# Ultrastructure of the Cyst Wall of *Sarcocystis* Species with Canine Final Host in Japan

Morihiro SAITO, Yutaka SHIBATA, Takayuki KOBAYASHI[1], Masaru KOBAYASHI[2], Masanori KUBO[2], and Hiroshi ITAGAKI[3]

*Kumagaya Meat Inspection Center Saitama Prefecture, 179–1 Shimomasuda, Kumagaya, Saitama 360, [1]Animal Clinic Kobayashi, 715–3, Sakai, Fukaya, Saitama 366, [2]National Institute of Animal Health, Tsukuba, Ibaraki 305, and [3]Department of Parasitology, Azabu University School of Veterinary Medicine, Fuchinobe, Sagamihara, Kanagawa 229, Japan*

(Received 19 February 1996/Accepted 14 May 1996)

ABSTRACT. The ultrastructural characteristics of cyst wall are a useful clue to the identification of *Sarcocystis* species. Among the eight species examined, *S. cruzi*, S. sp. 1 from sheep and *S. hircicanis* had the thin cyst wall with long, tapered hair-like villar protrusions. The protrusions arose from the dome-like bulges of cyst wall and ran parallel to the surface. No clear ramification was observed in the tip of protrusion of these three species. *S. tenella, S. capracanis, S. miescheriana, S. fayeri*, and *S.* sp. 2 from Japanese deer had the thick cyst wall. The villar protrusions of the former three species were palisade-like in shape, but those of *S. tenella* and *S. capracanis* were slightly thinner than those of *S. miescheriana*. The villar protrusions of *S. fayeri* and *S.* sp. 2 were finger-like, but those of the former species were shorter and thicker than those of the latter species. *S. fayeri* had many minute depressions on the surface of protrusion in a reticular pattern. Microtubules in the core of protrusion were seen in *S. miescheriana, S. fayeri* and *S.* sp. 2 but not in the other species. Microdepressions were observed on the surface of cyst wall among the protrusions in all the *Sarcocystis* species examined, but their function was not made clear. — KEY WORDS: cyst wall, *Sarcocystis*, ultrastructure, villar protrusion.

*J. Vet. Med. Sci.* 58(9): 861–867, 1996

The ultrastructural characteristics of cyst wall are considered to be a useful clue to identifying *Sarcocystis* species from a variety of hosts. The ultrastructure of cyst wall has been examined by light (LM) and transmission electron microscopies (TEM) [5, 9], but hardly by scanning electron microscopy (SEM).

In the present study the ultrastructure of cyst wall was compared by TEM and SEM between eight species of *Sarcocystis* detected from cattle, pigs, horses, sheep, goats and Japanese deer in Japan.

## MATERIALS AND METHODS

*Materials*: Muscles infected with sarcocysts were obtained from cattle, pigs, horses, sheep and goats slaughtered in some prefectures of Japan from April, 1994 to July 1995, and also from Japanese (shika) deer, *Cervus nippon centralis*, captured in Iwate Prefecture, Japan from December, 1994 to January, 1995

*Methods*: For SEM, fresh cysts were directly collected from the infected muscle [15], fixed in 10% formalin and postfixed in 1% osmic acid. The fixed specimens were dehydrated in a series of ethanol and dried at the critical point, and platinum was deposited on the surface. The specimens treated were observed with a scanning electron microscope (Nihon Denshi, Type JSM-35C) for villar protrusions on the cyst surface.

For TEM, the muscles infected with sarcocysts were cut into blocks with a size of 1 $cm^3$ and the blocks were fixed in 10% formalin and postfixed in 1% osmic acid. After dehydrated in ethanol series, the specimens were embedded in epoxy resin and ultrathin sections were made. The sections were observed with a transmission electron microscope (Nihon Denshi, Type 100 CX) after stained with uranyl acetate and lead citrate solutions.

## RESULTS

The *Sarcocystis* species from the above animals of livestock were identified as *S. cruzi, S. miescheriana, S. fayeri, S. tenella, S. capracanis*, and *S. hircicanis* respectively by morphological and biological characteristics, but that from Japanese deer and one of those from sheep could not be identified to species level in the present study although they were morphologically similar to *S. sybillencis* and *S. arieticanis* respectively and dogs were experimentally determined to be the final hosts of both species [16].

