Our results indicated that the 49-kDa soluble antigen was specific to the merozoites and that the antigen was located inside the membrane at the apical end. These findings were consistent with observations in a previous study (Omata et al., 2001), and it seems that the 49-kDa antigen is located on the microneme of the merozoites. Several apicomplexan parasites have micronemal proteins, which play important roles in gliding motility on solid substrates (in the case of those recognized as trail antigens) or in the penetration of host cells (Sibley et al., 1998). The microneme proteins have been found to conserve structural domains (Tomley and Soldati, 2001). However, in the present study, we have no data to show the molecular or genetic characteristics of the 49-kDa antigen. To clarify the similarity and specificity between these antigens, it will be necessary to determine the amino acid sequences of the 49-kDa antigen.

Immunization with the 49-kDa antigen provided protection against the infection. In immunized rabbits, GGT activity and ICG clearance activity showed no change throughout the experiment. This means that the rabbits had no cholestasis in their biliary ducts. Interestingly, a transient increase of ALT activity in the rabbits was observed on day 8 p.c. Histology of the rabbits revealed inflammatory reactions around the biliary ducts. These results suggest that hepatocellular damage may have occurred in the immunized rabbits. They also suggest that the inflammatory reaction around the biliary ducts may have inhibited the parasite's development. To clarify this hypothesis, further study is necessary to determine the immune responses to the 49-kDa antigen in the infection.

This work was supported in part by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science, Sports, and Culture, Japan.

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Ephemera strigata (Insecta: Ephemeroptera: Ephemeridae) is the Intermediate Host of the Nematodes *Rhabdochona denudata honshuensis* and *Rhabdochona coronacauda* in Japan

R. Hirasawa and M. Urabe*, Center for Ecological Research, Kyoto University, Otsuka, Hirano-cho, Kamitanakami, Otsu 520-2113, Japan; *Fukuoka University of Education, Akama 1, Munakata, Fukuoka 811-4192, Japan. *e-mail: riyo@ecology.kyoto-u.ac.jp*

ABSTRACT: Juvenile freshwater parasitic nematodes *Rhabdochona denudata honshuensis* Moravec and Nagasawa, 1989 and *Rhabdochona coronacauda* Belouss, 1965 (Spirurida: Thelazioidea: Rhabdochonidae) were found in mayfly nymphs collected in a mountain stream in Japan. Considering the relative density of mayfly nymphs, nematode prevalence, and intensity of parasitism, *Ephemera strigata* Eaton and *Potamanthus formosus* Ulmer (Ephemeroptera: Ephemeridae) are frequent natural intermediate hosts for *R. d. honshuensis* in this locality. The intermediate host of *R. coronacauda* also is the *E. strigata* nymph.

Nematodes in the genus *Rhabdochona* Railliet, 1916 (Spirurida: Thelazioidea: Rhabdochonidae) are parasites found in the digestive tracts of freshwater fishes. In this genus, eggs are deposited, and they hatch after they have been ingested by their intermediate hosts. The definitive hosts ingest infected intermediate hosts (Moravec, 1972, 1976). The larvae of some species have been recorded in mayflies and infrequently in stoneflies (Plecoptera) and caddisflies (Trichoptera) (Gustafson, 1939, 1942; Shtein, 1959; Vojatkova, 1971; Moravec, 1972, 1976, 1977, 1989, 1995; Byrne, 1992; Barger and Janovy, 1994; Shimazu, 1996).

In Japan, adult *Rhabdochona denudata honshuensis* Moravec and Nagasawa, 1989 have been recorded from *Zacco platypus* (Temminck & Schlegel), *Zacco temmincki* (Temminck & Schlegel), *Triborodon hakonensis* Günther, and *Phoxinus oxycephalus* (Jordan & Snyder) (Moravec and Nagasawa, 1989; Mori et al., 1998). However, the intermediate hosts of *R. d. honshuensis* remain unknown. Because there is no

TABLE I. Measurements (mm) of *Rhabdochona denudata* (n = 10) and *R. coronacauda* (n = 11) fourth-stage juveniles from *Ephemera strigata* (range is in parentheses). Only female larvae were measured.

