

RESEARCH NOTES

J. Parasitol., 88(2), 2002, pp. 403–404
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Presence of Genital Spines in a Male *Corynosoma cetaceum* Johnston and Best, 1942 (Acanthocephala)

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ABSTRACT: We collected 83 females and 80 males of *Corynosoma cetaceum* from 2 common dolphins, *Delphinus delphis*, collected in northern Patagonia (Argentina). Worms were most similar to specimens collected in other South American localities. However, 1 male had 2 spines adjacent to the genital pore and isolated from the rest of body spines. This finding confirms the recent reassignment of *C. cetaceum* to *Corynosoma*. Absence of genital spines is suggested to be avoided as the sole criterion to exclude specimens from *Corynosoma* or *Andracantha*.

The presence of genital spines is a major diagnostic trait for species of *Corynosoma* Lühe, 1904. In all but 3 species currently included within the genus, 1 or both genders have been described with genital spines (see references for original descriptions in Golvan, 1994; see also Aznar et al., 1999). In most species, genital spines are always observed in males, whereas in females, depending on the species, genital spines occur in all, part, or none of the specimens examined (Van Cleave, 1953; see also references in Golvan, 1994). Apparently, *Corynosoma cetaceum* Johnston and Best, 1942, is the only species in which females, but not males, possess genital spines (Aznar et al., 1999).

Recently, we had the opportunity to clarify the issue of genital spines in male *C. cetaceum* based on the examination of a sample of specimens collected from the stomach and duodenal ampulla of 2 common dolphins, *Delphinus delphis*, which accidentally drowned in nets of a fishing vessel operating in front of Golfo de San Matías, Northern Patagonia, Argentina (40–42°S, 60–61°W). Dolphins were frozen on board at –20 C and later thawed in the laboratory prior to examination. Acanthocephalans were washed in saline and stored in 70% alcohol. Morphological characters pertaining to the specific assignation were checked in all worms in the sample (83 females, 80 males) using a stereomicroscope. To reveal some anatomical details, some specimens were either dissected or cleared with lactophenol and observed with light microscopy. Morphometric measurements were taken as in Aznar et al. (1999) using 7 mature females and 3 mature males with the proboscides evaginated. One specimen was processed for scanning electron micrography (SEM) using standard methods. Voucher specimens are deposited in the U.S. National Parasite Collection (USNPC 91722, 91723).

Based on morphological data, these specimens were clearly assignable to *C. cetaceum*. Specimens of this species typically mature in the stomach and upper duodenum of cetaceans from the Southern Hemisphere (Aznar et al., 1999, 2001). Morphometric data indicated that specimens closely resembled individuals collected from cetaceans from other localities of the southwestern Atlantic (Aznar et al., 1999). However, 1 of the 80 males examined had 2 small spines adjacent to the genital pore and isolated from the rest of body spines, as typically occurs in males of most *Corynosoma*. One genital spine is pictured in Figure 1.

The finding of a male with genital spines definitively confirms the recent reassignment of *C. cetaceum* to *Corynosoma* (see Aznar et al., 1999). However, this is the first male with genital spines in over 3,000 males examined from different hosts in several geographic areas (Aznar et al., 1999). In other words, the expression of genital spines seems to be variable in male *C. cetaceum*. This was already acknowledged in the case of females of many *Corynosoma* species (Van Cleave, 1953; see also references in Golvan, 1994) and in specimens of 1 or both genders in *Andracantha* Schmidt, 1975 (Schmidt, 1975; Nickol and Kocan, 1982; Richardson and Cole, 1997). Commenting on the latter genus, Richardson and Cole (1997) claimed, “although the presence of genital spines seems to be variable in male *C. cetaceum*. This was already acknowledged in the case of females of many *Corynosoma* species (Van Cleave, 1953; see also references in Golvan, 1994) and in specimens of 1 or both genders in *Andracantha* Schmidt, 1975 (Schmidt, 1975; Nickol and Kocan, 1982; Richardson and Cole, 1997). Commenting on the latter genus, Richardson and Cole (1997) claimed, “although the presence of genital spines seems to be variable in male *C. cetaceum*. This was already acknowledged in the case of females of many *Corynosoma* species (Van Cleave, 1953; see also references in Golvan, 1994) and in specimens of 1 or both genders in *Andracantha* Schmidt, 1975 (Schmidt, 1975; Nickol and Kocan, 1982; Richardson and Cole, 1997).” Collectively, these observations also sug-

gest that the absence of genital spines should be avoided as the sole criterion to exclude specimens from *Corynosoma* or *Andracantha*.

