[Jpn. J. Parasitol., Vol. 44, No. 3, 218–221, June, 1995]

Morphology and Experimental Definitive Hosts of *Sarcocystis* sp. from Sika Deer, *Cervus nippon centralis*, in Japan

MORIHIRO SAITO[1], TADASHI ITAGAKI[2], YUTAKA SHIBATA[1] AND HIROSHI ITAGAKI[3]

[1]*Kumagaya Meat Inspection Center, Saitama Prefecture, 179-1 Shimomasuda, Saitama, 360, Japan.*
[2]*Department of Parasitology, Iwate University, Faculty of Agriculture, 3-18-8, Ueda, Morioka, Iwate, 020, Japan.*
[3]*Department of Parasitology, Azabu University School of Veterinary Medicine, 1-17-71 Fuchinobe, Sagamihara, Kanagawa, 229, Japan*

(Accepted June 1, 1995)

Abstract

Sarcocystis cysts were detected from the skeletal muscle of sika deer, *Cervus nippon centralis*. Sarcocysts were elongate, 400–600×40–72 μm in size and interiorly divided by the septa into compartments. The cyst wall was 6–10 μm thick and had hair-like surface protrusions. Transmission electron microscopy revealed that the primary cyst wall (pcw) consisted of a parasitophorous vacuole membrane (pvm) and an electron dense layer immediately beneath the pvm. The protrusions of the wall was more than 5 μm in length and had microtubules. Raccoon dogs and dogs experimentally fed with skeletal muscle of the deer infected with sarcocysts passed oocysts (19–20×18–18.5 μm) and sporocysts (15.5–16.5×11–12 μm) in the feces from day 11 to days 68–70 after ingestion respectively. No domestic cats fed with the same muscle excreted oocysts nor sporocysts throughout the experiments.

Key words: *Cervus nippon centralis*; dog; experimental infection; morphology; raccoon dog; *Sarcocystis*.

Introduction

Many species of *Sarcocystis* have been reported from the family Cervidae. The sika deer, *Cervus nippon*, is widely distributed in Japan and divided into 6 subspecies: *C. n. yesoensis, C. n. centralis, C. n. nippon, C. n. mageshimae, C. n. yakushimae* and *C. n. keramae*.

Sarcocysts were found in the skeletal and esophageal muscles of *C. n. centralis* in Iwate Prefecture, Japan. The purpose of the present study is to describe the morphological characteristics of *Sarcocystis* sp. detected from *C. n. centralis* and to determine definitive carnivore host by experimental infection of raccoon dogs, dogs and domestic cats with sarcocysts.

Correspondence: Morihiro Saito
斉藤守弘[1]，板垣　匡[2]，柴田　穣[1]，板垣　博[3]
([1]埼玉県食肉検査センター，[2]岩手大学農学部家畜寄生虫病学教室，[3]麻布大学獣医学部寄生虫学教室)

Materials and Methods

Sarcocysts

Sarcocysts were removed from the skeletal muscle of a male *C. n. centralis* slaughtered at a deer farm in Sanriku-cho, Iwate Prefecture, Japan.

Morphological observation of sarcocysts and bradyzoites

Fresh sarcocysts recovered from infected skeletal muscle and bradyzoites artificially removed from sarcocysts were measured with a micrometer under a light microscope. A portion of the infected skeletal muscle with sarcocysts was fixed in 10% formalin for histological examination. Paraffin-embedded muscle blocks were sectioned at 5 μm thick, and the sections were stained with hematoxylin-eosin for light microscopy. A part of the formalin-fixed skeletal muscle was postfixed in 1% osmium tetraoxide solution and then processed for ultrastructural observation.

Experimental infections of carnivores with sarcocysts

A portion of fresh skeletal muscle infected with sarcocysts was used for experimental infections of carnivorous animals. Two male raccoon dogs, *Nyctereutes procyonoides viverrinus*, about 3 years old, 2 male dogs 6 months to 1 year old, and 2 male mixed-bred domestic cats, 1 year of age, were each fed with 400 g of infected skeletal muscle. One raccoon dog, one dog and one cat were used as untreated controls. All the feces excreted by each animal were daily collected from days 5 to 70 after ingestion. Fecal samples were examined for oocysts and sporocysts by the flotation method with saturated NaCl solution.

