[Jpn. J. Parasitol., Vol. 45, No. 4, 290–294, August, 1996]

Sarcocystis arieticanis of Sheep in Japan (Protozoa; Apicomplexa)

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(Accepted July 19, 1996)

Abstract

Sarcocystis arieticanis was first recorded in Japan from the heart and diaphragm of 12 out of 100 adult sheep slaughtered in Saitama Prefecture from October 1994 to January 1996. Cysts were 1,700–2,400 × 90–126 μm in size and had the thin wall provided with hair-like villar protrusions with a size of 4–5 × 0.2–0.3 μm. Two dogs, 6 months old, fed with the muscle infected with cysts began to pass sporulated sporocysts, 15–16 × 10–11 μm in size, in the feces 12 days after ingestion.

Key words: dog; incidence; morphology; *Sarcocystis arieticanis*; sheep.

Introduction

Four species of *Sarcocystis* have been recorded from sheep: *S. arieticanis, S. gigantea, S. medusiformis* and *S. tenella* (Dubey *et al.* 1989). Of these species, only *S. tenella* has been reported from Japan (Imai *et al.* 1989). The final hosts of *S. tenella* and *S. arieticanis* are dogs and those of the other 2 species are cats (Erber, 1982; Dubey *et al.* 1982; Dubey *et al.* 1986; Heydorn and Mehlhorn, 1987; Obendorf and Munday, 1987; Dubey *et al.* 1988; Dubey *et al.* 1989).

In sheep slaughtered in Saitama Prefecture were found cysts different from those of *S. tenella* which has been recorded from Japan (Moulé, 1886; Dubey *et al.* 1982; Erber, 1982; Imai *et al.* 1989). Cysts from the diaphragm and heart were morphologically examined and fed to dogs and cats to identify the species and final host.

Materials and Methods

Cardial muscle and diaphragm, 50g each, were obtained from 100 adult sheep slaughtered in an abattoir of Saitama Prefecture from October 1994 to January 1996.

Fifty fresh sarcocysts each collected from both habitats were observed and measured with a micrometer (Saito *et al.* 1984). A part of fresh cysts were fixed with 10% formalin and post-fixed with 1% osmic acid. Then they were dehydrated in a series of ethanol and dried at the critical point. After platinum was deposited, the cyst samples were observed with a scanning electron microscope (Nihon Denshi, JSM-35C) for the surfacial structures such as villar protrusions.

For histopathological examination, samples each were removed from muscle infected with *Sarcocystis* cysts under a dissecting microscope and fixed with 10% formalin. The specimens were embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin and observed under a light microscope. Another part of the fixed material was post-fixed with 1% osmic acid and embedded in epoxy resin. The resultant ultrathin sections were stained with uranyl acetate and lead citrate solutions, and then observed for the ultrastructure of the cyst wall with a transmission electron microscope (100CX, Nihon Denshi, Japan).

Fifty fresh cysts each were fed to 2 female mongrel dogs, 6-months old, and 2 domestic cats, 6 and 12 months old, together with feed. Another female mogrel dog, 6 months old, and another female cat, 12 months old, were used as the control. All the inoculated and control animals were daily examined for sporocysts passed in the total amount of feces by the flotation method with satulated NaCl

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solution. Fifty sporocysts each excreted in the feces of the animals were measured with a micrometer and observed under a light microscope.

Results

Of the 100 sheep examined, 12 (12%) were positive for cysts which were detected from muscle of the diaphragm and heart.

Fresh cysts were 1,700–2,400 × 90–126 μm in size and had the thin wall, less than 1 μm in thickness, and long hair-like structures were found on its surface by light microscopy. Histopathological observation revealed that the cyst wall was thin and structureless. Transmission and scanning electron micrographs showed hair-like villar protrusions, 4–5 × 0.2–0.3 μm in size, on the cyst wall.

