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# PREVALENCE AND ULTRASTRUCTURE OF THREE TYPES OF *SARCOCYSTIS* IN MULE DEER, *ODOCOILEUS HEMIONUS* (RAFINESQUE), IN MONTANA

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ABSTRACT: Infection with *Sarcocystis* (Protozoa: Sarcocystidae) was diagnosed in 130 of 153 (85%) samples of muscle from mule deer around Bozeman, Montana. Three structurally distinct mature and microscopic sarcocysts with characteristic cyst walls were found. Cyst walls of type I sarcocysts were about 2 μm thick and had characteristic inverted tee-shaped villar projections; these cysts were considered to be *S. hemionilatrantis* Hudkins and Kistner, 1976. Cyst walls in type II sarcocysts were thick-walled (about 7 μm) and their villar projections were 6.7 × 1.1 μm. The core of the villar projections consisted of granular material and some filamentous structures. Bradyzoites were 11.6 × 2.8 μm and were tightly packed in compartments. Cyst walls of type III sarcocysts were also thick-walled (about 9 μm) but the villar projections were 8.5 × 4.7 μm. Bradyzoites were 13 × 3.3 μm and were loosely arranged in compartments.

## INTRODUCTION

Infections with cysts of species of *Sarcocystis* are common in many species of domestic and wild ruminants including mule deer. Most ruminants harbor more than one species of *Sarcocystis*, but only one species is thought to be present in mule deer (Dubey and Fayer, 1983). Ultrastructure of sarcocysts is one of several criteria that are useful in distinguishing among species of *Sarcocystis* within a single species of intermediate host (Mehlhorn et al., 1976). We report herein the prevalence of *Sarcocystis* spp. and describe the ultrastructural characteristics of three types of sarcocysts in the muscles of naturally infected mule deer in Montana.

## MATERIALS AND METHODS

From October 1978 to May 1982, samples of muscles (mostly diaphragm and abdominal muscle) from 153 mule deer were examined for *Sarcocystis* cysts at the Veterinary Research Laboratory (VRL), Bozeman, Montana. The deer were either road kills or shot legally by hunters around Bozeman. During the initial part of the survey, samples from 90 mule deer were examined by the pepsin digestion technique (Sharma and Dubey, 1981). During the latter part of the study, samples from 63 deer were examined by direct microscopic examination for cysts of *Sarcocystis*; six to 10 squash preparations of muscles from each mule deer were screened at 100× magnification. For histologic study, samples from four deer were fixed in Millonig's phosphate buffered 10% formalin. Of these four deer, Deer No. 1 came from an unknown source and age and sex were also not known. Deer No. 2 was an 18-mo-old buck and Deer No. 3 was a 12-mo-old doe; they were road kills found 16 km west of Bozeman. Deer No. 4 was a 4-yr-old buck shot at the National Bison Range (NBR), Moiese, Montana, and its esophagus had been refrigerated at 4 C for 24 hr before fixing in a mixture of 1% glutaraldehyde and 4% formaldehyde (GF). Selected muscles from Deer Nos. 1 and 3 were also embedded in glycol methacrylate and sectioned at 1 to 3 μm. Paraffin embedded tissues were cut at 5 μm. Sections were examined after staining with hematoxylin and eosin (H&E), periodic acid–Schiff hematoxylin (PASH), or Giemsa.

For ultrastructural study, skeletal muscle of Deer No. 3 fixed in formalin and esophagus of Deer No. 4 fixed in (GF) were processed for transmission electron microscopy and examined in a JEOL 100CX electron microscope. An additional eight mule deer bucks (Nos. 5–12) of unknown age from the NBR were examined for sarcocysts. Pieces of esophagus, diaphragm and skeletal muscle were excised, fixed in 2.5% glutaraldehyde in cacodylate buffer, rinsed in

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buffer, post-fixed in osmium tetroxide, rinsed in buffer, partially dehydrated in ethanol, prestained with 1% phosphotungstic acid and 1% uranyl acetate in 70% ethanol, completely dehydrated in ethanol and embedded in Spurr's low viscosity embedding medium. Thin sections and 1-μm-thick sections were cut with glass knives. Thick sections were mounted on microscope slides, stained with toluidine blue and examined by light microscopy. Thin sections were stained with lead citrate and examined with a Zeiss EM 9S-2 transmission electron microscope.

## RESULTS

*Sarcocystis* spp. infection was diagnosed in 130 of 153 (85%) deer around Bozeman; bradyzoites of undetermined species were seen in digests of 80 of 90 samples and sarcocysts of undetermined species were seen in muscle squashes of 50 of 63 samples.

Three structurally distinct mature sarcocysts were found. The sarcocysts were distinguished by the structure of their walls.

### Type I sarcocysts: *Sarcocystis hemionilatrantis* Hudkins and Kistner, 1976

Type I sarcocysts had characteristic inverted tee appearance and were considered to be *S. hemionilatrantis* based upon the light microscopic structure described by Dubey et al. (1983). Types II and III sarcocysts were thick-walled, and were distinguishable ultrastructurally. Sarcocysts of *S. hemionilatrantis* were found in all nine mule deer obtained from the NBR. Three of the mule deer from NBR also had type III sarcocysts.