The authors thank Celia Agustí for technical assistance with SEM and Al Bush for reviewing the paper. A. Pucci (CENPAT, CONICET), O. Padín (Dirección de Recursos Acuícolas e Ictícolas, Secretaría de Recursos Naturales y Desarrollo Sustentable), and the staff of the INIDEP and the Secretaría de Agricultura, Ganadería, Pesca y Alimentación from Argentina provided dolphins. Photographs were made thanks

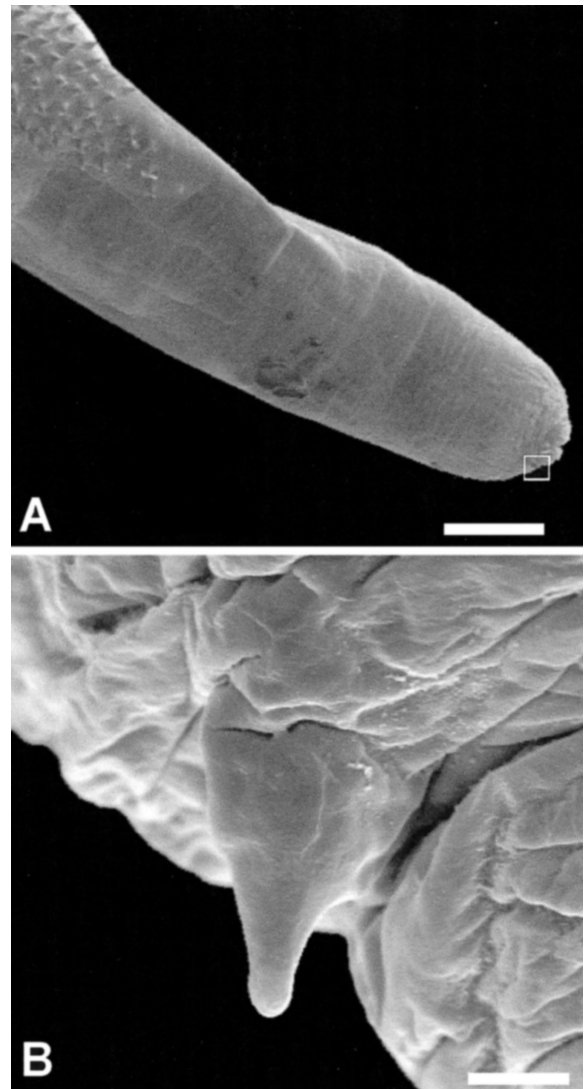


FIGURE 1. **A.** Ventral view of the hindtrunk of a male *Corynosoma cetaceum* collected from a common dolphin, *Delphinus delphis* in northern Patagonia, Argentina. The box encloses the area where 1 of the 2 genital spines observed in this specimen was found. Scale bar = 250 µm. **B.** Detail of the genital spine. Scale bar = 10 µm.

to the SCSIE, University of Valencia. Institutional support was provided by Instituto Cavanilles de Biodiversidad y Biología Evolutiva (U. Valencia, Spain) and Centro Nacional Patagónico (CONICET, Argentina). The study was supported by projects ANPCYT (PICT98 N° 01-04025), Argentina, and DGES PB96-0801 (MEC), Spain. F. J. Aznar benefits from a postdoctoral contract from the MEC of Spain.

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J. Parasitol., 88(2), 2002, pp. 404–405
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First Recovery of *Schistosoma mansoni* Eggs From a Latrine in Europe (15–16th Centuries)

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ABSTRACT: *Schistosoma mansoni* eggs were found in a latrine dated AD 1450–1550 in Montbéliard, France. This is the first record of intestinal schistosomiasis in archaeological material. Because the parasite has an African origin, its presence in Europe at that time may be due to African slaves. However, intestinal schistosomiasis has never been established in Europe.