	R. denudata		*R. coronacauda*	
	Mean	Range	Mean	Range
Body length	2.7212	(2.2946–2.9317)	1.3078	(0.8972–1.5773)
Body width	0.0860	(0.0756–0.0983)	0.0312	(0.0291–0.0356)
Muscular esophagus	0.2253	(0.1791–0.2923)	0.1640	—
Glandular esophagus	1.0266	—	0.3858	(0.3628–0.4000)
Prostom length	0.0085	(0.0078–0.0104)	0.0060	(0.0043–0.0077)
Prostom width	0.0073	(0.0044–0.0093)	0.0068	(0.0065–0.0074)
Vestibule length (including prostom)	0.0550	(0.0274–0.0820)	0.0434	(0.0191–0.0841)
Tail length	0.1112	(0.0759–0.1533)	0.0724	(0.0631–0.0807)

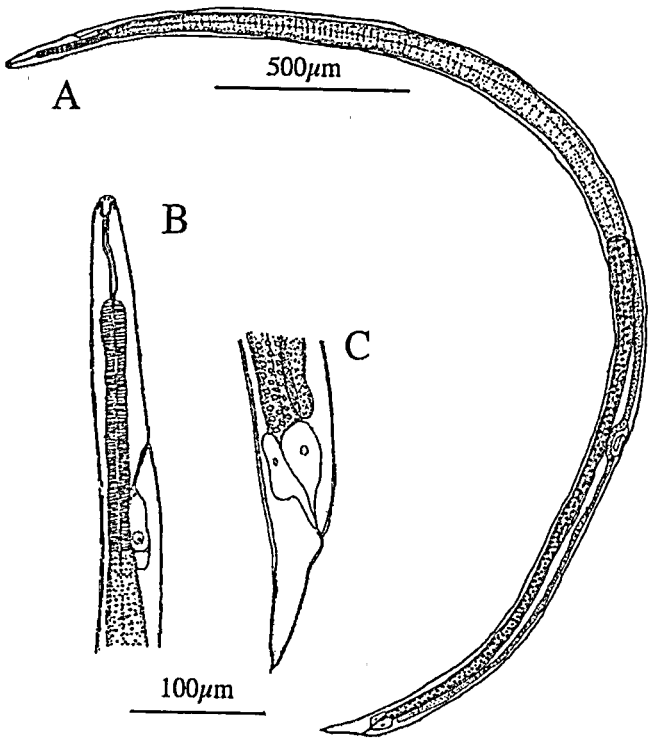

FIGURE 1. *Rhabdochona denudata honshuensis* Moravec and Nagasawa, 1989 fourth-stage juvenile (female) from intermediate hosts, *Ephemera strigata* Eaton. **A.** Entire body, lateral view. **B.** Anterior end of the body. **C.** Posterior end of the body.