More details of *S. hemionilatrantis* sarcocysts were visible in 1-μm sections of epoxy-embedded material stained with toluidine blue and photographed with phase-contrast microscopy (Fig. 1) than in 5-μm paraffin-embedded sections stained with H&E and photographed with bright-

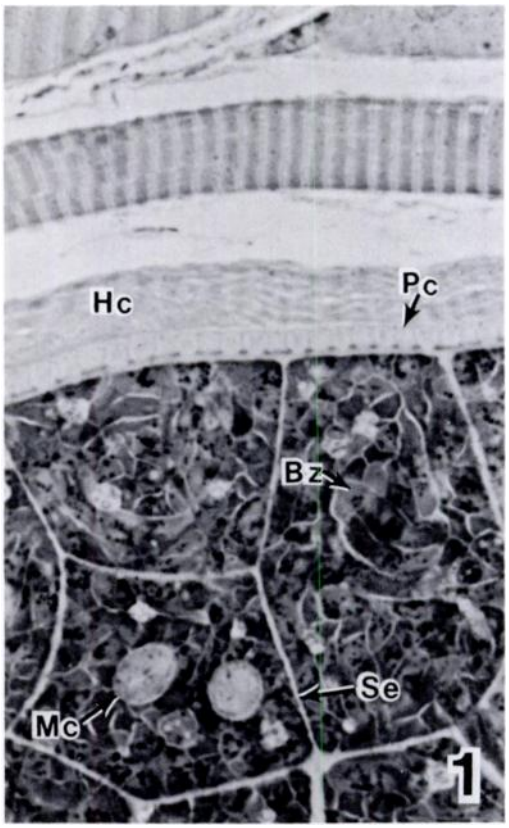


FIGURE 1. Photomicrograph of portion of a sarcocyst (type I) of *S. hemionilatrantis* in mule deer diaphragm. Abbreviations: Bz, bradyzoite; Hc, host cell cytoplasm; Mc, metrocyte; Pc, primary cyst wall; Se, septum. ×1,250.

