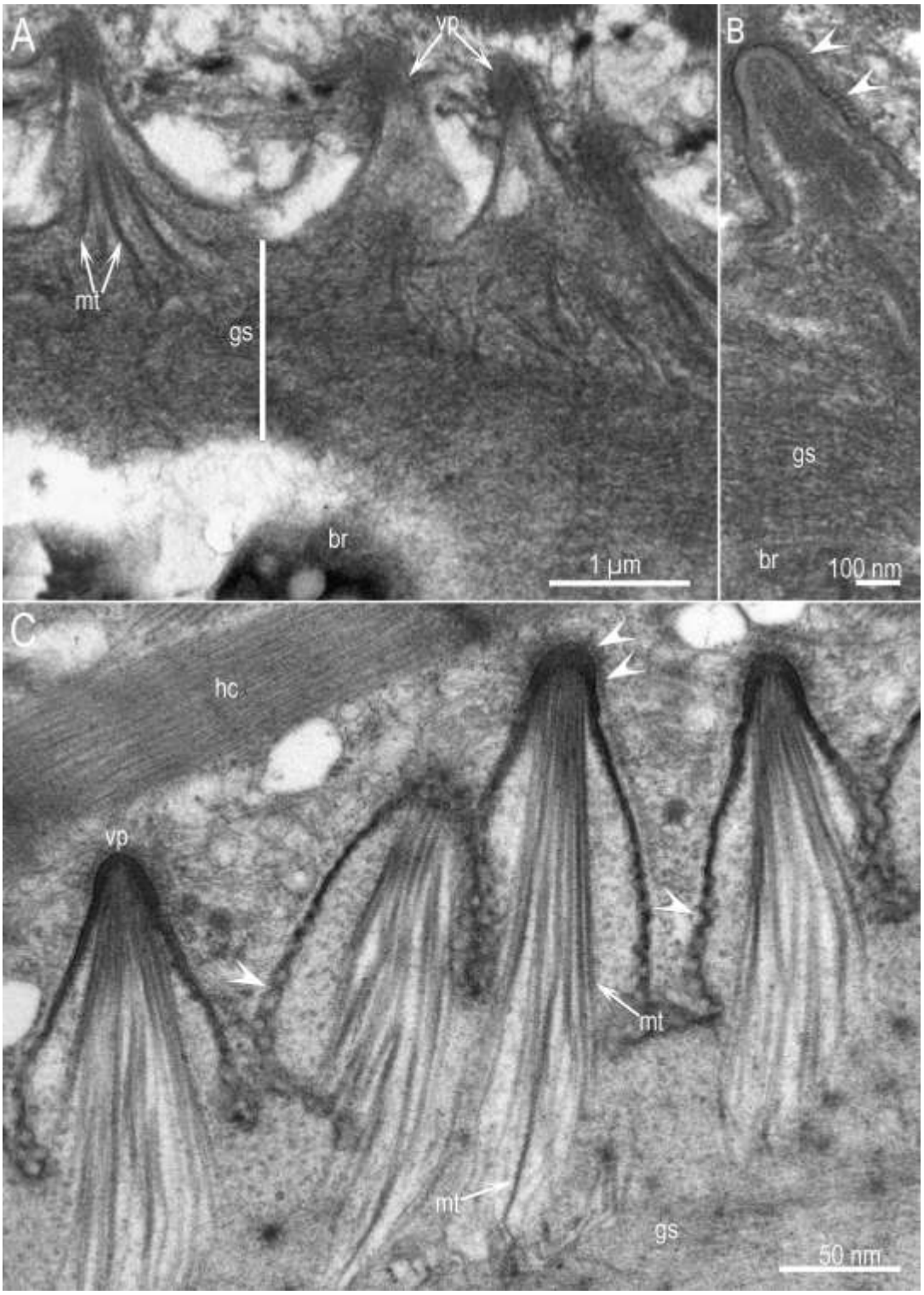

Fig. 6. Details of conical vp from two sarcocysts of *S. ippeni*. (**A**) Note criss-crossing microtubules (mt) and knob–like thickening of the vp. FF, cyst #3 (**B**) Details of part of the vp with a blunt tip. Arrowheads point to hair-like structures on the villar tip and sides. FF, cyst#3.-(**C**)**.** Note variable thickeness of the electron dense layer (edl). The edl is thicker at the villar tips and thinned at the base of villi (arrowheads). The microtules are of various density, smooth and without granules. GF, cyst # 7.

