Sarcocystis mihoensis n. sp. from Sheep in Japan

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ABSTRACT. *Sarcocystis mihoensis* n. sp., a heteroxenous coccidium was detected from sheep in Japan and dogs were experimentally determined as the definitive host. The cysts were 1,300–2,100 × 200–300 µm in size and had the thick cyst wall which was 10 to 12 µm thick and provided with radial striated and finger-like villar protrusions. Two 6-month old dogs fed with the cysts passed sporocysts, 15–16 × 8–9 µm in size, in the feces from day 11 to days 57–60 after ingestion. No domestic cats fed with the same cysts shed sporocysts throughout the experimental period. — **KEY WORDS:** experimental infection, morphology, *Sarcocystis mihoensis* n. sp., sheep.

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Four *Sarcocystis* species have been recorded from sheep such as *S. arieticanis*, *S. gigantea*, *S. medusiformis*, and *S. tenella* [1, 2, 5, 7, 12]. Of these species, the final hosts of *S. tenella* and *S. arieticanis* are the dog and those of *S. gigantea* and *S. medusiformis* are the cat [2–9, 13, 15–17]. We detected a new *Sarcocystis* species of which the cyst wall was morphologically different from that of the species reported from sheep.

The morphology and experimental final host of the present species are described in this paper.

MATERIALS AND METHODS

Materials: Sixteen Corriedale sheep, 2 to 3 years old, which were raised at a farm in Miho Village, Ibaraki Prefecture were slaughtered at an abattoir in Saitama Prefecture and striated muscle in different parts of body was examined for *Sarcocystis* cysts.

Methods: Twenty fresh cysts were measured with a micrometer under a light microscope. A part of the cysts were fixed with 10% formalin and postfixed with 1% osmic acid. The fixed specimens were then dehydrated in a series of ethanol and dried at the critical point. After platinum was deposited on the specimens, they were observed for the villar protrusions on cyst wall under a scanning electron microscope (Nihon Denshi, JSM-35C). A piece of striated muscle infected with cysts was dissected out and fixed in 10% formalin, embedded in paraffin and sectioned. Sections were stained with hematoxylin and eosin for histopathological examination. A part of the specimens fixed with formalin and post-fixed with 1% osmic acid were dehydrated in a series of ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate solutions and observed with a transmission electron microscope (Nihon Denshi, 100 CX).

Two female 6 months-old mongrel dogs and 2 female domestic cats, 6 and 12 months old respectively, were fed with 15 fresh cysts together with a small amount of food, while a female mongrel dog and a cat, 6 months old each, were used as control.

All the inoculated and control animals were daily examined for sporocysts by the sedimentation method using the total amount of feces, with saturated NaCl solution. Fifty sporocysts just after passed in the feces were measured with a micrometer under a light microscope.

RESULTS

In 2 of 16 sheep examined, cysts were detected in the diaphragm, masseter, and muscles in the neck, dorsal region, brachium and thigh, especially in the diaphragm in great numbers.

Fresh cysts measured 1,300–2,100 × 200–300 µm in size. The cyst wall was thick, 10–12 µm in thickness, and of a palisade-like structure. Clear compartments were observed inside the cyst. Histopathological examination revealed that the cyst wall was thick and the villar protrusions were radially arranged. A great number of bradyzoites were observed in the cyst together with a small number of merozoites which were situated along the cyst wall (Figs. 1 and 2).

Although light microscopy showed that the cyst wall was thick and of “palisade-like” structure, scanning electron micrographs showed that villar protrusions on the cyst wall were finger-like and 8–10 × 2–2.5 µm in size, and were attenuated gradually from the base to about two thirds of length and then abruptly from two thirds to the tip. There were many folds on the surface of villar protrusion from the base to about two thirds of length (Figs. 3 and 4). This structure of villar protrusion was also shown by transmission electron microscopy, but the transverse section of protrusion was occasionally mushroom-like when cut at some angle. Microtubules were observed in the villar protrusion (Figs. 5–7).

All the dogs orally inoculated with the muscle infected with cysts began to pass sporulated sporocysts in the feces on day 11 after inoculation. Sporocysts measured 15–16 × 8–9 µm in size. Patent period was 57 to 60 days. None of the control animals shed sporocysts in the feces (Fig. 8).

Fig. 1. Cross section of a thick-walled cyst of *Sarcocystis mihoensis* n. sp. in striated muscle of a sheep. Arrow shows metrocytes. Bar=10 μ m. H E stain, $\times$ 400.

Fig. 2. A fresh cyst removed from striated muscle of a sheep. Bar=10 μ m. $\times$ 400.

DISCUSSION

Of the 4 species of *Sarcocystis* that have been reported from sheep, *S. tenella* and *S. arieticanis* utilize the dog as the final host and *S. gigantea* and *S. medusiformis* do the cat. The species with the sheep-canid life cycle have far smaller cysts than the sheep-felid species. The cyst of the present species was also smaller than those of the sheep-felid species, but it was the largest among the known

Fig. 3. Scanning electron micrograph (SEM) of a cyst. Bar=10 μ m. $\times$ 1,000.

Fig. 4. SEM of cyst, showing finger-like villar protrusions on cyst wall. Bar=1 μ m.

sheep-canid species (Table 1) [2–9, 13, 16, 17].

The cyst wall of *S. tenella* is observed thick by light microscopy but thin in the other 3 species, *S. arieticanis*, *S. gigantea* and *S. medusiformis*. The present species had the far thicker cyst wall than all the above 4 species, so by this feature, the present species can be distinguished from all the other known species (Table 1) [2, 3, 5, 9–11, 16, 17].