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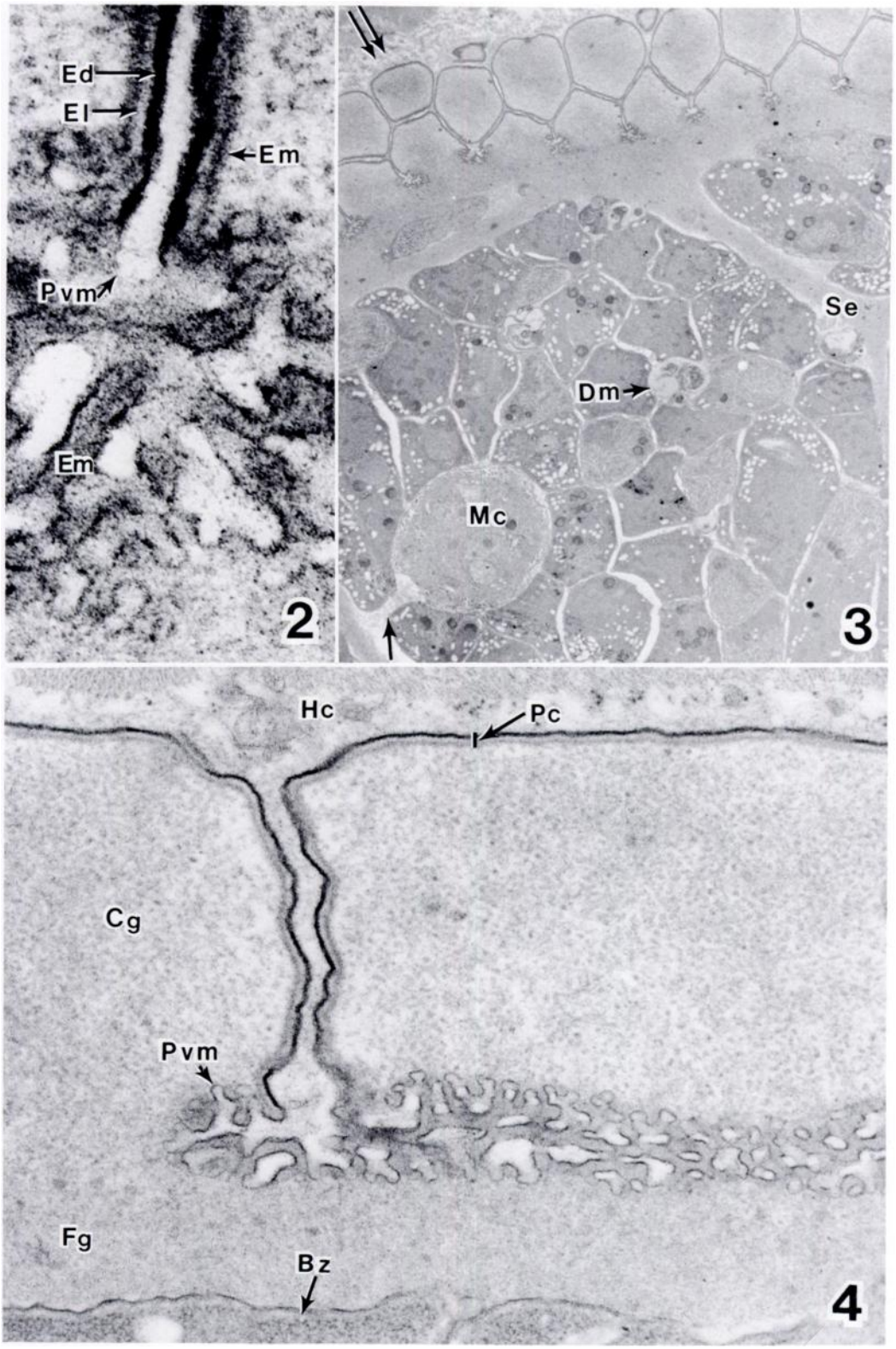
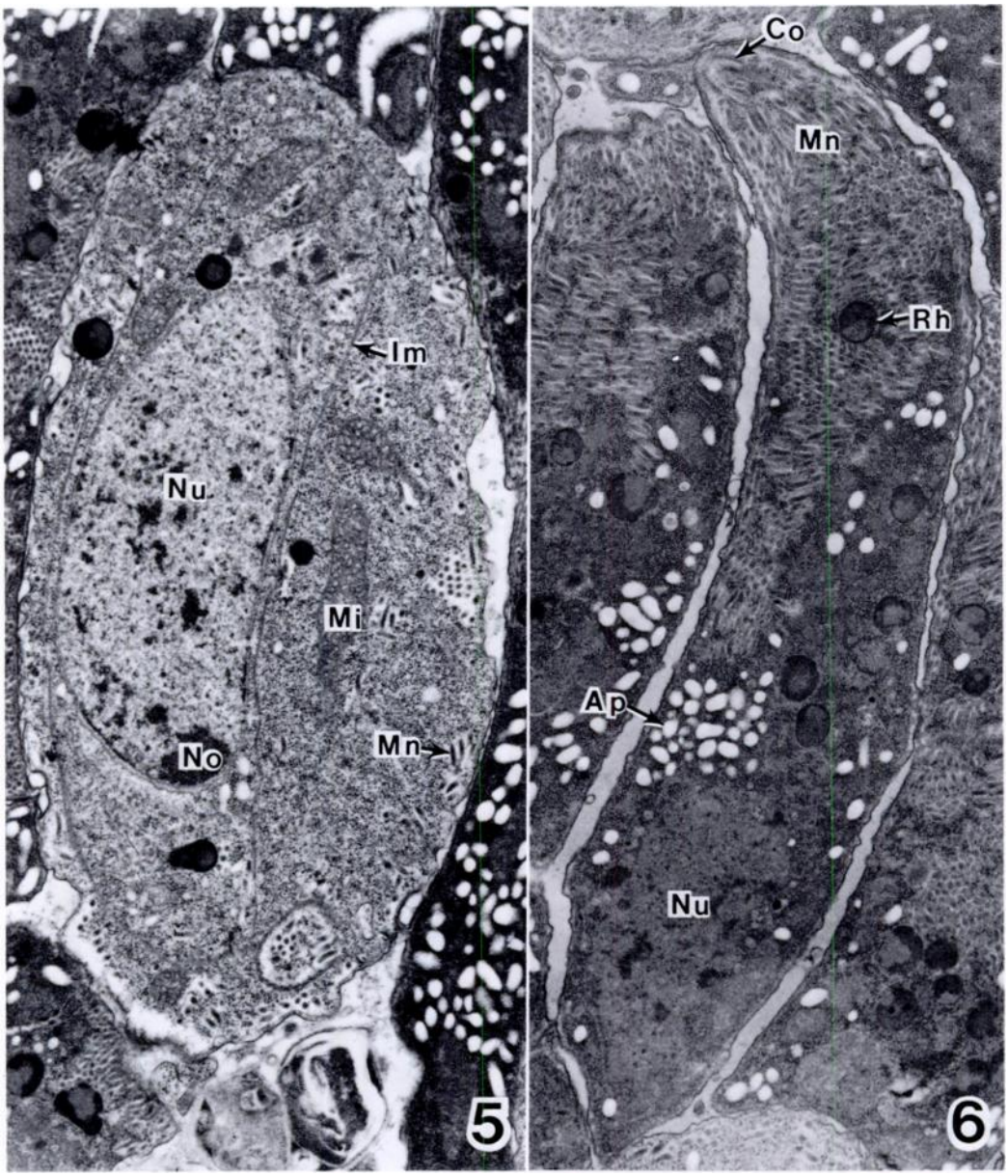
FIGURES 2–4. Transmission electron micrographs of type I sarcocysts of *S. hemionilatrantis* in mule deer diaphragm. Abbreviations: Bz, bradyzoite; Cg, coarsely granular core of villar projections; Dm, degenerate material; Ed, electron-dense layer of primary cyst wall; El, electron-lucent layer of primary cyst wall; Em, moderately electron-dense layer of primary cyst wall; Fg, finely granular layer; Mc, metrocyte; Pc, primary cyst wall; Pvm, parasitophorous vacuolar membrane. 2. High magnification of base of villar projection. ×100,000. 3. Tangential section through margin of sarcocyst showing honeycomb appearance of villar projections; note connection (arrow) between septa (Se) and metrocyte (Mc). ×4,565. 4. Margin of sarcocyst; note the coarsely granular core (Cg) of the villar projections and the finely granular layer (Fg) between the bradyzoites (Bz) and the villar projections. ×37,140.