*Sarcocystis cruzi, S.* sp. 1 from sheep and *S. hircicanis* were similar to each other in the shape and size of villar protrusion and the presence of dome-shaped bulges in cyst wall. All the three species had the thin cyst wall, by LM, with the hair-like villar protrusions on it, which tapered from the base to tip and measured 4–5 × 0.2–0.3 $\mu$m. Villar protrusions arose from the bulges of cyst wall and ran parallel to the long axis of cyst. The protrusions were not forked at least at the tip and included no microtubules in the core. A great number of minute depressions or microdepressions were reticulatedly distributed on the surface of cyst wall among the protrusions.

*S. tenella, S. capracanis* and *S. miescheriana* had the thick cyst wall by LM and their villar protrusions were similar to each other in shape but slightly differed in size. The villar protrusions were palisade-like in contour and had almost the same breadth from the base to tip. They measured 4–5 × 0.5–1 $\mu$m, 3–4 × 0.5–1 $\mu$m and 3–4 × 0.7–1 $\mu$m in *S. tenella, S. capracanis* and *S. miescheriana* respectively. A small number of microtubules were seen in the core of protrusion in *S. miescheriana*, but not in the

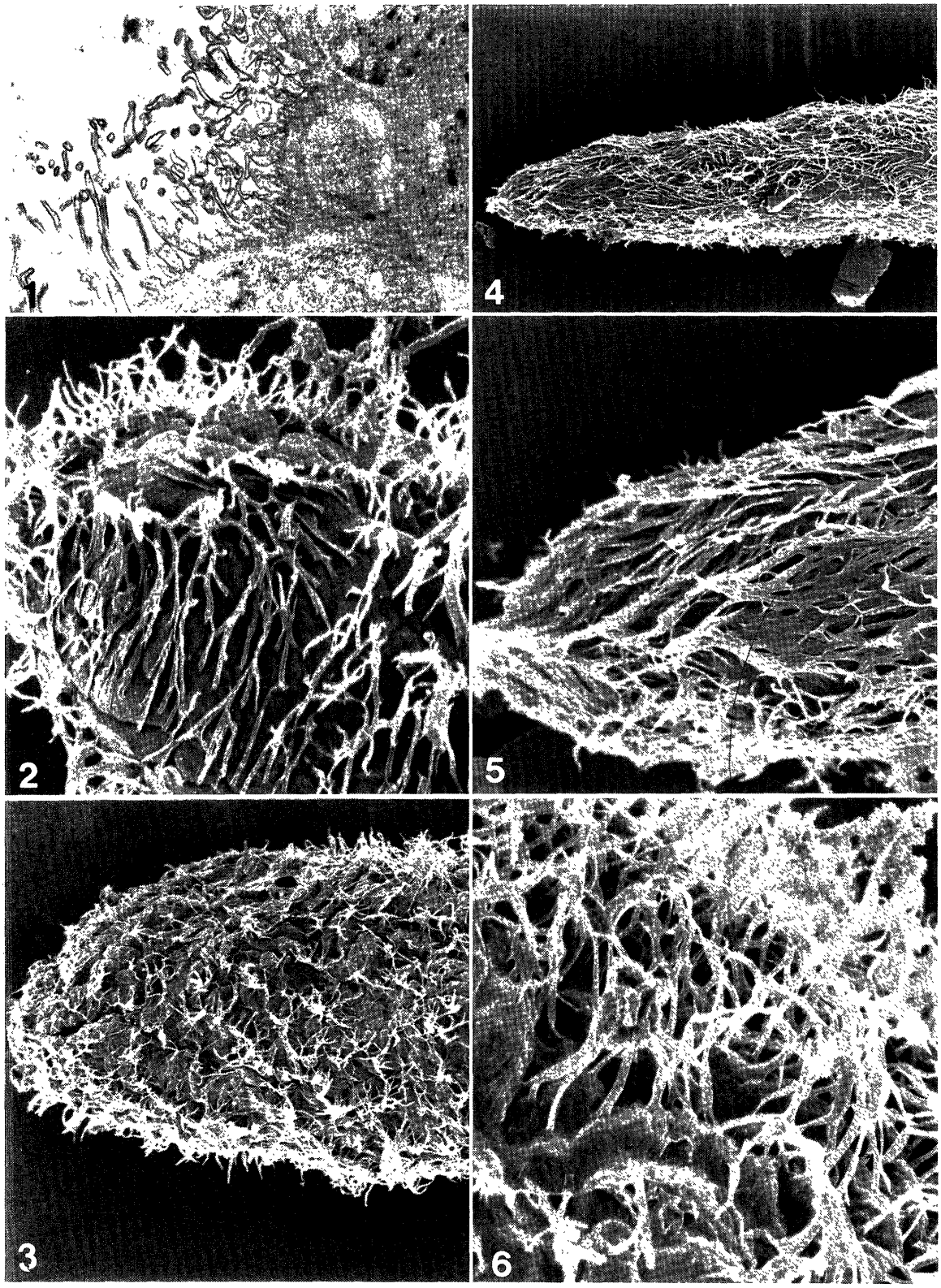


Fig. 1. Transmission electron micrograph (TEM) of *S. cruzi* cyst in skeletal muscle of cattle. × 10,000.
Fig. 2. Scanning electron mirograph (SEM) of *S. cruzi* cyst. Note hair-like villar protrusions on cyst wall. × 2,500.
Fig. 3. SEM of *S.* sp. 1 cyst from sheep. Note hair-like villar protrusions on cyst wall. × 850.
Fig. 4. SEM of *S. hircicanis* cyst. Note hair-like villar protrusions on cyst wall. × 400.
Fig. 5. Magnification of an end of cyst in Fig. 4. Note protrusions running parallel to cyst surface. × 1,800.
Fig. 6. Higher magnification of Fig. 4. Note villar protrusions arising from bulge of cyst wall. × 3,300.