Human and other animal intestinal parasite eggs have been recorded from a latrine dating AD 1450–1550 in Montbéliard, France (Bouchet and Paicheler, 1995). Reviewing the same material, helminth eggs with a typical *Schistosoma mansoni* lateral spine were also found.

The material studied was composed of coprolites and sediment collected from a latrine found in a house built during the second half of the 15th century. The latrine (2.35 × 2.30 × 1.65 m) is located in an inner courtyard, which was completely closed from the first half of the 16th century up to the recent archaeological excavations. Three stratigraphic units were identified, but only samples of the middle one



FIGURE 1. *Schistosoma mansoni* egg with lateral spine (142 × 58 μm) in archaeological material (US 544) in Montbéliard site.

(US544) were sent for paleoparasitological analysis. The sediment is macroscopically rich in organic remains, fecal material, and artifacts. Dishware and other cultural remains point to very rich dwellings (Cantrelle et al., 2000).

The recovered parasite eggs were found to be very well preserved by the anaerobic and humidity conditions. Thus, the material was not rehydrated by current methods used in palaeoparasitology (Reinhard et al., 1988). Samples were directly submitted to ultrasound for 20 sec then run through graduated sieves (315, 160, 50, and 25 μm). The final sediment recovered from the last 2 sieves was observed under a microscope. Eggs and other organic remains found were measured and photographed.

The eggs presented here were identified by their morphology as *S. mansoni*, size (142 × 58 μm), and lateral spine (Fig. 1). All 4 species classified in the *S. mansoni* group have eggs with a lateral spine. Apart from *S. mansoni*, these species are found only in Africa. *Schistosoma rodhaini* is a parasite found in African rodents and carnivores; *Schistosoma edwardiense*, which have eggs with a rudimentary lateral spine, is a parasite of *Hippopotamus amphibius*; *Schistosoma hippopotami*, in the same host, might be a synonym of *S. mansoni* (Rollinson and Simpson, 1987).

The palaeoparasitology of human schistosomiasis has been discussed by several authors. Wei (1973) described a *Schistosoma japonicum* infection in a mummified body of the Han Dynasty. *Schistosoma japonicum* eggs were found in China in a 2,100-yr-old male corpse (Liangbiao and Hung, 1981). *Schistosoma haematobium* was the first parasite found in an Egyptian mummy dated 5,200-yr-old (Ruffer, 1910). Later, as Egyptian mummy necropsies were performed more frequently, this parasite was found at different periods (Jonckheere, 1942; Reyman, 1973; Cockburn et al., 1975; Lewin, 1977; Capron, 1993; Contis and David, 1996). The advent of new techniques allowed the diagnosis of other cases (Deelder et al., 1990; David, 1997), and a paleoepidemiology of schistosome infection was tentatively drawn (Miller et al., 1992; Araújo and Ferreira, 1997).

There are no records of human schistosomiasis infection outside Asian or African prehistoric populations (Araújo and Ferreira, 1997). *Schistosoma haematobium* infection was reported in Europe in the 19th century but vanished in a short period of time (Brumpt, 1936). *Schistosoma haematobium* eggs dated to the 15th century also were found in France (Bouchet and Paicheler, 1995).

Schistosoma mansoni infection can only have originated from Africa because there are no intermediate hosts in Europe. A returning infected European voyager or a foreigner are 2 possibilities to be examined. Archaeological evidence showed that the house was occupied by a very rich family, which could have afforded a voyage to an epidemic region. However, there is no archaeological evidence that such a voyage was made. No souvenirs or exotic artifacts have been found. On the other hand, a slave could have introduced it. Since 1441, African slaves were taken to Portugal, and a few years after, they could be found in many European countries (Zurara, 1937). In France, there are records of African slaves by the middle of the 16th century (d'Avenel, 1910).