FIGURES 5, 6. Transmission electron micrographs of *S. hemionilatrantis* from mule deer esophagus. Abbreviations: Ap, amylopectin; Co, conoid; Im, inner membrane of developing zoite; Mi, mitochondrion; Mn, microneme; No, nucleolus; Nu, nucleus; Rh, rhoptry-like organelle. 5. Metrocyte; note nucleus and inner membrane of developing zoite within metrocyte. ×16,250. 6. Bradyzoite. ×16,175.

field microscopy. In 1-$\mu$m sections, the cyst-wall had inverted tee-like projections, whereas in 5-$\mu$m-thick sections the cyst wall appeared to be cross-striated. Ultrastructurally, the primary cyst wall was 50.9 nm thick (40.7–61.5 nm; $n = 23$) and consisted of an outermost unit membrane (the parasitophorous vacuolar membrane,

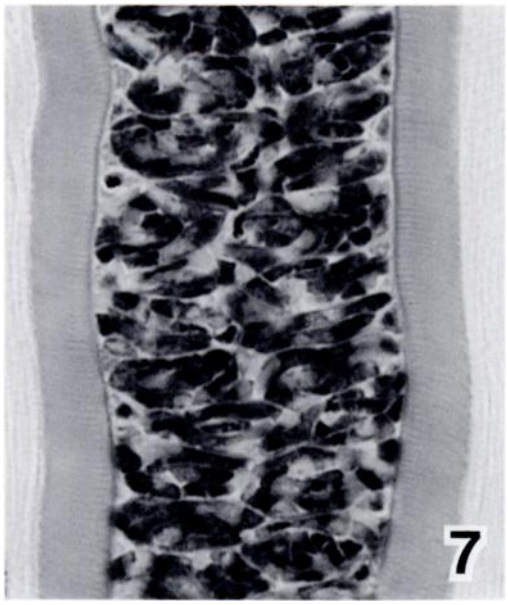


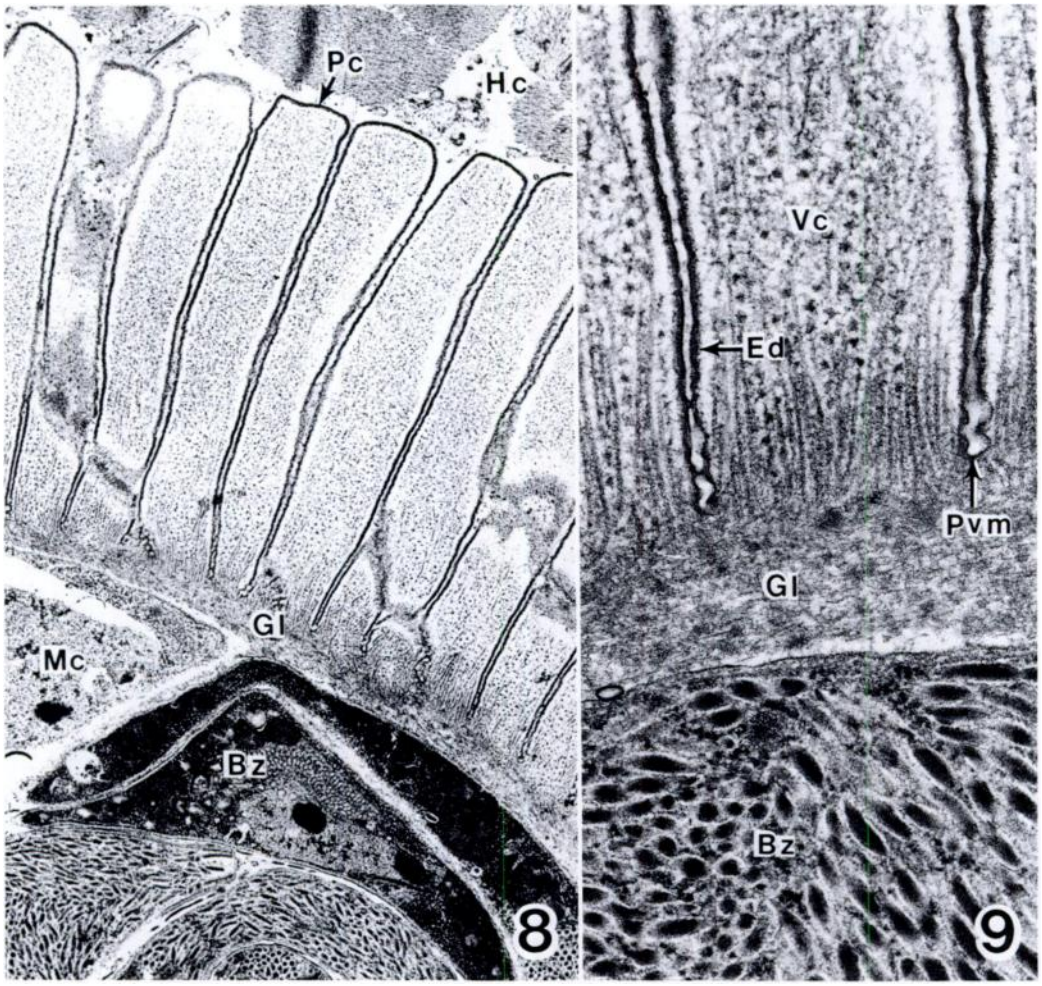

FIGURE 7. Photomicrograph of a type II sarcocyst in mule deer esophagus. ×610.