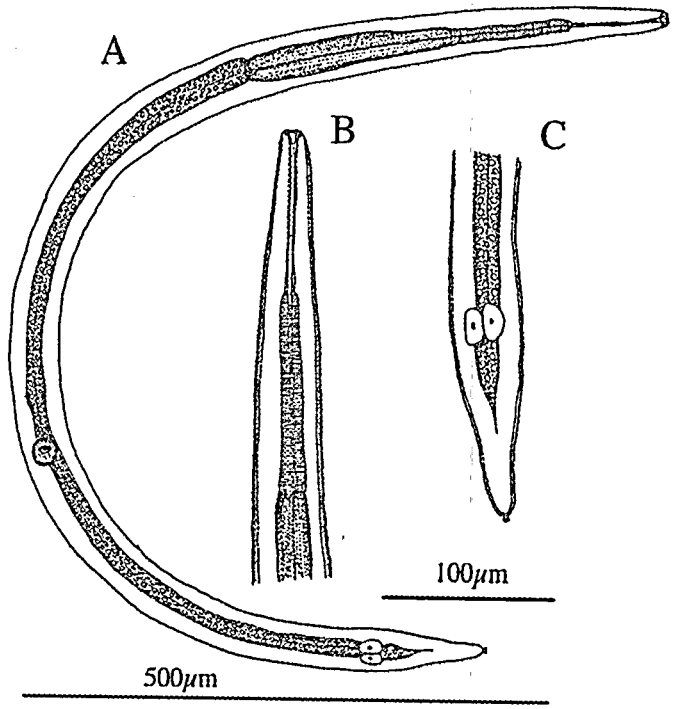

FIGURE 2. *Rhabdochona coronacauda* Belouss, 1965 fourth-stage juvenile (female) from intermediate hosts, *Ephemera strigata* Eaton. **A.** Entire body, lateral view. **B.** Anterior end of the body. **C.** Posterior end of the body.

other subspecies of this species in Japan, we refer to the nematode with the binomial *R. denudata*. In nature, the nominate subspecies, *R. d. denudata* (Dujardin, 1845), uses mayfly nymphs of *Heptagenia* spp. and *Ephemerella* spp. as intermediate hosts in the Republic of Karelia (Shtein, 1959) and *Ephemera danica* Müller, *Ecdyonurus aurantiacus* (Burmeister), and *Caenis macrura* Stephens in the Czech Republic (Moravec, 1989).

Rhabdochona coronacauda Belouss, 1965 was redescribed by Moravec et al. (1981) from the cyprinid *Opsariichthys uncirostris* (Schlegel) in Japan. Another definitive host is the catfish *Liobagrus reini* Hilgendorf (Moravec and Nagasawa, 1998). The intermediate hosts of *R. coronacauda* remain unknown.

Mayfly nymphs were collected in the Takami River (the Yoshino River drainage system), Higashi-Yoshino, Nara Prefecture, central Japan, from June 1997 to May 1998. The mayfly nymphs were identified to species following the study by Gose (1985). They were dissected under a stereoscopic microscope to look for nematode infection. Drawings were made with the aid of a Nomarski interference microscope drawing attachment. Scanning electron microscopy (SEM) was used to observe organs that were too small to be seen with the optical microscope. Fourth-stage juveniles of *R. denudata* and *R. coronacauda* were found in *E. strigata* Eaton. Voucher specimens of these nematodes and the *E. strigata* nymphs were deposited in the Lake Biwa Museum, Kusatsu City, Shiga Prefecture, Japan (accession nos. LBM Misc. Invert. FY2002-24 and LBM Aq45–47).

Rhabdochona denudata juveniles were found in the host hemocoel (Fig. 1; for measurements, see Table I), where they formed spiral coils in thin-walled, hyaline, lens–shaped capsules. The body resembled that of the adult nematode. The cuticle is smooth, and the head end is rounded. Deirids are situated at midvestibule and are less than 1 μm in length and are observable only by SEM. The sclerotized vestibule (stoma) is straight and narrow, and it widens at the anterior end to form a funnel-shaped prostom. The esophagus is rather long, and is clearly divided into an anterior, shorter, narrower, muscular portion and a posterior, much wider glandular portion. The tail is conical and pointed at the tip without any caudal accessories. The presence of the vulva, vagina, tubular ovaries, and uterus characterized female juveniles, and some had many eggs in their ovaries. All 10 nematodes observed were female; the male characteristics in the larval stage remain unknown.

Mori et al. (1998) reported that the prevalence of *R. denudata* in the fish definitive host, *Z. temmincki*, increased from spring to early summer. This shows that the intermediate hosts were present at that time. Twenty-eight species of mayfly nymphs were collected in April, June, July, and August 1997 using a Surber net sampler (30 × 30 cm; mesh size, 350 μm) to determine the prevalence and mean intensity of infection of fourth-stage juveniles of *R. denudata* in mayfly nymphs. In most cases, *E. strigata* were parasitized by *R. denudata* (n = 59; prevalence, 55.9%; mean intensity, 2.6) (Table II). In addition to *E. strigata*, nymphs

TABLE II. Prevalence and mean intensity of *Rhabdochona denudata* and *R. coronacauda* in mayfly nymphs from the Takami River. Mayflies were collected quantitatively in April, June, July, and August in 1997. n, sample size; %, prevalence; $\bar{x}$, mean intensity.