Together with the finding of *S. haematobium* (Bouchet and Paicheler, 1995) and *S. mansoni* eggs from the same archaeological material, evidence supports the presence of an infected human host, probably of African origin at this 15th–16th century European site. Nevertheless, wherever it came from and whichever host it came in, schistosomiasis was never established in Europe as an endemic disease.

The authors thank C. Goy (AFAN) for allowing us to study the archaeological material. Supported by CNRS, AFAN, CAPES/COFECUB, and CNPq/PRONEX.

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J. Parasitol., 88(2), 2002, pp. 405–407
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Dexamethasone Inhibition of the Cellular Immune Response of *Drosophila melanogaster* Against a Parasitoid

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ABSTRACT: Host larvae of *Drosophila melanogaster* injected with the eicosanoid biosynthesis inhibitor, dexamethasone, prior to parasitization by the wasp *Leptopilina boulardi*, exhibited significantly reduced rates of melanotic encapsulation in comparison with control and saline-injected larvae. The results of this investigation suggest that prostaglandins and other eicosanoids are involved as cell-signaling molecules in the hemocytic encapsulation reaction of *D. melanogaster* larvae.

Insects express 2 broad categories of innate immunity, humoral and cellular (Strand and Pech, 1995; Gillespie et al., 1997). Humoral defense reactions include the biosynthesis of various antimicrobial peptides (Meister et al., 1997, 2000). Cellular reactions involve blood cells or hemocytes, which form melanotic, multilayered capsules around parasites that are too large for individual cells to internalize (Carton and Nappi, 1997; Vass and Nappi, 2000). Although insect immune cell effector responses have been well documented (Nappi and Ottaviani, 2000), little is known of the signaling mechanisms mediating insect cellular defense reactions.

In the larvae of *Drosophila melanogaster*, the innate cellular immune

response that results in the formation of melanotic capsules around intrahemocoelic wasps (parasitoids) is accompanied by elevated levels of nitric oxide, superoxide anion, and hydrogen peroxide, molecules implicated along with certain reactive quinonoid intermediates of melanin in the killing process (Nappi et al., 1995; Nappi and Vass, 1998; Carton and Nappi, 2001). Collectively, these host responses not only destroy the parasitoids, but also confine the cytotoxic activity within pigmented capsules, thereby protecting endogenous tissues from the biochemically hostile reactions that are directed against nonself entities. Of interest is the fact that some of the reactive intermediates of oxygen (ROIs) and reactive intermediates of nitrogen (RNIs) generated during encapsulation are known to be cell messengers (Nappi et al., 2000), and may thus serve a dual role in insect immunity, functioning as both cytotoxic and signaling molecules (Fig. 1). Other known signaling molecules that may be involved in insect cell-mediated innate immunity include eicosanoids. Eicosanoids are derived from arachidonic acid (20:4n – 6), which is hydrolyzed from cellular phospholipids via the activation of phospholipase A₂ (PLA₂) (Fig. 1). Whereas there is substantial evidence that eicosanoids are involved in the defense reactions of insects against

bacteria (Stanley-Samuelson et al., 1991, 1997; Miller et al., 1994, 1996, 1999; Jurenka et al., 1997; Mandato et al., 1997; Stanley et al., 1999; Tunaz and Stanley, 1999; Tunaz et al., 1999; Bedick et al., 2000; Stanley, 2000), virtually nothing is known of the possible involvement of these molecules in hemocytic encapsulation.

To test the hypothesis that eicosanoids mediate insect cellular innate immunity, we examined the effects of dexamethasone, a known inhibitor of eicosanoid biosynthesis, on the cellular encapsulation of the larvae of 2 immune-reactive strains of *D. melanogaster* ($R = 940$ and 1,088) against the wasp parasitoid *Leptopilina boulardi* (Brazzaville strain G486). Adult flies were reared in half-pint culture jars on standard cornmeal and yeast medium, and adult wasps were maintained on a 50% honey solution. An Eppendorf transjector was used to introduce 0.05 μl of varying doses (0, 1, 5, and 8 $\mu\text{g}/\mu\text{l}$) of the eicosanoid biosynthesis inhibitor into the hemocoel of *Drosophila* larvae (72 hr old; 25 C). Mean ($n = 25$) hemolymph volume (2 μl) was used to calculate the concentrations of the inhibitor (Fig. 2). Control larvae were either untreated or injected with saline. At 3 hr after injection, the larvae were exposed to gravid adults of *L. boulardi* for 6 hr. After a 24-hr incubation period, the percentage of encapsulated parasitoid eggs was determined by dissecting the hosts and calculating the percentage of solitary parasitized larvae bearing a melanotic capsule (Carton and Nappi, 1997).