PVM, 3.5 nm thick), an electron-dense layer (16.1 nm thick; 15.3–17.8 nm; $n$ = 20), an electron-lucent layer (15.7 nm thick; 12.7–17.8 mn; $n$ = 23) and a moderately electron-dense, innermost layer (19.6 nm thick; 12.7–22.9 nm; $n$ = 23) (Fig. 2). The primary cyst wall was folded into villar projections, the margins and bases of which appeared as inverted tees (Fig. 4). The villar projections were 1.92 μm (1.78–2.42 μm; $n$ = 14) long and 2.65 μm (1.95–2.78 μm; $n$ = 14) wide. Tangential sections of the cyst wall exhibited a honeycomb-like appearance (Fig. 3). The electron-dense and electron-lucent layers of the primary cyst wall terminated at the bases of the villar projections where the moderately electron-dense layer was situated adjacent to the PVM, which was highly convoluted and branched (Figs. 2, 4). The core of the villar projections consisted of a coarsely granular material situated between the bases of the villar projections and the sarcocyst zoites (Fig. 4). The fine granular layer was continuous with the septa (Fig. 3) that traversed the sarcocyst completely. Serial sections showed that the septa branched to each metrocyte (Fig. 3). Septa, which were 483.8 nm thick (419.3–548.3 nm; $n$ = 18), separated the sarcocyst into compartments that contained closely packed bradyzoites which were 14.4 × 2.8 μm (13.3–15.8 × 2.6–3.2 μm; $n$ = 15), metrocytes which were 7.4 × 5.3 μm (5.8–11 × 4.3–6.4 μm; $n$ = 12) and spheroid masses of degenerate material (Figs. 3, 5, 6). Usually, the most centrally located compartments contained only degenerate material and degenerate zoites. Metrocytes were usually most numerous near the margins of compartments and near the periphery of the sarcocyst (Fig. 3).

Cytopathologic effects (CPE) were restricted to the host cells harboring sarcocysts. The host cell nucleus was pyknotic and the myofilaments were disrupted near the PVM (Figs. 3, 4). No cellular infiltration or other tissue changes were evident in tissue close to infected cells.

#### Type II sarcocysts

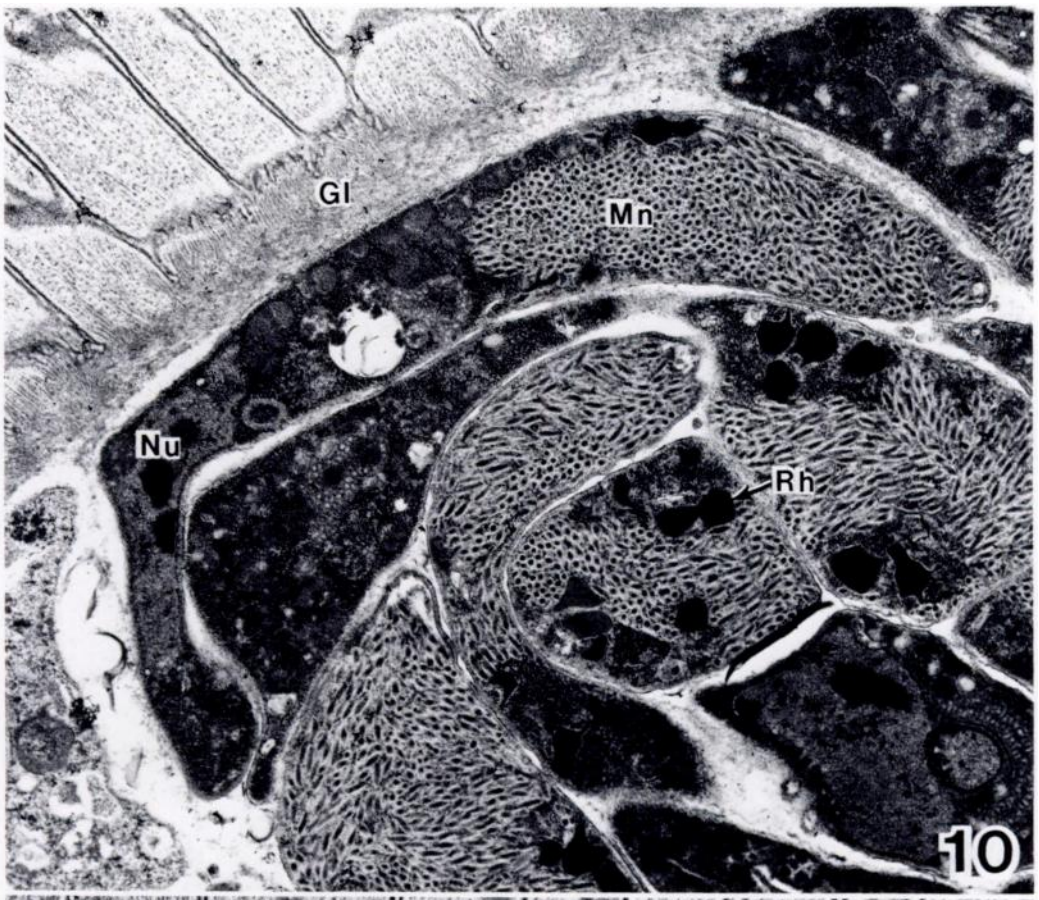
The description is from Deer No. 3. Histologically, sarcocysts were 220 × 60 μm (65–400 × 45–80 μm; $n$ = 18) (Fig. 7). The wall was 7.4 μm (5.5–8.5 μm; $n$ = 15) thick. Two sarcocysts from limb muscles were examined ultrastructurally. The primary cyst wall was 4.6 nm thick (2.6–5.7 nm; $n$ = 15) and consisted of a PVM (1.3 nm thick) and an electron-dense layer (3.3 nm thick; 1.3–4.4 nm; $n$ = 15) which was situated immediately beneath the PVM (Fig. 10). The primary cyst wall was folded into villar projections (Fig. 8) which were 6.7 μm (6.2–7.1 μm; $n$ = 7) long and 1.1 μm (0.7–1.3 μm; $n$ = 12) wide. The core of the villar projections consisted of a coarsely granular material plus some filamentous structures that were present at the bases of the projections (Figs. 8, 9). A finely granular and filamentous layer 60.3 nm thick (55.6–66.7 nm; $n$ = 12) was situated between the sarcocyst zoites and the bases of the villar projections (Figs. 8–11).