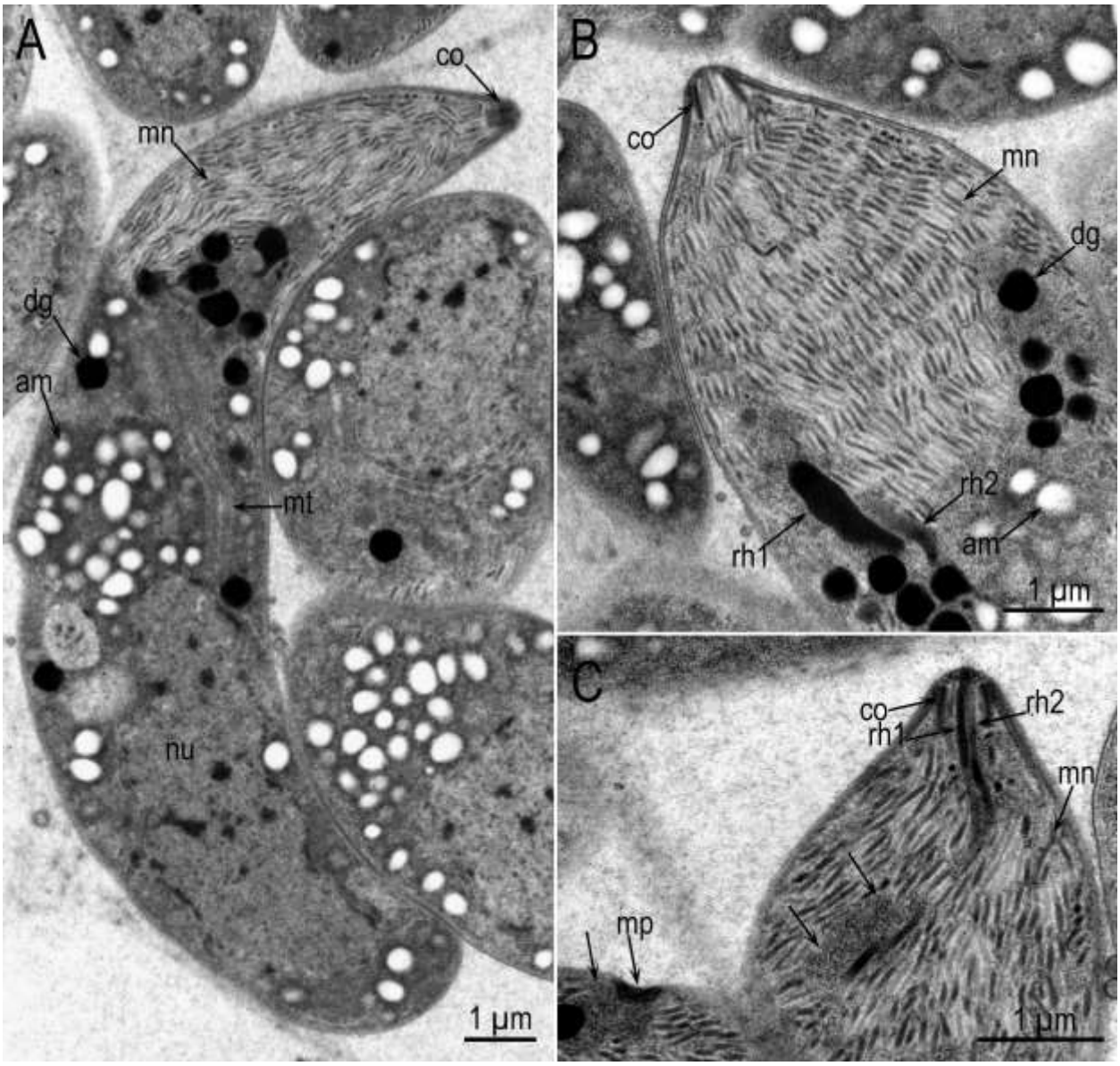

Fig. 7. TEM of bradyzoites of *S. ippeni*. GF, cyst #7. Note conoid (co), numerous micronemes (mn), 2 rhoptries (rh1, rh2), a convoluted mitochondrion (mc), amylopectin granules (am), and a nucleus (nu). (**A**) Longitudinally cut bradyzoite with elongated nucleus. (**B**) Coinoidal part. Note electron dense contents of rhoptries, and dense granules. (**C**) Conoidal part of a bradyzoite showing two rhoptries opening in conoid. Also note micropore (mp) of another bradyzoite. Dense flocular material surrounds the micropore.