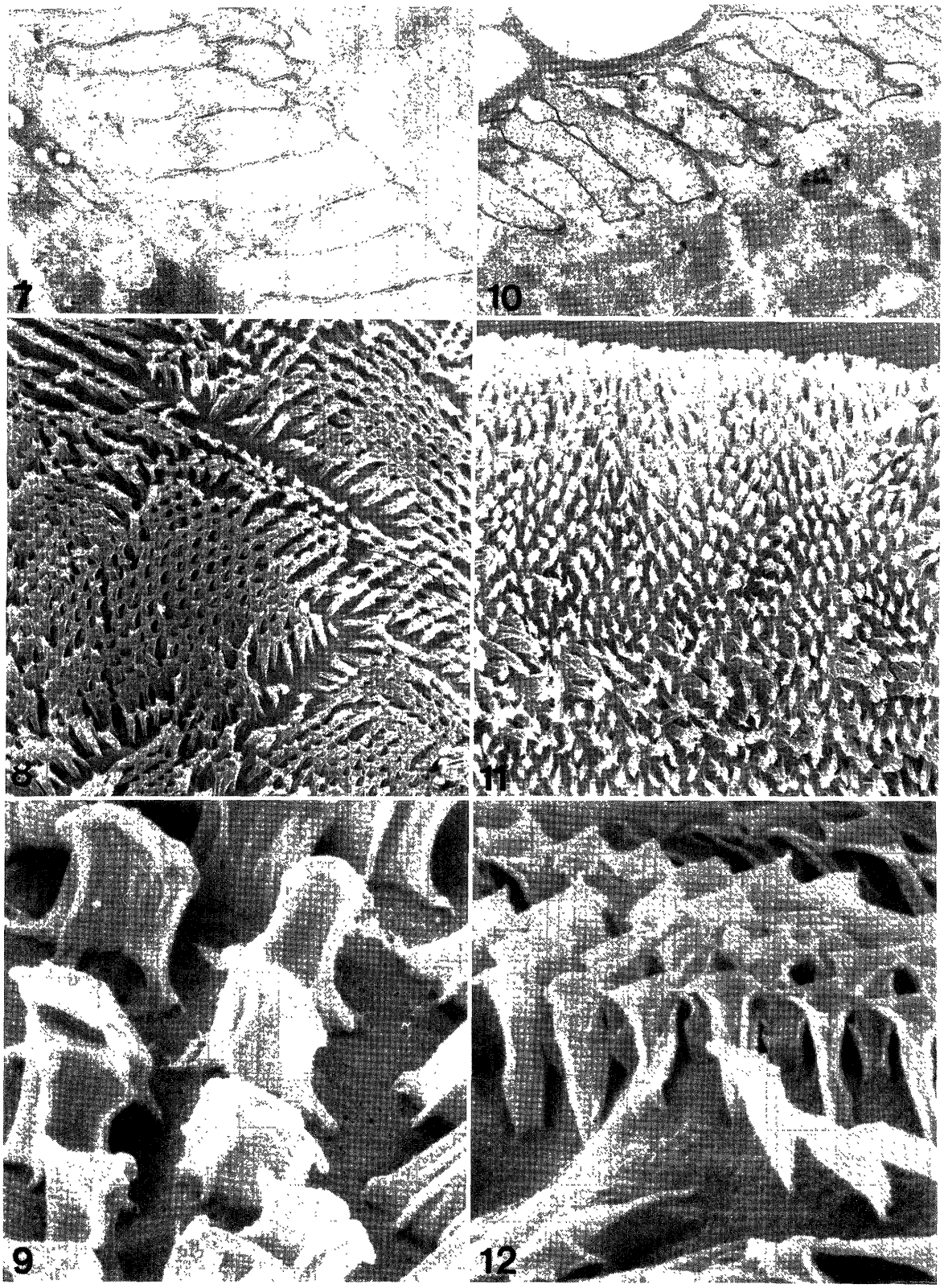


Fig. 7. TEM of *S. miescheriana* cyst in skeletal muscle of pig. × 8,000.
Fig. 8. SEM of *S. miescheriana* cysts. Note palisade-like villar protrusions on cyst wall. × 2,000.
Fig. 9. Higher magnification of Fig. 8. × 10,000.
Fig. 10. TEM of *S. capracanis* cyst in skeletal muscle of goat. × 8,000.
Fig. 11. SEM of S. *capracanis* cyst. × 1,200.
Fig. 12. Higher magnification of Fig. 11. Note palisade-like villar protrusions on cyst wall. × 10,000.