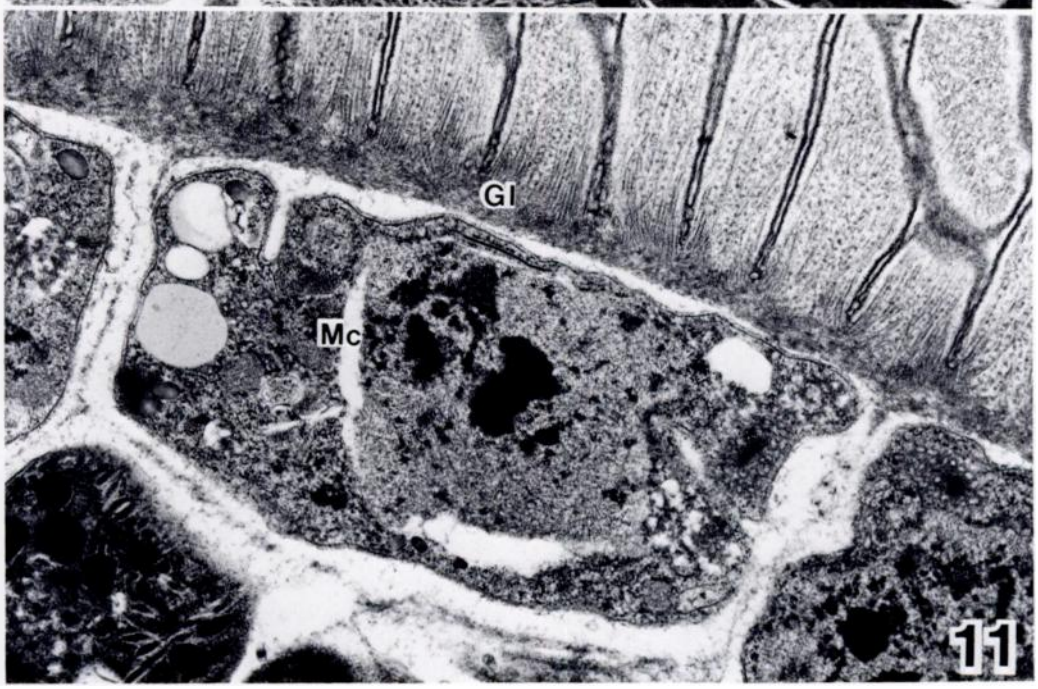



FIGURES 8, 9. Type II sarcocysts in mule deer esophagus. 8. Margin of sarcocyst showing villar projections, primary cyst wall (Pc), granular layer (Gl), and portions of a metrocyte (Mc) and bradyzoites (Bz). ×11,330. 9. Higher magnification of base of villar projection showing villar core (Vc), electron-dense layer (Ed) and parasitophorous vacuolar membrane (Pvm) of the primary cyst wall, granular layer (Gl) and portion of bradyzoite (Bz). ×45,000.

This layer was continuous with the septa which were granular (28 nm thick; 21–48 nm; $n = 8$) and separated the sarcocyst into compartments containing tightly packed bradyzoites (11.6 × 2.8 μm; 10–13.7 × 2.2–4.6 μm; $n = 7$) and metrocytes (6.7 × 3.5 μm; 5.5–8.3 × 3.3–4 μm; $n = 4$) (Figs. 10, 11). Metrocytes were few and located near the margin of the sarcocyst. The CPE was restricted to host cells harboring sarcocysts and was similar to that of type I sarcocysts.