The ultrastructures of villar protrusions detected by scanning and transmission electron microscopy were more useful in distinguishing the present species from the other species. The villar protrusion was finger-like in the present species, whereas it is palisade-like, hair-like, cauliflower-like, and snake-like in *S. tenella*, *S. arieticanis*, *S. gigantea* and *S. medusiformis* respectively. The presence of microtubule in the villar protrusion also afforded a clue to the identification. The microtubule was found not only in the present species but also in *S. gigantea* and *S. medusiformis*, whereas *S. tenella* and *S. arieticanis* have no

Fig. 5. Transmission electron micrograph (TEM) of a cyst in striated muscle of sheep, showing finger-like villar protrusions and many bradyzoites on and inside of a cyst. Bar=1 μm . $\times 2,000$.

Fig. 7. TEM of cross sections of villar protrusions. Bar=1 μm $\times 12,000$.

Fig. 6. TEM of a cross section through the finger-like villar protrusions. Note mushroom-like sections of some protrusions. Bar=1 μm . $\times 10,000$.

Fig. 8. A fresh sporocyst passed in the feces of dog. Bar=10 μm . $\times 400$.

microtubule (Table 1) [2–5, 9–11, 16, 17].

The prepatent period was not markedly different among the species parasitic in sheep although in *S. tenella* the period is shorter than those of the other species. Sporocysts are larger in the species with the sheep-canid life cycle than those in the sheep-felid species, but any difference was hardly found in the size among the sheep-canid species [2,

4, 5, 7, 8, 16].

From the above characteristics of the morphology of cyst wall, especially of villar protrusion, and of the life cycle, the present species could be discriminated from all the known *Sarcocystis* species parasitic in sheep, so that the present species was considered to be new to science and was designated as *Sarcocystis mihoensis* n. sp.

Table 1. Comparison of biological characteristics of *Sarcocystis* species parasitic in sheep

Species	Cyst length (μm)	Cyst wall thickness (μm)	Villar protrusion		Microtubule	Final host	Prepatent period (in days)	Sporocyst (μm)
			Shape	Size (μm)				
<i>S. tenella</i>	700	1–3	palisade-like	3.5×0.5	–	dog	8–9	$14\text{--}15 \times 8\text{--}10.5$
<i>S. arieticanis</i>	900	< 1	hair-like	5–10	–	dog	12	$15\text{--}16 \times 9\text{--}10$
<i>S. gigantea</i>	10000	< 2	cauliflower-like	–	+	cat	10–12	$10.5\text{--}14.0 \times 8.0\text{--}9.7$
<i>S. medusiformis</i>	8000	< 2	snake-like	–	+	cat	12–21	$10.3\text{--}13.0 \times 7.3\text{--}8.8$
<i>S. mihoensis</i> n. sp.	1300–2100	10–12	finger-like	$8\text{--}10 \times 2.2\text{--}2.5$	+	dog	11	$15\text{--}16 \times 8\text{--}9$

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REFERENCES

- Ashford, R. W. 1977. The fox, *vulpes vulpes*, as a final host for *Sarcocystis* of sheep. *Ann. Trop. Med. Parasitol.* 71: 29–34.
- Collins, G. H., Atkinson, E. M., and Charleston, W. A. G. 1979. Studies on *Sarcocystis* species. 3. The macrocystic species of sheep. *N. Z. Vet. J.* 27: 204–206.
- 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.
- 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.
- Dubey, J. P., Speer, C. A., and Fayer, R. 1989. pp. 113–120, 146–147, *Sarcocystosis of Animals and Man*. CRC Press, Boca Raton, Florida.
- Erber, M. 1982. Life cycle of *Sarcocystis tenella* in sheep and dog. *Z. Parasitenkd.* 68: 171–180.
- Heydorn, A. O. 1985. Zur Entwicklung von *Sarcocystis arieticanis* n. sp. *Berl. Muench. Tieraerztl. Wochenschr.* 98: 231–241.
- Heydorn, A. O., Gestrich, R., Mehlhorn, H., and Rommel, M. 1975. Proposal for a new nomenclature of the Sarcosporidia. *Z. Parasitenkd.* 48: 73–82.
- Heydorn, A. O. and Mehlhorn, H. 1987. Fine structure of *Sarcocystis arieticanis* Heydorn, 1985 in its intermediate and final host (sheep and dog). *Zentralbl. Bakteriол. Parasitenkd. Infektionskr. Hyg., I Abt. Orig., A* 264: 353–362.
- Mehlhorn, H., Heydorn, A. O., and Gestrich, R. 1975. Lichtund elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis ovis* (Heydorn et al. 1975) in der Muskulatur von Schafen. *Z. Parasitenkd.* 48: 83–93.
- 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.
- Moulé, L. T. 1886. Psorospermies du tissu musculaire du mouton. *Bull. Mem. Soc. Centr. Med. Vet.* 40: 125–129.
- Obendorf, P. L. and Munday, B. L. 1987. Experimental infection with *Sarcocystis medusiformis* in sheep. *Vet. Parasitol.* 24: 59–65.
- Pomroy, W. E. and Charleston, W. A. G. 1987. Prevalence of dog-derived *Sarcocystis* spp. in some New Zealand lambs. *N. Z. Vet. J.* 35: 141–142.
- 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).
- Saito, M., Shibata, Y., and Itagaki, H. 1996. *Sarcocystis arieticanis* of sheep in Japan. *Jpn. J. Parasitol.* 45: 290–294.
- Saito, M., Shibata, Y., Kobayashi, T., Kobayashi, M., Kubo, M., and Itagaki, H. 1996. Ultrastructure of the cyst wall of *Sarcocystis* species with canine final host in Japan. *J. Vet. Med. Sci.* 58: 861–867.