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Identification of Macroscopic Sarcocysts of *Sarcocystis cameli* from One-Humped Camel (*Camelus dromedarius*) in Iraq

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ABSTRACT: There is considerable confusion concerning the identity of macroscopic *Sarcocystis* species in camels. Currently 2 species, *Sarcocystis cameli* and *Sarcocystis ippeni*, are recognized from 1-humped camel (*Camelus dromedarius*), and sarcocysts of both species are microscopic. Here, we report the identity of macroscopic sarcocysts from the *C. dromedarius* in Iraq as *S. cameli*. Five sarcocysts from the muscle of 2 adult camels collected in 1999 and stored in 10% formalin were studied by transmission electron microscopy (TEM). Sarcocysts were 1.5–5.0 mm long and 200–400 μm wide. By TEM, all 5 sarcocysts had thin sarcocyst walls. Ultrastructurally, the sarcocyst wall had "type 9j" villar protrusions similar to those of *S. cameli*. This is the first confirmation of macroscopic sarcocysts from 1-humped camel as *S. cameli*.

Camels are economically important for several countries, especially those in Asia and the Middle East. A recent review of *Sarcocystis* infections in camels concluded that there are 2 species of *Sarcocystis* in camels, *Sarcocystis cameli* Mason, 1910, and *Sarcocystis ippeni* Odening, 1997, both amended by Dubey et al. (2015). There was no morphologic description of *S. cameli* named by Mason (1910), but he noted macroscopic sarcocysts in camel meat. There was also considerable confusion concerning the identity of *S. ippeni*. Therefore both *S. cameli* and *S. ippeni* were redescribed based on specimens from Egypt and their names amended (Dubey et al., 2015). In this redescription the sarcocysts of both species were microscopic. There are no archived specimens from the reports of Mason (1910) and Odening (1997).

Worldwide reports of *Sarcocystis* spp. from camels, including reports from Afghanistan, Egypt, Ethiopia, India, Iran, Jordan, Mongolia, Saudi Arabia, Somalia, Sudan, and the former USSR, were recently reviewed (Dubey et al., 2015, 2016). There is only 1 report of *Sarcocystis* sp. from Iraq (Latif et al., 1999). *Sarcocystis*-like bradyzoites were detected in pepsin digests of muscle from 33 of 36 (91.6%) camels; the identity of *Sarcocystis* species was not determined. Here we report definitive identification of macroscopic sarcocysts from camels in Iraq as *Sarcocystis cameli*.

Esophageal tissues were collected from 2 adult camels (*Camelus dromedarius*) in 1999 from an abattoir in Iraq. Tissues were fixed and stored in 10% formalin. In 2016, these sarcocysts were transported from Iraq to Beltsville Agricultural Research Center, U.S. Department of Agriculture (USDA), Beltsville, Maryland, under an Animal Plant Inspection Service (APHIS), USDA import permit.

For TEM, formalin-fixed pieces were refixed for 2 hr at room temperature in 2.5% glutaraldehyde, 0.05 M NaCacodylate, 0.005 M $CaCl_2$ (pH 7.0), then refrigerated at 4 C overnight, rinsed 6 times with 0.05 M NaCacodylate, 0.005 M $CaCl_2$ buffer, and post-fixed in 1% buffered osmium tetroxide for 2 hr at room temperature. The tissue was then rinsed 6 times in the same buffer, dehydrated in a graded ethanol series followed by 2 exchanges of propylene oxide, infiltrated in a graded series of LX-112 resin/propylene oxide, and polymerized in LX-112 resin at 65 C for 24 hr. Then 60–90 nm silver-gold sections were cut on a Reichert/AO Ultracut ultramicrotome (Leica Microsystems, Wetzlar, Germany) with a Diatome diamond knife and mounted onto 200 mesh formvar-coated copper grids and slot-grids. Grids were stained with 4% uranyl acetate and 3% lead citrate and imaged at 80 kV with a Hitachi HT-7700 transmission electron microscope (Hitachi High Technologies America Inc., Dallas, Texas).