FIGURES 10, 11. Type II sarcocysts in mule deer diaphragm. 10. Several bradyzoites located near the margin of the sarcocyst; note nucleus (Nu), micronemes (Mn) and rhoptry-like organelles (Rh). ×14,920. 11. Metrocyte (Mc) located immediately beneath granular layer (Gl). ×17,430.



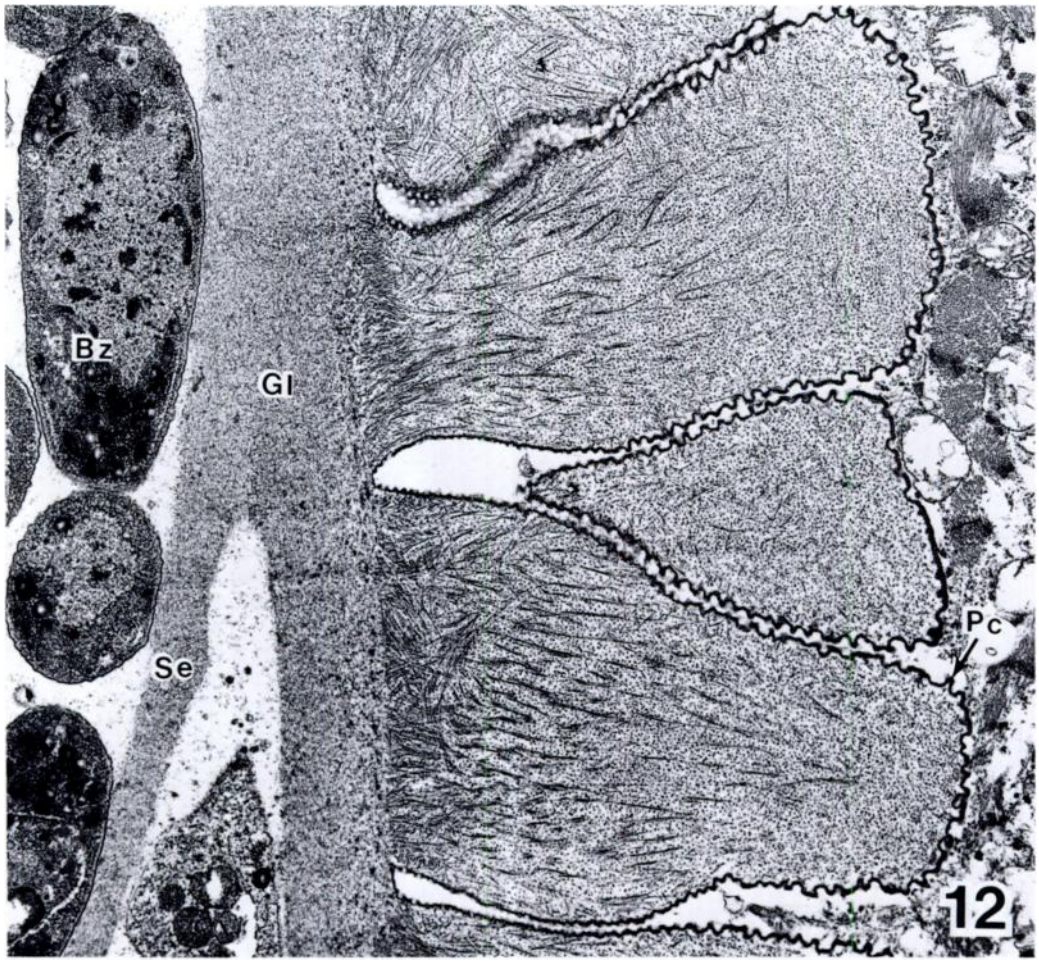



FIGURE 12. Type III sarcocyst from mule deer diaphragm showing villar projections of primary cyst wall (Pc), granular layer (Gl), septum (Se) and several bradyzoites (Bz). ×10,000.

**Type III sarcocysts**

One sarcocyst from the esophagus of Deer No. 4 and several sarcocysts in the esophagus and diaphragm of two mule deer (Nos. 5 and 6) from the NBR were studied ultrastructurally (Figs. 12, 13). The primary cyst wall was 83.7 nm (71–103 nm; $n$ = 15) thick and consisted of the PVM and an electron-dense layer which was located immediately beneath the PVM. The PVM and electron-dense layer were arranged as stubby, finger-like invaginations which were 113 nm (62–143 nm; $n$ = 10) long and were separated from each other by 75 nm (41–566 nm; $n$ = 12). In tangential sections of the primary cyst wall, the finger-like invaginations appeared as vesicles (Fig. 12). The primary cyst wall was folded into villar projections (Fig. 12) which were 8.5 μm (8.1–9.0 μm; $n$ = 7) long and 4.7 μm (3.5–5.6 μm; $n$ = 6) wide. The villar core consisted of two alternating shapes; they were broad distally and narrow centrally or vice versa (Fig. 12). The villar core consisted of coarse granules, that were more numerous in the outer ⅔ of the projections, and filamentous structures which were more nu-