		Nematode species			
		R. denudata		*R. coronacauda*	
Mayfly species (nymph)	n	%	$\bar{x}$	%	$\bar{x}$
Siphlonuridae					
(*Siphlonurs binotatus*)	4	25.0	2.0	0	—
Leptophlebiidae					
(*Choroterpes trifurcata*)	31	0.9	1.0	0	—
Ephemerellidae					
(*Ephemerella setigera*)	30	0.9	2.0	0.9	2.0
Potamanthidae					
(*Potamanthus formosus*)	304	10.7	3.7	0	—
Ephemeridae					
(*Ephemera strigata*)	59	55.9	2.6	18.6	3.2

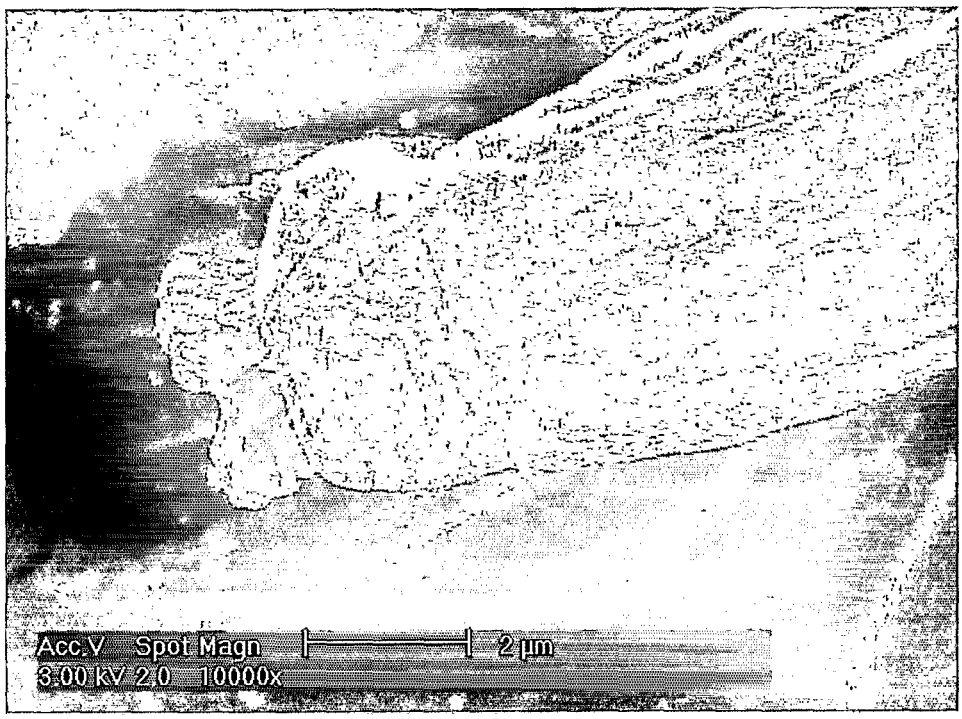

FIGURE 3. SEM of the tail end of *Rhabdochona coronacauda* fourth-stage juvenile from intermediate hosts, *Ephemera strigata* Eaton. Bar = 2 µm.

of 4 species of mayfly were infected with *R. denudata*: *Potamanthus formosus* Ulmer, *Siphlonurus binotatus* Eaton, *Choroterpes trifurcata* Uéno, and *Ephemerella setigera* Bajkova. Considering the abundance of nematodes in each mayfly species (prevalence × mean intensity × number of mayflies), it is thought that *P. formosus* is the most important intermediate host of this nematode. In light of the mayfly life cycle and the sampling period, however, the relative density of *E. strigata* nymphs was underestimated in these months. Although *E. strigata* is an annual species and emerges during a short period in late spring, few nymphs were collected in the stream from June to August because of their small size in early instars. Conversely, *P. formosus* emerges during summer. Considering the relative density of mayfly nymphs, nematode prevalence, and intensity of parasitism, *E. strigata* and *P. formosus* seem to be the main natural intermediate hosts in this locality.