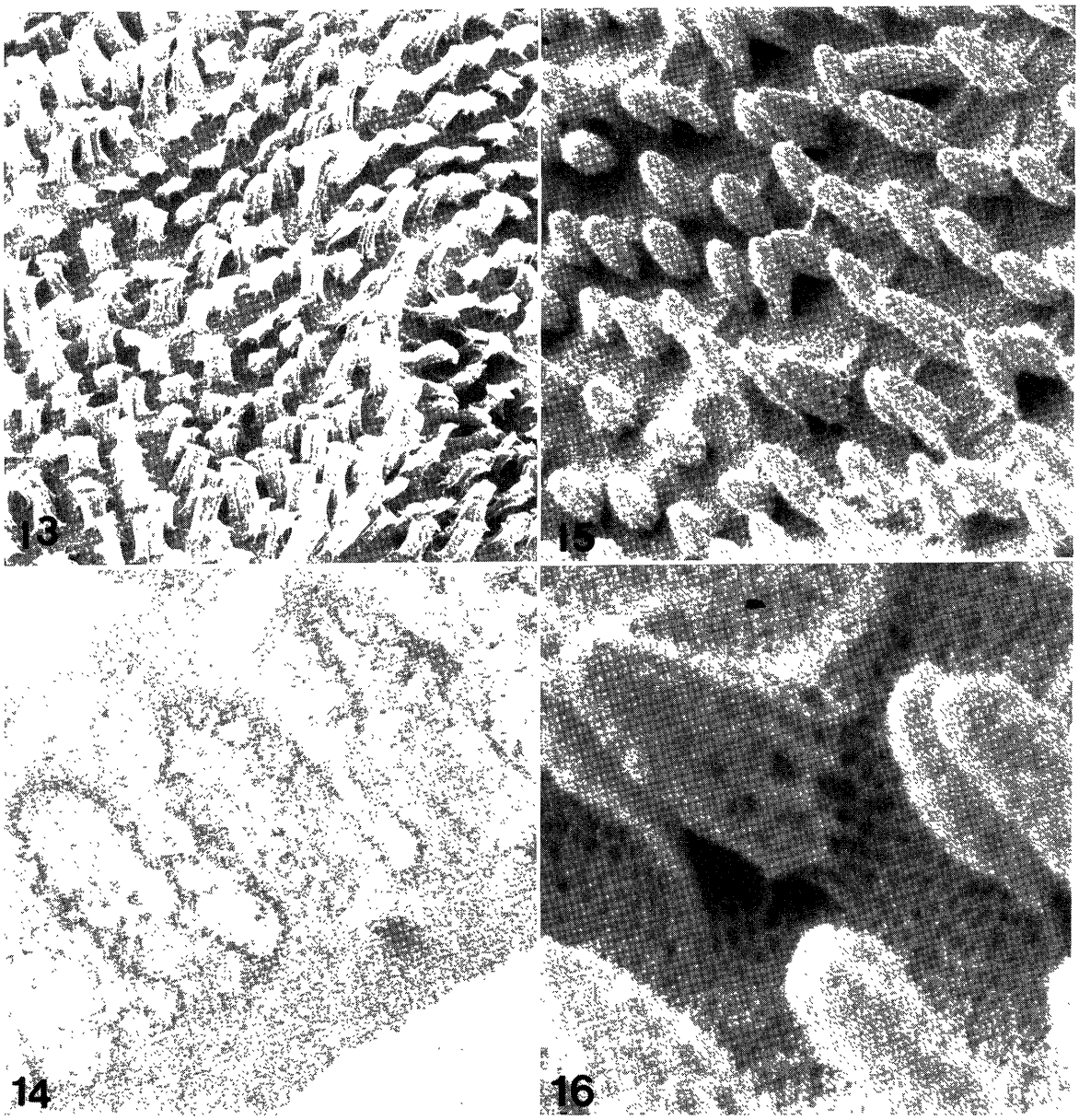


Fig. 13. SEM of *S. tenella* cyst. Note palisade-like villar protrusions on cyst wall. × 2,200.
Fig. 14. TEM of *S. fayeri* cyst in skeletal muscle of horse. × 20,000.
Fig. 15. SEM of *S. fayeri* cyst. × 8,500.
Fig. 16. Higher magnification of Fig. 15. Note short finger-like villar protrusions on cyst wall. × 30,000.

other two species. The protrusions were regularly arranged on the surface of cyst wall. In *S. miescheriana* the neighboring tips of protrusion made a honeycomb structure. A vast number of microdepressions were distributed in a reticular pattern on the surface of cyst among the protrusions.

The villar protrusions of *S. fayeri*, rather sparsely distributed on the cyst wall, were short finger-like in contour and 1.2–2 × 0.5–0.8 $\mu$m in size, slightly tapered from the base to blunt tip. The protrusion included a small number of microtubules in the core. Numberless microdepressions were reticulately distributed on the surface of cyst wall among the protrusions.

In *Sarcocystis* sp. 2 originated from Japanese deer, villar protrusions were slender finger-like and 7–9 × 0.3–0.4 $\mu$m in size. Villar protrusions were densely distributed and arranged in a distinct direction. The protrusions included a small number of microtubules in the core and were not forked at the tip. Innumerable microdepressions were located on the surface of cyst wall among the protrusions.