Morphological observation of oocysts, sporocysts and sporozoites

Oocysts and sporocysts detected by fecal examination and sporozoites artificially excysted from sporocysts were observed morphologically and measured with a micrometer under a light microscope.

Results

Morphology of sarcocysts

Light microscopy revealed that fresh sarcocysts were elongated and measured 510.5±57.4×57.6±8.7 μm (n=50) (Fig. 1). Sarcocysts had the thick hairy wall of 6–10 μm thick and were interiorly divided by the septa into the compartments which contained a great number of elongate crescent bradyzoites and a small number of metrocytes (Fig. 2). Bradyzoites measured 12.9±1.0×3.5±0.5 μm (n=50) and had a

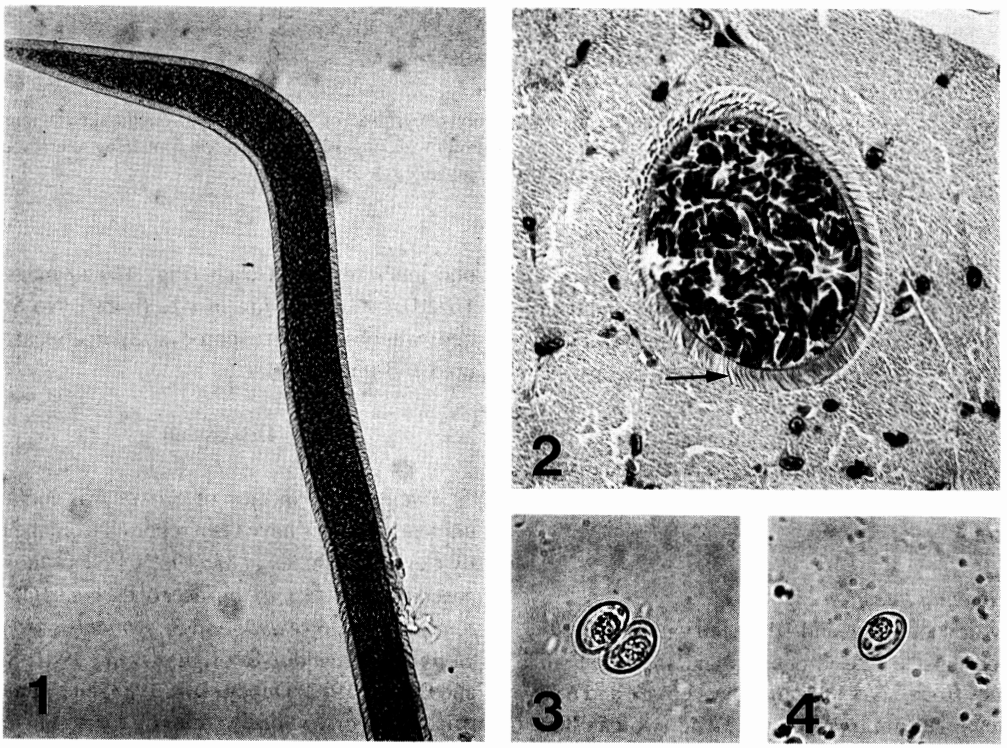

Fig. 1 A fresh elongate sarcocyst recovered from the skeletal muscle of a sika deer. ×100

Fig. 2 Light micrograph of a *Sarcocystis* cyst from the skeletal muscle of a sika deer. Note the cyst wall with hairy protrusions (arrow). H&E stain. ×200

Fig. 3 An oocyst of *Sarcocystis* sp. excreted in the feces of a raccoon dog. ×400

Fig. 4 A sporocyst of *Sarcocystis* sp. excreted in the feces of a raccoon dog. ×400

Fig. 5 Transmission electron micrograph of the peripheral portion of cyst wall of *Sarcocystis* sp. from the skeletal muscle of a sika deer, showing host cell (Hc), primary cyst wall (Pcw) with finger-like protrusions containing microtubules (arrows), granular electron dense layer (G), septa (S) and bradyzoites (B). ×5,000

round nucleus. Ultrastructurally the primary cyst wall (Pcw) consisted of a parasitophorous vacuole membrane and an electron dense layer immediately beneath the membrane. The wall had finger-like protrusions, more than 5 μm in length, provided with microtubules (arrows). The granular electron dense layer (G) was found beneath the Pcw and extended interiorly into the cyst as the thin septa (S) (Fig. 5).