All experiments were replicated twice, and the data statistically analyzed using the Student's *t*-test.

In the control experiments with untreated *Drosophila* larvae, approximately 92% of these hosts encapsulated parasitoid eggs (Fig. 2). Saline-injected control larvae and experimental larvae treated with 1 $\mu\text{g}/\mu\text{l}$ dexamethasone yielded similar rates of encapsulation. Significantly reduced rates of encapsulation ($P < 0.05$) were obtained with larvae treated with higher doses of dexamethasone. Approximately 33% of the host larvae treated with 5 $\mu\text{g}/\mu\text{l}$ dexamethasone and only about 14% of the host larvae treated with 8 $\mu\text{g}/\mu\text{l}$ dexamethasone encapsulated eggs of *L. boulardi*.

The results of this investigation show a significant dose-dependent influence of dexamethasone on the encapsulation capacity of *D. melanogaster* larvae, and suggest that the cellular innate immune response of this insect against eukaryotic parasites is mediated in part by eicosanoids. Although eicosanoid biosynthesis is known to be inhibited by dexamethasone, additional studies are warranted to ascertain if other processes are also adversely affected by this substance. In other insect models, "rescue" experiments involving the injection of arachidonic acid have been shown to reverse the inhibitory effect of dexamethasone on antimicrobial responses (Stanley et al., 1999, 2000; Tunaz and Stanley, 1999; Tunaz et al., 1999; Bedick et al., 2000). Unfortunately, rescue