Rhabdochona coronacauda were found in the host hemocoel (Fig. 2; for measurements, see Table I), where they formed spiral coils in thin-walled, hyaline, lens–shaped capsules that were smaller than those of *R. denudata* juveniles. The body resembled that of the adult nematode, but the organs were not developed except for the intestine. Deirids are situated at midvestibule. They are less than 1 µm in length and observable only by SEM. The cuticle is smooth and the head end is rounded. The tail is conical, with an undetermined number of minute processes encircling its truncated tip to form the corona (Fig. 3). Although the shape of the corona varied among individuals, each process was sharper than that of the adult. The presence of the vulva and vagina characterized female juveniles. All 11 nematodes observed were female, and male characteristics in the larval stage remain unknown.

Of the mayfly nymphs collected in April, June, July, and August 1997, fourth-stage juveniles of *R. coronacauda* were found mostly in *E. strigata* nymphs (n = 59; prevalence, 18.6%; mean intensity, 3.2) (Table II). Besides *E. strigata*, nymphs of *E. setigera* were infected with *R. coronacauda*. Considering the relative density of mayfly nymphs, nematode prevalence, and intensity of parasitism, *E. strigata* seems to be the main natural intermediate host in this locality.

We thank Dr. František Moravec (Institute of Parasitology, Academy of Sciences of the Czech Republic) for assisting with the identification of these nematodes.

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Development of Patent Infection in Immunosuppressed C57BL/6 Mice with a Single *Cryptosporidium meleagridis* Oocyst

Kehe Huang, Donna E. Akiyoshi*†, Xiaochuan Feng*, and Saul Tzipori*, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu Province, People's Republic of China. *Department of Biomedical Sciences, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts 01536. †To whom correspondence should be addressed. *e-mail: donna.akiyoshi@tufts.edu*

ABSTRACT: The ability of *Cryptosporidium meleagridis* to produce patent infection was studied in adult C57BL/6 mice that were immunosuppressed with dexamethasone phosphate provided in the drinking water at a dosage of 16 μg/ml. Four days after the onset of immunosuppression, mice were orally challenged with 1, 3, 10, or 1,000 *C. meleagridis* TU1867 oocysts per mouse. The mice were monitored daily for 18 days postinoculation for oocyst shedding. Five of 10 mice given a single oocyst, 4 of 5 mice given 3 oocysts, and all 9 mice given either 10 or 1,000 oocysts became infected and began shedding oocysts 5–7 days after challenge and continued to shed oocysts until the end of the experiment on day 18 postchallenge. Approximately 10^7 oocysts per mouse per day were excreted, regardless of the challenge dose. Neither the noninfected, immunosuppressed nor the inoculated, nonimmunosuppressed control mice shed oocysts. The excreted oocysts were confirmed to be those of *C. meleagridis* by polymerase chain reaction–restriction fragment length polymorphism analysis. We show that *C. meleagridis*, originally classified as an avian pathogen but recently found in humans with cryptosporidiosis, can produce patent infection in mice infected with a single oocyst. Moreover, we demonstrate that the immunosuppressed C57BL/6 adult mouse is an ideal host for the propagation of clonal populations of *C. meleagridis* isolates for laboratory studies.