Dogs orally inoculated with cysts began to pass sporulated oocysts and sporocysts in the feces from day 12 after inoculation through the examination period of 60 days. Sporocysts were ellipsoidal and measured 15–16 × 10–11 μm in size, and they included 4 sporozoites and 1 large intraresidual body but no Stieda body. The control animals shed neither sporocysts nor oocysts in the feces. None of the cats inoculated, however, shed sporocysts in the feces.

Discussion

Sarcocystis species that utilize sheep as the intermediate host are *S. arieticanis, S. gigantea, S. medusiformis* and *S. tenella* (Dubey *et al.* 1982; Erber, 1982; Heydorn, 1985; Dubey *et al.* 1986; Heydorn and Mehlhorn, 1987; Obendorf and Munday, 1987; Dubey *et al.* 1988; Dubey *et al.* 1989; Imai *et al.* 1989) Cysts of *S. arieticanis* are up to 900 μm in length and have the thin cyst wall which is provided with hair-like villar protrusions (Heydorn and Mehlhorn, 1987; Dubey *et al.* 1988; Dubey *et al.* 1989). Cysts of the present species, on the other hand, had the thin wall provided with hair-like protrusions. These morphological characteristics were also confirmed by histopathological examination and electron microscopy. Consequently, the present species was identical to *S. arieticanis* in the morphological features except that the length of cyst was far greater than that of the species reported. The

Fig. 1 Transverse section of a thin-walled cyst of *Sarcocystis arieticanis* in striated muscle of a sheep. HE stain. ×400

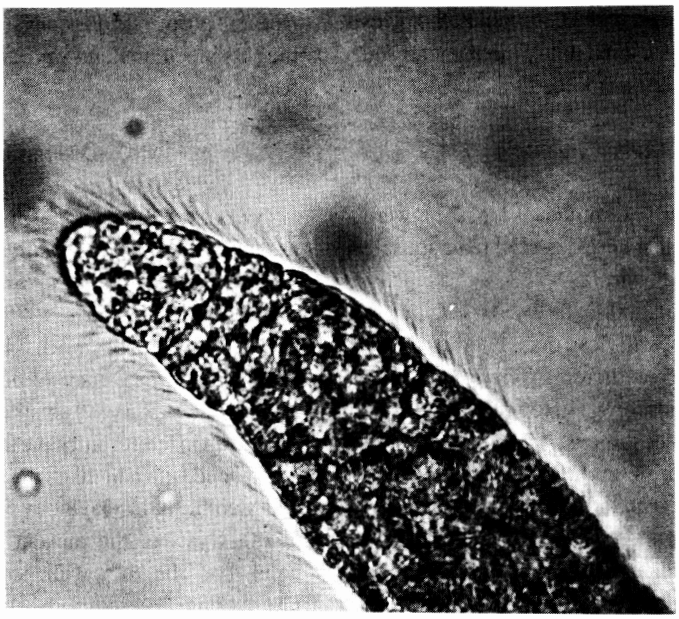

Fig. 2 A fresh cyst removed from striated muscle of a sheep. ×200

Fig. 3 Transmission electron micrograph of a cyst in striated muscle of a sheep. ×2,000