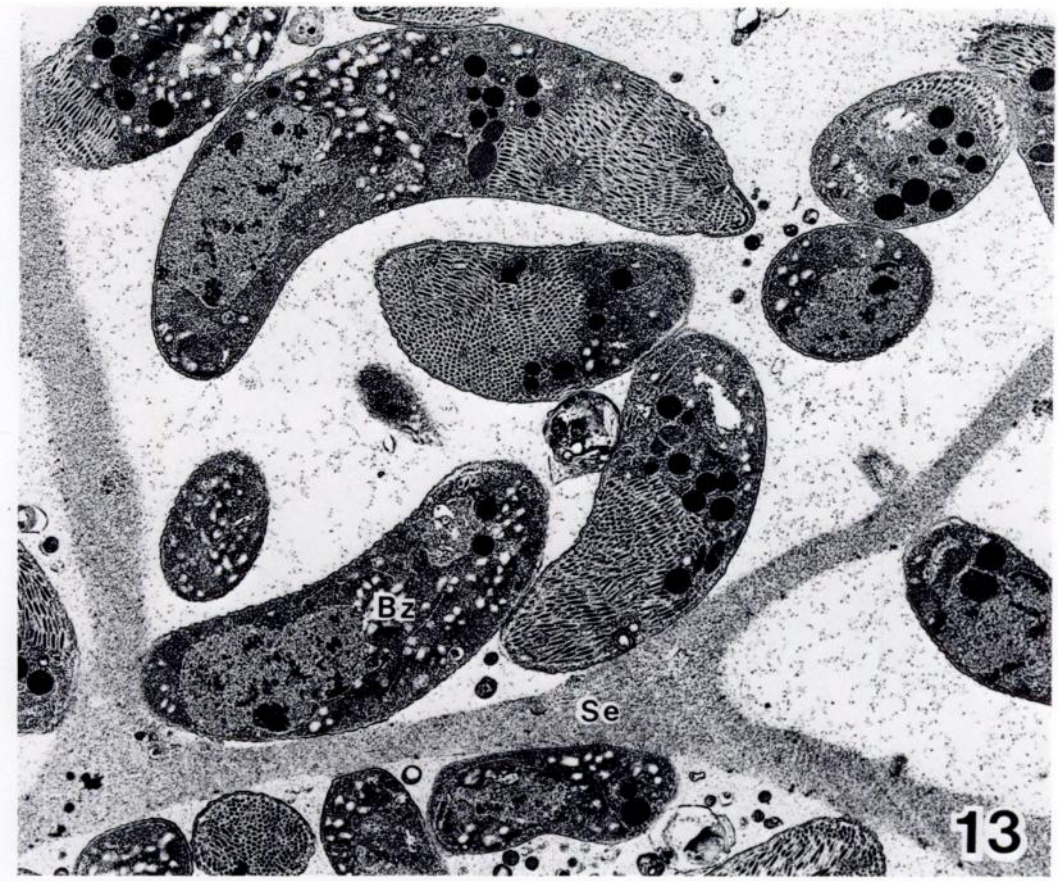



FIGURE 13. Portions of several compartments of a type III sarcocyst from mule deer diaphragm; note that the bradyzoites (Bz) are loosely arranged in compartments separated by septa (Se). ×6,600.

merous near the villar bases. The filamentous structures were arranged parallel to the long axis of the villar projections.

A moderately electron-lucent, finely granular layer 1.57 μm (1.0–1.9 μm; $n$ = 6) thick, was situated immediately beneath the villar projections (Fig. 12). Septa arose from the granular layer and traversed the sarcocyst completely separating it into compartments (Fig. 13). Septa were 0.69 μm (0.34–1.02 μm; $n$ = 9) thick. Each compartment contained numerous bradyzoites, which were loosely arranged (Fig. 13), a few metrocytes and spheroid degenerate material. Bradyzoites were 13 × 3.3 μm (10.7–15.8 × 3.2–3.6 μm; $n$ = 12). The CPE was similar to that described for type I sarcocysts.

## DISCUSSION

Ultrastructure of sarcocyst walls appears to be a reliable criterion for differentiating *Sarcocystis* species within a given host (Mehlhorn et al., 1976) but not between hosts. For example, *S. capracanis* of goats although ultrastructurally similar to *S. tenella* of sheep are two distinct species based on life cycle studies. Similarly, sarcocysts of the three species of *Sarcocystis* found in mule deer in Montana are basically similar in ultrastructure to those of white-tailed deer (*Odocoileus virginianus* Zimmermann) (Dubey and Lozier, 1982; Entzeroth et al., 1982). The three species of *Sarcocystis* in white-tailed deer are *S. odocoileocanis*, *S. odoi* and *Sarcocystis* sp. (Dubey and Lozier, 1982).

Sarcocysts of *S. hemionilatrantis* of mule deer are similar to those of *S. odocoileocanis* of white-tailed deer, the type II sarcocyst appears similar to that of *S. odoi* and the type III of mule deer appears similar to the *Sarcocystis* sp. in white-tailed deer. Whether the species in mule deer are indeed distinct from those in white-tailed deer or other cervids can only be determined by cross-transmission studies.

## ACKNOWLEDGMENTS

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