Sarcocysts were 1.5–5.0 mm long and 200–400 μm wide. By TEM, the sarcocyst wall was smooth, <1 μm thick, and without any visible protrusions (Fig. 1). The sarcocyst wall consisted of an outermost parasitophorous vacuolar membrane (pvm) that was lined by an electron dense layer that was up to 50 nm thick (Fig. 2). The pvm had numerous villar protrusions (vp), approximately 2.5 μm apart from each other (Fig. 2). The host myocyte was degenerated along the vp to a varying degree, giving the impression that vp were apart (Fig. 2). The vp were slender, approximately 2.5 μm long from the base to the tip (Fig. 2). 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, but had fine cross-striations. On each

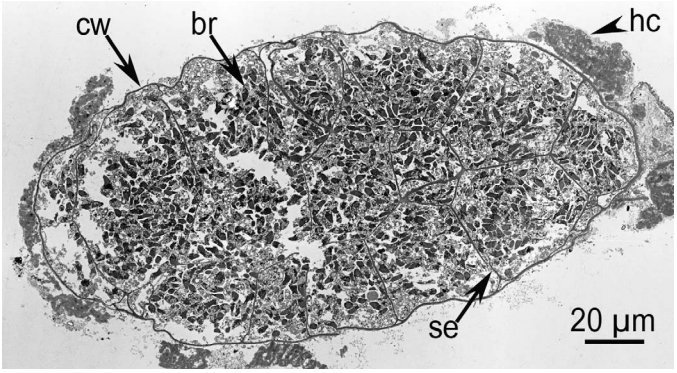

FIGURE 1. TEM section of a *Sarcocystis cameli* sarcocyst from a camel. Note thin smooth cyst wall (cw) without projections, prominent septa (se), and bradyzoites (br) in compartments. Note host cell (hc).

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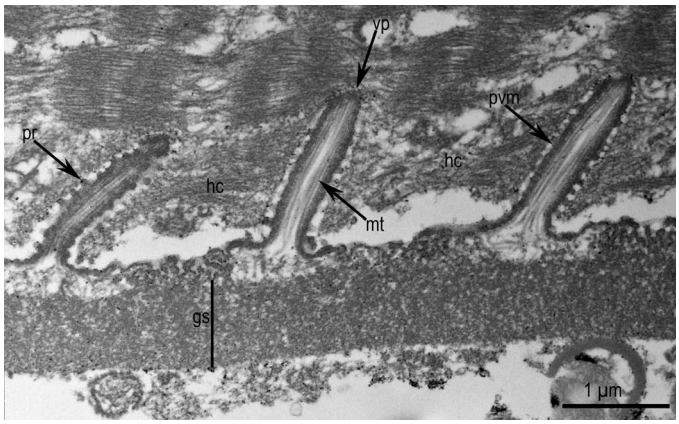

FIGURE 2. TEM of *Sarcocystis cameli* sarcocyst wall. Note parasitophorous vacuolar membrane (pvm), villar protrusions (vp), ground substance layer (gs), projections (pr), microtubules (mt), and host cell (hc). The vp are interspersed with vacuolated (degenerated) hc.

villus there were several rows of knob-like projections (pr). The gs was 0.5–1.0 µm thick (Fig. 2). The gs continued into the interior of sarcocyst as septa, and thus the gs at the origin of septa appeared thicker than in other areas. Bradyzoites were 12–14 µm long. Although most bradyzoites were poorly preserved, enormous numbers of micronemes, rhoptries, conoids, amylopectin granules, and nuclei were visible.

Sarcocysts in the present study were identified as *S. cameli* and not *S. ippeni*, based on their structure (Dubey et al., 2016). *Sarcocystis cameli* has characteristic villar protrusions of "type 9j" (Dubey et al., 2016). The villar protrusions is one of the criterion of speciation of *Sarcocystis* species within a given host (Dubey et al., 2016). The structure of microscopic and macroscopic sarcocysts of *S. cameli* is the same. *Sarcocystis ippeni* has unique "type 32" vp with electron dense knobs and microtubules from villar tips to the middle of the gs layer. The life cycles of both *Sarcocystis* spp. are unknown. All evidence currently available indicates that domestic dogs are the definitive hosts for both species of *Sarcocystis* in camels. Further studies are needed to molecularly characterize sarcocysts of camels.

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