## DISCUSSION

*Sarcocystis* species from a variety of animals have been studied for the structure of cyst wall mainly by LM and TEM, and 24 types of *Sarcocystis* cyst have been

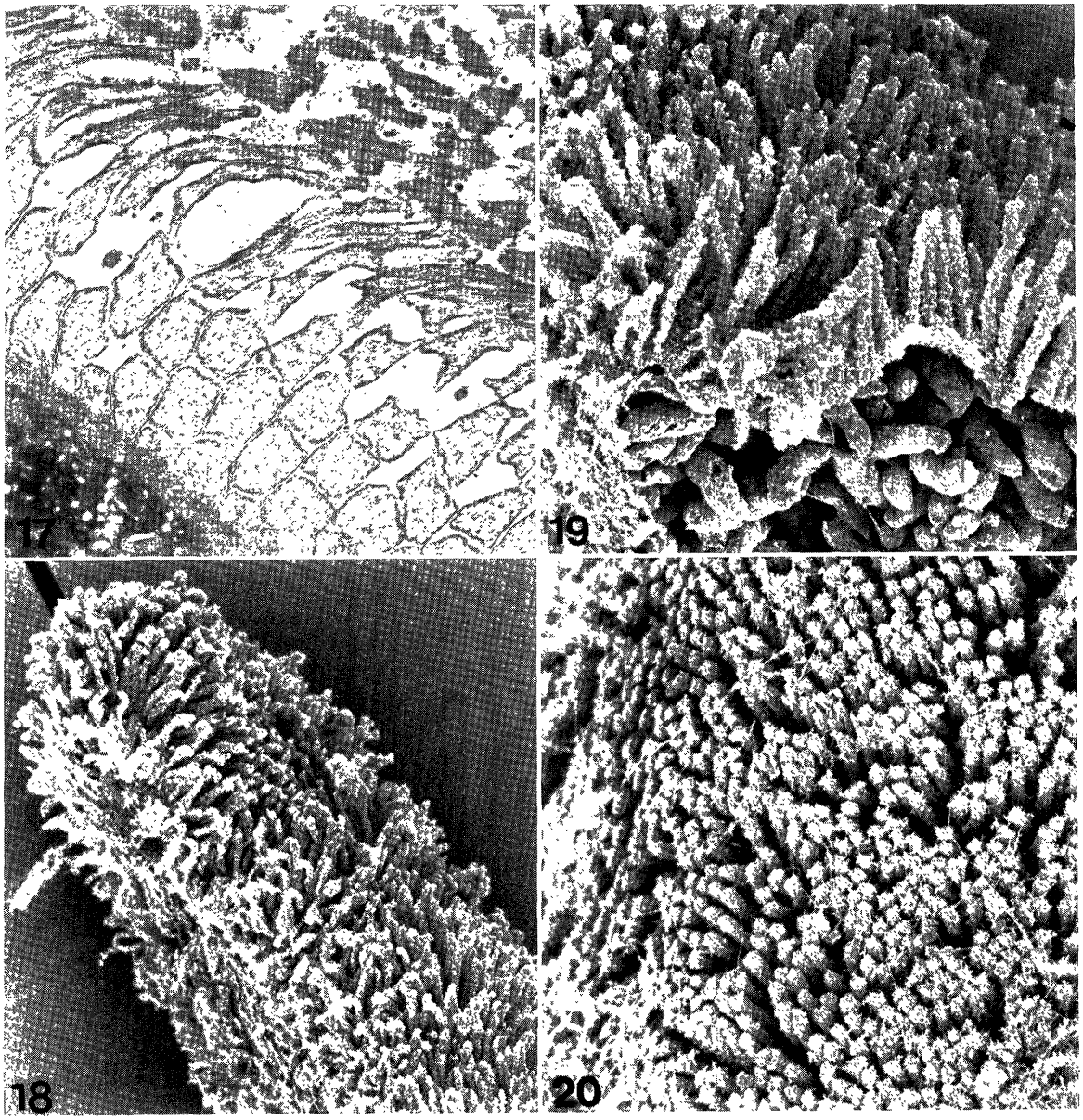


Fig. 17. TEM of *Sarcocysis* sp. 2 cyst in skeletal muscle of Japanese (shika) deer. × 5,000.
Fig. 18. SEM of *S.* sp. 2 cyst from Japanese deer. Note slender finger-like villar protrusions on cyst wall. × 600.
Fig. 19. SEM of *S.* sp. 2 cyst from deer, showing villar protrusions on cyst wall and bradyzoites included in a cyst. × 1,200.
Fig. 20. Magnification of Fig. 18. × 1,600.

discriminated by TEM observation of the cyst wall and the cyst structure is considered useful for the identification of *Sarcocystis* [5].