Specimens deposited

Voucher specimens of histological sections stained with Toluidine blue and H and E from camels 4 and 5 are deposited in the United States National Parasite Collection in the Division of

Invertebrate Zoology and National Museum of Natural History, Smithsonian Institution, Washington, D.C. under (USNM-)

DISCUSSION

From the review of literature and the findings presented here, it is clear that there are two structurally distinct *Sarcocystis* species in the one-humped camel. Before the discovery of the life cycle of *Sarcocystis* in 1972, *Sarcocystis* species were often named for the host species and often only one species was thought to parasitize a given host. Heydorn *et al*. (1975) conclusively showed that more than one structurally distinct species may exist in each host. They proposed new names for *Sarcocystis* species based on the intermediate host and the definitive host (e.g. *Sarcocystis bovicanis* for the species with cattle and dog as intermediate hosts). They suggested to replace old names with new names because the original descriptions were inadequate, and no type specimens were available (Dubey *et al.* 1989). Their application to the International Code of Zoological Nomenclature was rejected and with a view «A name is or remains available even though it is found that the original description relates to more than one taxonomic unit. The species must be simply redescribed» (Levine, 1977).

This scenario is now applicable to *Sarcocystis* species in camel. There are no type specimens deposited for any *Sarcocystis* species in camel. Mason (1910) who first reported *Sarcocystis* in camel did not describe the parasite adequately and the name *S. cameli* that he proposed was only briefly mentioned in the discussion. This name was largely ignored until Dubey *et al.* (1989) arbitrarily assigned one sarcocyst species to be named *S. cameli*; Abdel-Ghaffar *et al.* (1979) had reported unique structure of this parasite but they did not name it. Odening (1997) proposed a new name, *S. ippeni*, for the parasite that Entzeroth *et al.* (1981) had

described. Abdel-Ghaffar *et al.* (2009) ignored all previously assigned names and called the parasite they studied as *S. camelicanis*, continuing with the earlier philosophy of Heydorn *et al.* (1975). An additional problem with the description of the sarcocysts was that there was no correlation of description by LM and TEM, and specimens are not available for verification. We have now fulfilled this vacuum and properly described the two *Sarcocystis* species, and deposited specimens in a museum available to all scientists.

Taxonomic summary

In the present study, *S. camelicanis* is synonymized with *S. cameli*. The names *S. camelocanis* and *S. miescheri* are declared invalid because of the inadequate description or erroneous identification of sporocysts, and without description of sarcocysts. Two species *S. cameli* and *S. ippeni* are redescribed.

The taxonomical position is summarized below :

***Sarcocystis cameli* (Mason, 1910) amended Dubey, Hilali, Van Wilpe, Calero-Bernal, Verma, and Abbas**

(Syn. *S. camelicanis* Abdel-Ghaffar, Mehlhorn, Bashtar, Al-Rasheid, Sakran, and Fayoumi, 2009)

Diagnosis: Sarcocysts microscopic, appear thin walled by LM. By TEM sarcocyst wall has unique villar protrusions (vp), type 9j (Dubey *et al*. 2015), these are upright, slender, up to 3.0 μm long and 0.5 μm wide, with knob-like protrusions that appeared to be interconnected in a mesh-like structure, microtubules in vp are smooth, originate at midpoint of the gs and continue

up to the tip. Total thickness of the sarcocyst wall with ground substance layer (gs) 3.5 μm. Bradyzoites were approximately 14-15 x 3-4 μm in size. Dog is most likely definitive host.

***Sarcocystis ippeni* (Odening, 1997) amended Dubey, Hilali, Van Wilpe, Calero-Bernal, Verma, and Abbas**

Diagnosis: Sarcocysts microscopic, appearing thin walled by LM. By TEM sarcocyst wall has unique type 32 (Dubey *et al*. 2015) conical villar protrusions (vp) with an electron dense knob. The vp approximately 1.0 μm long, 1.2 μm wide at the base and 0.25 μm at the tip, microtubules in vp originate at midpoint of gs and continue up to tip, criss-crossed, smooth and without granules or dense areas. The total thickness of the sarcocyst wall (from the base of gs to vp tip) was 2.3-3.0 μm. Bradyzoites 12.0-13.5 x 2.0-3.0 μm in size.

The status of thick walled and macroscopic sarcocysts in camels needs further investigation. Nothing is known of the *Sarcocystis* infection in bactrian camel (*Camelus bactrianus*).

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