Sarcocystis suihominis Detected for the First Time from Pigs in Japan

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ABSTRACT. *Sarcocystis suihominis* was detected for the first time in Japan from the heart and diaphragm of 5 out of 600 older culled breeding pigs slaughtered in Saitama Prefecture, Japan. Fresh cysts were 1,080–2,040 × 106–170 μm in size. Bradyzoites measured 15 × 4 μm on average. The cyst wall was usually observed thick, 4–6 μm, and striated, but occasionally thin and smooth according to the difference in sectioning angle and in portion of cysts. Scanning electron microscopy showed that many palisade-like villar protrusions, 6–7 × 0.3–0.5 μm in size, were closely folded onto the surface of cyst. A small number of microtubules were seen in the core of protrusion. No dogs nor domestic cats fed with 20 fresh cysts each excreted oocysts or sporocysts in the feces throughout the experimental period of 30 days. — KEY WORDS: identification, morphology, *Sarcocystis suihominis*, swine.

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Three *Sarcocystis* species have been recorded from pigs: *S. miescheriana, S. porcifelis*, and *S. suihominis*. Of these species, only *S. suihominis* has not been reported from Japan [7, 9]. The final host of *S. miescheriana, S. porciferis* and *S. suihominis* is dogs, cats, and humans respectively [1–6, 9, 11].

Cysts detected from pigs slaughtered in Saitama Prefecture were different in morphology from those of *S. miescheriana* [2, 3, 9] which has been recorded from Japan. Cysts obtained from the diaphragm and heart were morphologically examined and fed to dogs and domestic cats to identify the species.

MATERIALS AND METHODS

Blocks of cardiac muscle and diaphragm, 100 g each, were obtained from 600 older culled breeding pigs slaughtered at an abattoir in Saitama Prefecture from October, 1996 to January, 1997. All the pigs were bred in the same piggery, not introcuded from abroad.

Fifty fresh cysts each collected from both habitats were observed by light and electron microscopy and measured with a micrometer [8]. Part of fresh cysts were fixed with 10% formalin and postfixed with 1% osmic acid. After that they were dehydrated in a series of ethanol and dried at the critical point. After platinum was deposited on the surface, the cyst samples were observed with a scanning electron microscope (Nihon Denshi, JSM-35C, Japan) for the surfacial fine structure such as villar protrusions.

For histopathological examination, small muscle blocks containing *Sarcocystis* cysts were removed under a dissecting microscope and fixed with 10% formalin. The fixed specimens were embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin and observed under a light microscope. Part of the fixed specimens were postfixed 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 ultrastructures of cyst wall with a transmission electron microscope (Nihon Denshi 100 CX, Japan).

Fresh bradyzoites were measured with a micrometer after released from a cyst on a slide glass with a dissecting needle.

Twenty fresh cysts each were fed to 2 female mongrel dogs, 6 months old, and 2 female domestic cats, 4 months old, together with a small amount of pet food. Another female mongrel dog, 6 months old, and female cat, 4 months old, were used as control. All the animals fed with cysts and controls were examined for sporocysts passed in the daily total amount of feces by the flotation method with saturated NaCl solution.

RESULTS

Of the 600 pigs examined, 5 animals (0.83%) were positive for cysts in muscle of the diaphragam and/or heart.

Fresh cysts measured 1,080–2,040 × 106–107 μm in size and had the thick wall, 4–6 μm thick. The cyst wall had many protrusions. Fresh bradyzoites measured an average of 15 × 4 μm (n=50).

Histopathological observation showed that the cyst wall was usually observed thick and striated, but occasionally thin and smooth according the difference in sectioning angle and portion of cysts. Cysts contained many bradyzoides and a small number of metrocytes. No inflammatory reaction was observed in the tissue around cysts.

Scanning electron microscopy showed that palisade-like villar protrusions, 6–7 × 0.3–0.5 μm in size, were closely folded onto the surface of cyst. Innumerable microdepressions were located on the surface of cyst wall among the protrusions.

No dogs nor domestic cats fed with fresh cysts passed oocysts or sporocysts in the feces throughout the experimental period of 30 days.