Cryptosporidium spp., a coccidian protozoan parasite, is a major cause of diarrheal disease in humans and other animals worldwide. Whereas infection is usually self-limiting in immunocompetent individuals, it can be life threatening in immunocompromised individuals (Fayer et al., 1997). Currently, there is no effective treatment against the infection in humans and other animals (Tzipori, 1998). In recent years, there has been an increased awareness of the waterborne transmission of this parasite as a result of several recent outbreaks in the United States, including the 1993 outbreak in Milwaukee, Wisconsin, where over 400,000 people were infected with *Cryptosporidium parvum* (Mackenzie et al., 1994). *Cryptosporidium parvum* remains the major *Cryptosporidium* species that infects mammals including humans and comprises 2 major genotypes (Widmer et al., 1998; Morgan et al., 1999). Other species of *Cryptosporidium*, including *C. meleagridis*, have also been isolated from humans (Pieniazek et al., 1999; Morgan et al., 2000; Pedraza-Díaz et al., 2000; Sreter et al., 2000; Guyot et al., 2001). *Cryptosporidium meleagridis* was first isolated in turkeys in 1955 and was thought to infect only avian species (Slavin, 1955). However, a recent study reported that the causative agent of avian cryptosporidiosis was more likely to be *C. baileyi* and not *C. meleagridis* (Sreter and Varga, 2000). For laboratory studies, *C. meleagridis* is routinely propagated in young chicken or turkey poults. Our laboratory has also propagated *C. meleagridis* in gnotobiotic piglets and interferon-gamma knockout mice (γGKO; Akiyoshi et al., in press). The establishment of infection and propagation of *C. meleagridis* in mammalian hosts is a biological curiosity because it is the only known species that consistently infects several avian and mammalian species. The association with human cryptosporidiosis elevates *C. meleagridis* to a pathogen that warrants further investigation.

In this study, we report the infection of immunosuppressed adult C57BL/6 mice with a single *C. meleagridis* TU1867 oocyst using the dexamethasone phosphate (DEXp) immunosuppression model originally used to study *C. parvum* infections (Yang and Healey, 1993; Yang et al., 2000). Thirty-two female C57BL/6 mice, aged 6–8 wk, obtained from Jackson Laboratories (Bar Harbor, Maine), were randomly divided into 6 groups. In groups 1–3, mice were housed individually in microisolators, whereas in groups 4–6, 4 mice were housed per microisolator. All manipulations were done in a Class II biological safety cabinet, and the microisolators, food, water, and bedding were autoclaved before use. All mice, except those in group 6, were immunosuppressed with DEXp (16 μg/ml; Sigma–Aldrich Chemical Co., St. Louis, Missouri) provided ad libitum in the drinking water for the duration of the experiment (Yang et al., 2000). After 4 days of immunosuppression, mice were orally challenged with 1 oocyst (group 1, 10 mice), 3 oocysts (group 2, 5 mice), 10 oocysts (group 3, 5 mice), or 1,000 oocysts (group 4, 4 mice) of *C. meleagridis*. Mice in group 5 (4 mice) served as the immunosuppressed, uninoculated control group. Mice in group 6 (4 mice) served as the nonimmunosuppressed control group and were orally inoculated with 1,000 oocysts. The *C. meleagridis* TU1867 isolate was originally isolated from a Ugandan child with cryptosporidiosis (Akiyoshi et al., in press). This isolate was propagated by several passages in newborn poults of turkey and then in chicken, from which oocysts were purified and used as inoculum for this study. The oocysts were purified as described by Yang et al. (2000). The procedure of single-oocyst isolation was a modification of the gel-capture technique of Shirley and Harvey (1996). Briefly, oocysts were serially diluted to 1,000 oocysts/ml in sterile water. The suspension was pipetted onto a slide in a volume of 1 μl for a quick microscopic observation. After the presence of 1 oocyst per slide was confirmed using phase-contrast microscopy, 12 μl sterile distilled water was immediately added to overlay the oocyst and mixed by pipetting the suspension several times. Suspensions containing 1 oocyst were used to orally inoculate each mouse. The removal of the single oocyst from the slide was confirmed by phase-contrast microscopy. The same procedure was used for the 3 and 10 oocyst doses.

Mice were monitored daily for infection for 18 days postinoculation (PI). A fecal pellet from each mouse was collected daily per rectum starting on day 3 PI and smeared onto a microscope slide, and the oocysts were enumerated by microscopic examination after modified acid-fast staining (Tzipori et al., 1994). Fecal pellets from each mouse (from day 3 PI to the end of the experiment) were also collected daily. Table I summarizes the results of the study of oocyst dose versus infectivity. Five of 10 mice inoculated with a single oocyst (group 1) and 4 of 5 mice inoculated with 3 oocysts (group 2) developed patent infections. All the mice inoculated with either 10 (group 3) or 1,000 (group 4) oocysts became infected. The uninfected group (group 5) and the nonimmuno-