TEM micrographs of the six species of *Sarcocystis* from cattle, pigs, sheep, horses, and goats showed the characteristic ultrastructures of cyst wall of *S. cruzi, S. miescheriana, S. tenella, S. Fayeri, S. capracanis* and *S. hircicanis* respectively [1, 3–14, 17, 18]. *Sarcocystis* sp. 2 from Japanese deer was similar to *S. sybillensis* in the thick cyst wall by LM and finger-like protrusion by TEM and SEM but different from it in the absence of the forked tip of villar protrusion and S. sp. 1 from sheep was skin to *S. arieticanis* in the structure of cyst wall by LM and TEM [2, 5].

TEM and SEM micrographs showed, as previously described by Dubey *et al.* and Odening *et al.* [5, 14], that *S. cruzi, S.* sp. 1 from sheep, and *S. hircicanis* with the thin cyst wall by LM had tapering hair-like villar protrusions which arose from the dome-shape bulges of cyst wall.

All the other five species had the thick cyst wall by LM but varied in the morphology of villar protrusion. *S. tenella, S. capracanis*, and *S. miescheriana* were provided with palisade-like villar protrusions but the protrusion of *S. tenella* was slightly longer than those of the other two

Table 1. Comparative morphology of *Sarcocystis* cysts

| Species of *Sarcocystis* | Thickness of cyst wall by LM* | Bulge of cyst wall | Shape of protrusion | Depression on protrusion | Microtubule in protrusion | Ramification of protrusion |
|---|---|---|---|---|---|---|
| *cruzi* | thin | + | hair-like | – | – | ± |
| sp. 1** | thin | + | hair-like | – | – | ± |
| *hircicanis* | thin | + | hair-like | – | – | ± |
| *tenella* | thick | – | palisade-like | – | – | – |
| *capracanis* | thick | – | palisade-like | – | – | – |
| *miescheriana* | thick | – | palisade-like | – | + | – |
| *fayeri* | thick | – | short finger-like | + | + | – |
| sp. 2*** | thick | – | slender finger-like | – | + | – |

* LM: light microscopy, **: from sheep, ***: from Japanese deer

species. Microtubules in the core were present in *S. miescheriana* but absent in *S. tenella* and *S. capracanis*.

*S. fayeri* and *S.* sp. 2 from Japanese deer also had the thick wall by LM but their finger-like protrusions were different from these of the above three species. The protrusion of *S. fayeri* was short finger-like, covered with minute depressions on the surface. The villar protrusions of *S.* sp. 2, however, were slender finger-like. The granular appearance of protrusion shown in the micrographs was produced by the small particles adherent to the surface during the preparation of samples (Figs. 18–20).

The presence of microtubules in the core of protrusion is a discriminative feature of sarcocysts and *S. miescheriana*, *S. fayeri* and *S.* sp. 2 had microtubules [5, 13].

*S. cruzi* and *S. hircicanis* were reported to have the villar protrusions with the forked tip [5], but this was not ascertained in the present study.

The present observation revealed that innumerable microdepressions were reticulately distributed over the surface of cyst wall among villar protrusions in all the *Sarcocystis* species examined. The presence of microdepressions has not been reported and their function could not be made clear in the present study.

SEM has been hardly applied to observation of *Sarcocystis* cysts although this electron microscopic technique is advantageous for observation and measurement of the superficial ultrastructures on cyst surface, whereas TEM can reveal the internal ultrastructures of cyst wall which are also useful for identification of *Sarcocystis* species. One of the reasons why SEM has not been applied will come from the difficulty in preparing intact fresh sarcocyst samples for SEM. These intact samples can be obtained by the direct method [15] from the diaphragm but not from the cardic muscle, because the cardiac muscular fibers are complicately entangled to each other and intact sarcocysts can be hardly obtained.