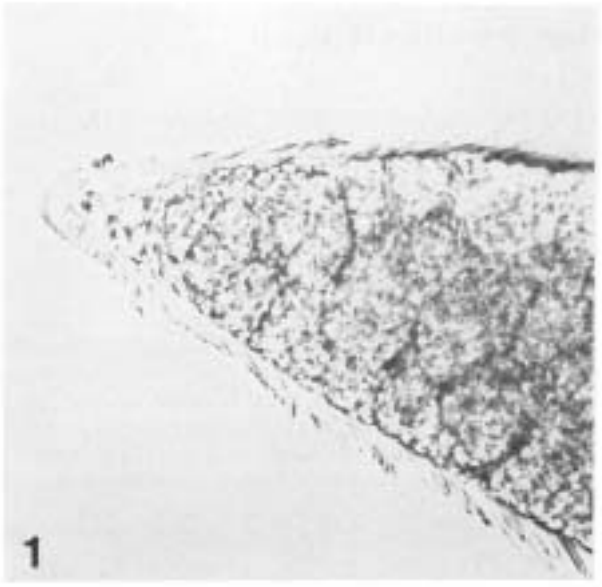

Fig. 1. A fresh *Sarcocystis suihominis* cyst removed from striated muscle of a pig. × 200.

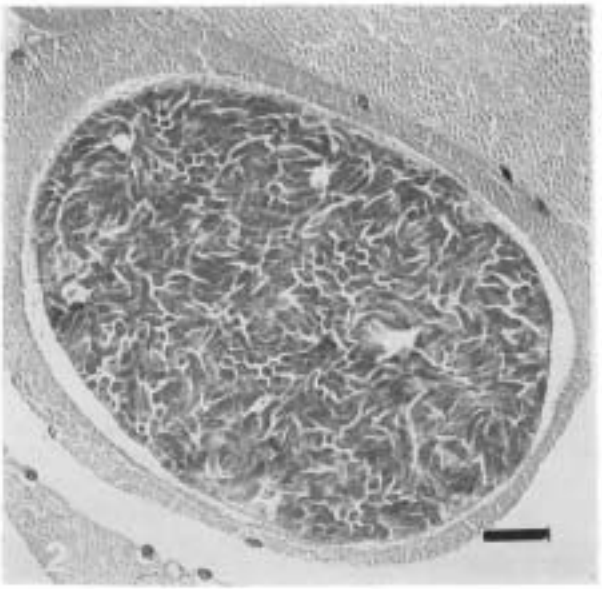

Fig. 2. Transverse section of a cyst near the extremity, located in striated muscle of a pig. Note the thick wall with long protrusions which in general are folded over. H. E. stain, Bar = 10 μm, × 200.

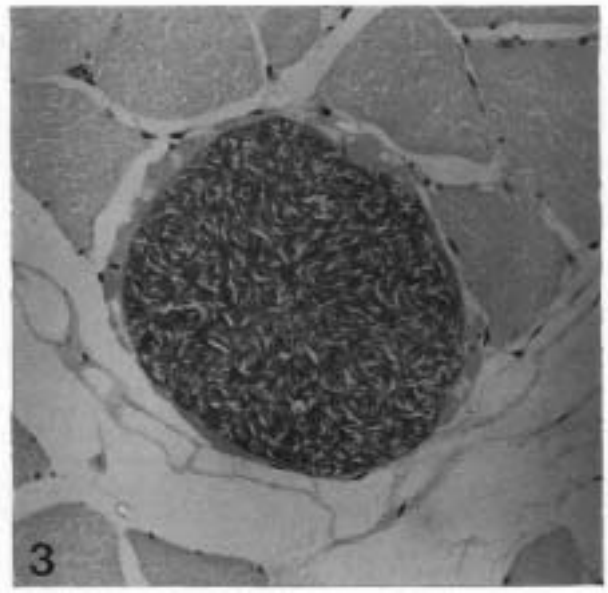

Fig. 3. Transverse section of a cyst near the center, located in striated muscle. H. E. stain, × 200.

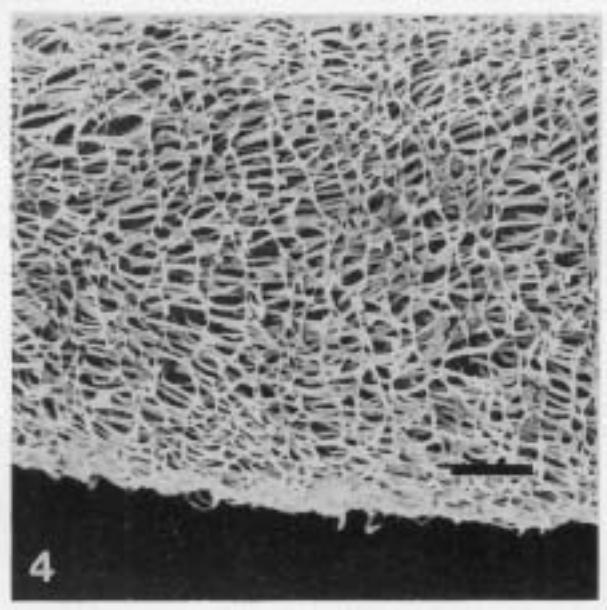

Fig. 4. Scanning electron micrograph (SEM) of a cyst, showing protrusions. Bar=10 μm, × 2,000.