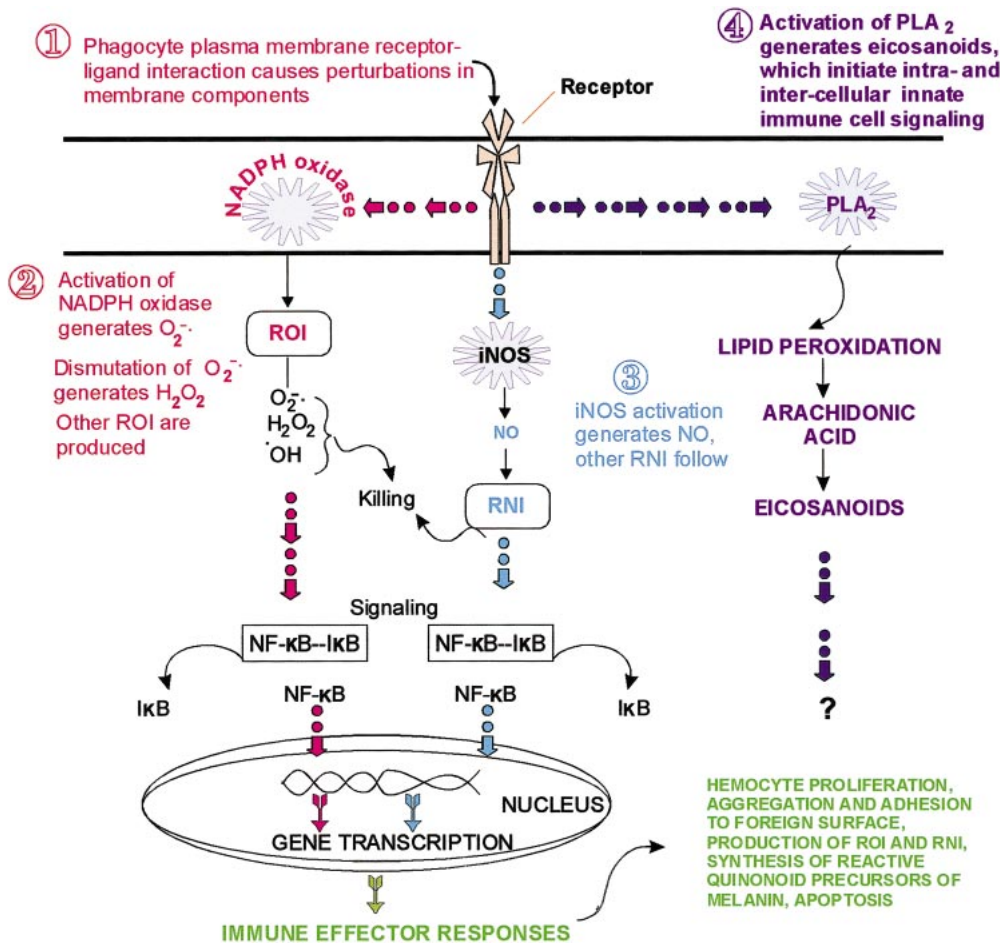


FIGURE 1. Some of the signaling pathways proposed to be involved in the innate cellular immune response of *Drosophila* against eggs of the parasitoid *Leptopilina boulardi*. Non-self recognition of the parasitoid, perhaps by some plasma membrane receptor(s), results in the activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, inducible nitric oxide synthase (iNOS), and PLA₂, responses that generate ROIs and RNIs, and eicosanoids, respectively. Previous studies of *Drosophila* immunity against parasitoids established the production of superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and nitric oxide (NO) in immune reactive larvae (Nappi et al., 1995, 2000; Nappi and Vass, 1998). These molecules can be cytotoxic, and can activate the NF-κB signaling cascade, leading to gene transcription and the ensuing immune effector responses of hemocytes. The current study showing decreased rates of encapsulation following dexamethasone-mediated inhibition of eicosanoid synthesis suggests a role for the PLA₂-arachidonic acid signaling pathway in the innate cellular immune response of *Drosophila* against parasitoids.

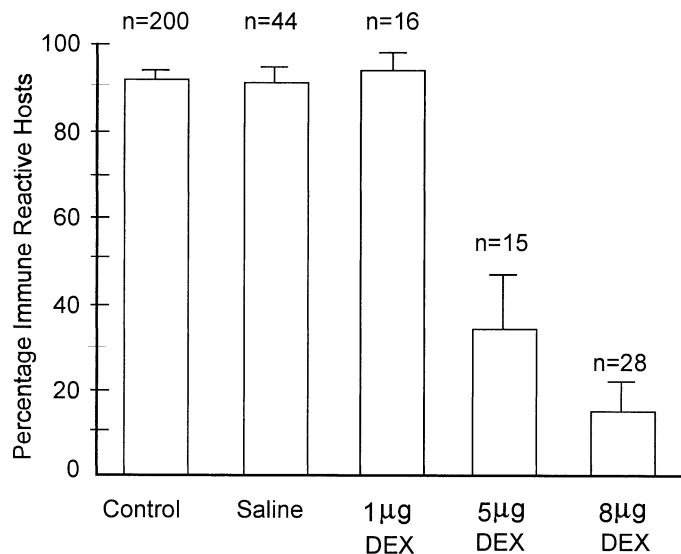


FIGURE 2. Concentration-dependent effects of dexamethasone (DEX) injection on the cellular encapsulation response of *Drosophila*. Host age 72 hr when infected by *Leptopilina boulardi*. Standard deviations are represented by error bars. The concentrations of dexamethasone for the doses injected (1, 5, and 8 µg/µl) were 25, 125, and 200 ng/µl hemolymph, respectively.

experiments involving the injection of arachidonic acid into second-stage *Drosophila* larvae previously injected with dexamethasone and parasitized are technically unfeasible. Establishing the involvement of eicosanoids in the cellular innate immune response of *Drosophila* would identify a critical biochemical signaling system mediating hemocyte encapsulation. Undoubtedly, several signaling pathways are involved in the complex cascade of responses that comprise cellular melanotic encapsulation in insects. Determining which pathways are crucial to the process of encapsulation remains a formidable task, as different signaling pathways frequently intersect synergistically at multiple levels, with common transcription factors, such as NF-κB, activated to elicit or amplify, or both, a given immune effector response. The elucidation of the involvement of eicosanoids in the process of encapsulation provides an exciting new focus for studies concerned with non-self recognition and cell signaling in the innate immune mechanisms of insects. The possibility that eicosanoids collaborate with RNI and ROI to induce insect defense gene expression warrants future consideration. Indeed, it has been suggested that eicosanoids mediate the expression of 2 fat body genes for antibacterial proteins, specifically cecropin and lysozyme, in silkworms (*Bombyx mori*) (Morishima et al., 1997).