## REFERENCES


1. Dubey, J. P. 1976. A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs. *J. Am. Vet. Med. Assoc.* 169: 1061–1078.
2. Dubey, J. P., Jolley, W. R., and Thorne, E. T. 1983. *Sarcocystis syillensis* sp. nov. from the north American elk *(Cervus elaphus). Can. J. Zool.* 61: 737–736.
3. Dubey, J. P., Lindsay, D. S., Speer, C. A., Fayer, R., and Livingston, C. W. Jr. 1988. *Sarcocystis arieticanis* and other *Sarcocystis* species in sheep in the United States. *J. Parasitol.* 74: 1033–1038.
4. Dubey, J. P., Speer, C. A., Callis, G., and Blixt, J. A. 1982. Development of sheep-canid cycle of *Sarcocystis tenella. Can. J. Zool.* 60: 2464–2477.
5. Dubey, J. P., Speer, C. A., and Fayer, R. 1989. pp. 30–156. *Sarcocystis* of Animals and Man. CRC Press, Boca Raton, Florida.
6. Dubey, J. P., Streitel, R. H., Stromberg, P. C., and Toussant, M. J. 1977. *Sarcocystis fayeri* sp. n. from the horse. *J. Parasitol.* 63: 443–447.
7. Erber, M. 1977. Moeglichkeiten des Nachweises und Differenzierung von Zwei *Sarcocystis*-Arten des Schweines. *Berl. Muench. Tieraerztl. Wochenschr.* 90: 480–482.
8. Erbre, M. and Geisel, O. 1981. Vorkommen und Entwicklung von 2 Sarkosporidienarten des Pferde. *Z. Parasitenkd.* 65: 283–291.
9. Heydorn, A. O. and Mehlhorn, H. 1987. Fine structure of *Sarcocystis arieticanis* Heydorn, 1985 in its intermediate and final hosts (sheep and dog). *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. I. Abt. Orig., A* 264: 353–362.
10. Mehlhorn, H., Hartley, W. J., and Heydorn, A. O. 1976. A comparative ultrastructural study of the cyst wall of 13 *Sarcocystis* species. *Protistologica* 12: 451–467.
11. Mehlhorn, H. and Heydorn, A. O. 1978. The Sarcosporidia (Protozoa, Sporozoa): life cycle and fine structure. *Adv. Parasitol.* 16: 43–91.
12. Mehlhorn, H., Heydorn, A. O., and Gestrich, R. 1975. Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis ovicanis* Heydorn, *et al.* (1975) in der Muskulatur von Schafen. *Z. Parasitenkd.* 48: 83–93.
13. Odening, K., Wesenmeier, H.-H., Walter, G., and Bockhardt, I. 1995. Ultrastructure of sarcocysts from equids. *Acta Parasitologica* 40: 12–20.
14. Odening, K., Wesenmeier, H.-H., Walter, G., and Bockhardt, I. 1995. On the morphological diagnostics and host specificity of the *Sarcocystis* species of some domesticated and wild Bovini (cattle, banteng and bison). *Appl. Parasitol.* 36: 161–178.

15. Saito, M., Hachisu, K., Iwasaki, K., Nakajima, T., Watanabe, A., Moriya, H., and Itagaki, H. 1984. A new simple method for detection of bovine *Sarcocystis* cysts. *J. Jpn. Vet. Med. Assoc.* 37: 158–162 (in Japanese with English summary).
16. Saito, M., Itagaki, T., Shibata, Y., and Itagaki, H. 1995. Morphology and experimental definitive hosts of *Sarcocystis* sp. from sika deer, *Cervus nippon centralis* in Japan. *Jpn. J. Parasitol.* 44: 218–221.
17. Tinling, S. P., Cardinet, G. H., III, Blythe, L. L., Cohen, M., and Vonderfecht, S. L. 1980. A light and electron microscopic study of *Sarcocystis* in horse. *J. Parasitol.* 66: 458–465.
18. Yamada, M., Yukawa, H., Sekikawa, H., Kenmotsu, M., and Mochizuki, K. 1993. Studies on the morphology of sarcocysts in throughbred horses in Japan. *J. Protozool. Res.* 3: 14–19.