Experimental infection

Two raccoon dogs and 2 dogs fed with sarcocyst-infected muscle passed oocysts and sporocysts in the feces from day 11 through days 68–69 and 68–70 after ingestion, respectively. The two cats fed with the same muscle and non-inoculated control animals excreted no oocysts nor sporocysts in the feces throughout the experiments. Oocysts were colorless, thin-walled and 19.5±0.5×18.3±0.3 μm in size (n=50) and contained 2 mature sporocysts each (Fig. 3). Sporocysts had 4 mature sporozoites and one inner residuum each (Fig. 4) and measured 16.0±0.4×11.5±0.5 μm in size (n=50). No Stieda body was observed in a sporocyst. Sporozoites measured 7×3 μm (n=50).

Discussion

Twenty five species of *Sarcocystis* including unidentified ones have been reported from the family Cervidae (Dubey *et al.*, 1989). Five of those 25 species were detected from deer of the genus *Cervus*: *S. cervicanis, S. wapiti* and *S. sybillensis* from *C. elaphus* (Hernandez-Rodriguez *et al.*, 1981: Speer and Dubey, 1982: Dubey *et al.*, 1983) and 2 unidentified *Sarcocystis* species from *C. dama* (Entzeroth *et al.*, 1985; Poli *et al.*, 1988). Compared with those 5 species, the present *Sarcocystis* species detected from *C. n. centralis* had the following morphological characteristics that the cyst wall is thick (<10 μm) as *S. sybillensis* in contrast to those of *S. cervicanis, S. wapiti*, and *Sarcocystis* spp. from *C.*

dama. Furthermore, the protrusions of cyst wall are elongate and finger-like in the present species and *S. sybillensis*, whereas they are stubby in *S. cervicanis* and sheet-like in *Sarcocystis* spp. from *C. dama*. Since the structure of the cyst wall seems to be a reliable criterion for distinguishing the species of *Sarcocystis* in a given host (Mehlhorn *et al.*, 1976), the present species of *Sarcocystis* is obviously different from *S. cervicanis, S. wapiti* and *Sarcocystis* spp. from *C. dama*. The present *Sarcocystis* sp. from *C. n. centralis*, on the other hand, almost coincided with *S. sybillensis* in respect of the structure of cyst wall and the measurements of bradyzoites, oocysts, and sporozoites. From these morphological features the present species from *C. n. centralis* closely resembled *S. sybillensis*.

Further studies such as cross transmission experiments between *S. sybillensis* from *C. elaphus* and the present species from *C. n. centralis* and phylogenetic study based on nucleotid sequence will be needed for accurate identification of the species from *C. n. centralis*.

References

1) Dubey, J. P., Jolley, W. R. and Thorne, E. T. (1983): *Sarcocystis sybillensis* sp. nov. from the North American elk (*Cervus elaphus*). Can. J. Zool., 61, 737–742.
2) Dubey, J. P., Speer, C. A. and Fayer, R. (1989): Sarcocystosis of Animals and Man. CRC Press, Inc., Boca Raton, Florida, 215 pp.
3) Entzeroth, R., Chobotar, B., Scholtyseck, E. and Nemeseri, L. (1985): Light and electron microscope study of *Sarcocystis* sp. from the fallow deer (*Cervus dama*). Z. Parasitenkd., 71, 33–39.
4) Hernandez-Rodriguez, S., Martinez-Gomez, F., Navarrete, I. and Acosta-Garcia, I. (1981): Estudio al microscopio optico y electronico del quiste de *Sarcocystis cervicanis*. Rev. Iber. Parasitol., 41, 351–361.
5) 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.
6) Poli, A., Mancianti, F., Marconcini, A., Nigro, M. and Colagreco, R. (1988): Prevalence, ultrastructure of the cyst wall and infectivity for dog and cat of *Sarcocystis* sp. from fallow deer (*Cervus dama*). J. Wildl. Dis., 24, 97–104.
7) Speer, C. A. and Dubey, J. P. (1982): *Sarcocystis wapiti* sp. nov. from the North American wapiti (*Cervus elaphus*). Can. J. Zool., 60, 881–888.