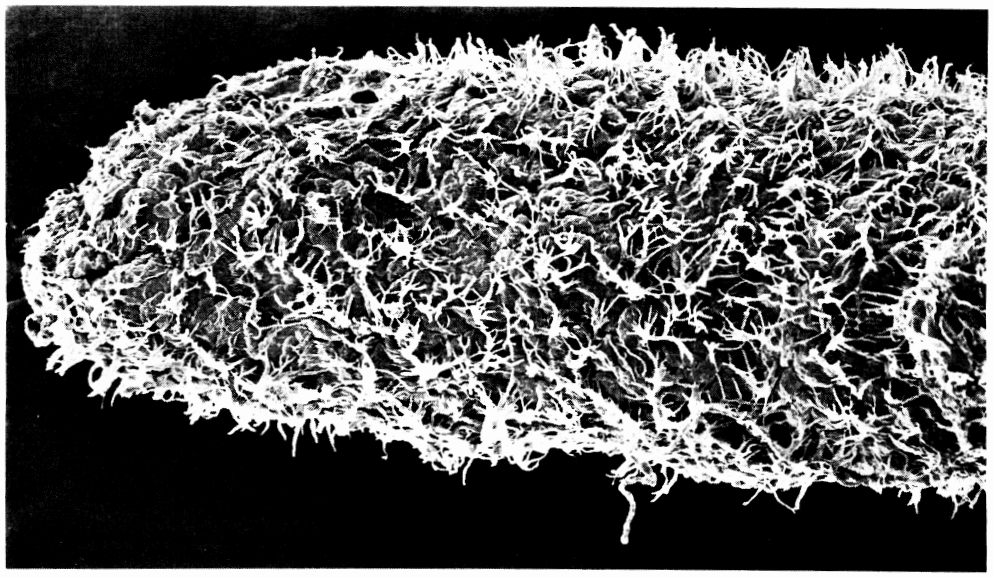

Fig. 4 Scanning electron micrograph of a cyst. ×800

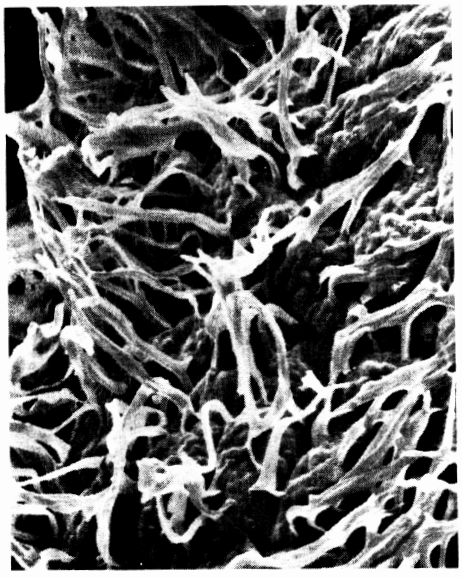

Fig. 5 Magnification of Fig. 4, showing hair-like villar protrusions on the surface of cyst. ×3,500

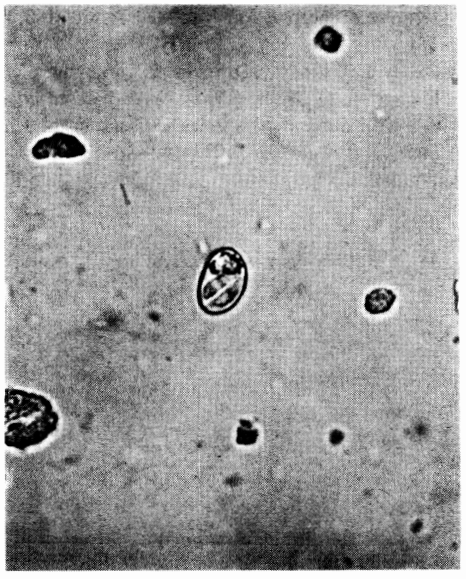

Fig. 6 Sporocyst excreted in the feces of a dog inoculated with cysts. ×400

difference in the length will result from that in the materials used for measurement, histopathological specimens and fresh cysts, and further that in different developmental stages of the parasite.

Oral inoculation of dogs with fresh cysts of the present species resulted in shedding of oocysts and sporocysts in the feces, but not in cats. The prepatent period in the present study was almost the same as

previously reported. The size of sporocysts passed in the feces was also the same although the lower limit of the length was greater than that described previously. This difference will result from that in the time of measurement in life cycle of the parasite (Heydorn, 1985; Dubey *et al.* 1989).

From the above morphological and developmental features, the present species was identified as *S. arieticanis* Heydorn, 1985 and its incidence in Japan was established by the present study.

Of the 4 *Sarcocystis* species from sheep (Dubey *et al.* 1989), *S. tenella* has been the only species reported from Japan (Imai *et al.* 1989). In the present survey also, *S. tenella* was identified, in addition to *S. arieticanis*, by light- and electron-microscopy and successful inoculation to dogs and found in 45% of 100 sheep examined.

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