DISCUSSION

Sarcocystis species reported from pigs can be identified by the morphological features of cysts, especially thickness and fine structure of the wall, and specificity to final host in addition to prepatent period. Cysts have the distinct morphology and maximum size characteristic of each species, and the wall of mature cyst is smooth or has numerous protrusions, of which size, morphology and arrangement are distinct between the species [11].

Cysts of *S. suihominis* have been reported to have a length up to 1,500 μm and the wall is thick, up to 6 μm, and provided with palisade-like villar protrusions, up to 13 μm in length, or thin and smooth. The villar protrusions are closely folded onto the surface of cyst and each contain microtubules arranged in pairs [6].

The cyst wall of the present species was usually observed

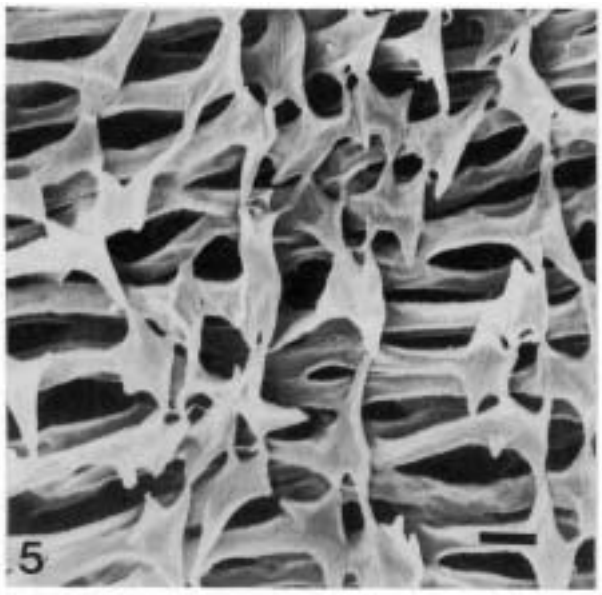

Fig. 5. SEM of a cyst, showing palisade-like villar protrusions on cyst wall. Bar=1 μm, × 4,500.

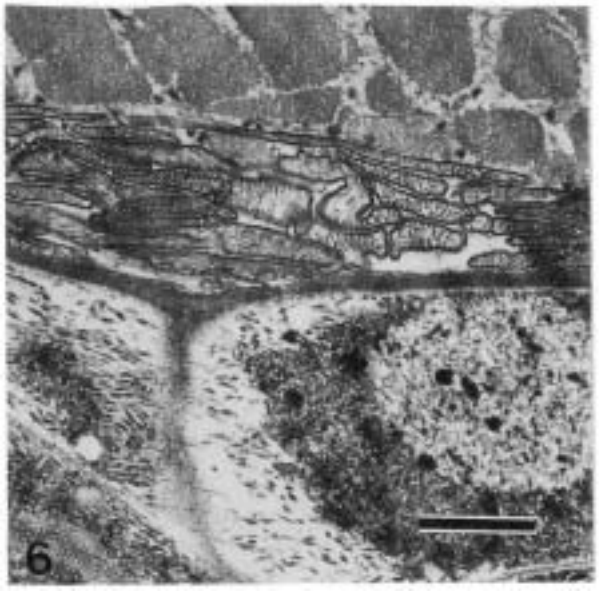

Fig. 6. Transmission electron micrograph of a cyst in striated muscle. Bar=1 μm, × 6,000.

thick and striated, but occasionally thin and smooth by light microscopy. In *Sarcocystis* species parasitic in domestic animals, this difference of the wall by light microscopy causes not only by that in the sectioning angle of cysts but also by presence of either the palisade-like or hair-like protrusions on the wall [10]. Additionally, thickness of the wall will vary in different developmental stages of cysts used, as shown in the difference between Mehlhorn and Heydorn [6] and the present authors.

Heydorn [4] clarified the life cycle of *S. suihominis*. Humans excrete sporocysts in the feces but no dogs nor domestic cats pass sporocysts, when fed with muscular tissue of experimentally infected pigs containing mature *S. suihominis* cysts. In the present experiment also, any of the dogs and cats fed with muscle containing cysts did not passed sporocysts in the feces although experimental infection with volunteers or primates could not be carried out.

By the above morphological features and ingestion experiments, the present species was identified as *S. suihominis* Heydorn, 1977 and its incidence in Japan was established by the present study.

In the present study another porcine *Sarcocysts* species, *S. miescheriana*, was also detected in 7% of the 600 pigs examined in addition to *S. suihominis*.

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