This work was supported by ACC French Universities and Research Ministry (SV7-95007023), the French CNRS-INRA program Biodiversité-Interactions Durables, the French CNRS GDR 2153, the National Institutes of Health (GM59774), the National Science Foundation (IBN 0095421), the Research Services at Loyola University, Chicago, and the Agricultural Research Division at the University of Nebraska, Lincoln. This paper is publication no. 13,641, Nebraska Agricultural Research Division.

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Prevalence of *Neospora caninum* Antibodies in Dogs From Dairy Cattle Farms in Parana, Brazil

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ABSTRACT: Serum samples from 134 dogs from 22 cattle dairy farms in the northern region of Parana State, Brazil, were tested for antibodies to *Neospora caninum* in an indirect fluorescent antibody test. Antibodies ($\geq 1:50$) to *N. caninum* were found in 29 (21.6%) of the 134 dogs, and seropositive dogs were found on 14 (63.6%) of the 22 dairy cattle farms. The antibody titers of dogs were 1:50 (3 dogs), 1:100 (7 dogs), 1:200 (7 dogs), 1:400 (6 dogs), and $\geq 1:800$ (6 dogs). The low prevalence (9%) in <1-yr-old dogs compared with the 2- to 3-fold higher prevalence in older dogs (17–29%) suggests postnatal exposure to *N. caninum* infection.

Neospora caninum is an important parasite of dogs and livestock. Worldwide reports of clinical and subclinical infections in dogs have been summarized (Dubey and Lindsay, 1996; Lindsay and Dubey, 2000). In addition to clinical infections in dogs, subclinical *N. caninum* infections are important epidemiologically because the domestic dog (*Canis familiaris*) is the only known definitive host of *N. caninum* that can shed the environmentally resistant oocysts. Dogs fed tissues of experimentally infected mice shed *N. caninum* oocysts in their feces (McAllister et al., 1998; Lindsay et al., 1999). Recently, *N. caninum* oocysts were found in the feces of a naturally infected dog (Basso, Venturini L, Venturini MC, Hill et al., 2001). Although the role of the dog in the natural epidemiology of *N. caninum*-induced abortions and subclinical infections in cattle is uncertain, epizootics of bovine abortions have been linked to postnatal infections in cattle (Yaeger et al., 1994; McAllister et al., 1996, 2000; Anderson et al., 2000; Dijkstra, Barkema et al. 2001). Studies from Canada, Japan, and the Netherlands, have reported a positive relationship between dogs and bovine neosporosis (Paré et al., 1998; Sawada et al., 1998; Wouda et al., 1999).

Little is known of the seroprevalence of *N. caninum* in dogs in Brazil. The objective of this study was to determine the seroprevalence of *N. caninum* in dogs from cattle dairy farms in the rural areas of Brazil.

Blood samples were collected from 22 dairy farms from the northern region of Parana State, Brazil, over a period of 2 mo. These dairy farms produce high-quality milk. The dogs were of different breeds, and there were 47 (35.0%) females and 87 (65%) males.

Sera were separated and stored at $-20\text{ }^{\circ}\text{C}$ until tested for *N. caninum* antibodies, using an indirect fluorescent antibody test (IFAT). For IFAT, culture-derived tachyzoites of the NC-1 isolate and the rabbit anti-canine IgG conjugate (Sigma, St. Louis, Missouri) were used (Dubey et al., 1988). Sera were tested by IFAT at 2-fold dilutions, beginning at a 1:50 dilution.

The effects of sex and age were analyzed using the testing of differences among proportions, and the Cochran-Armitage Trend Test was conducted to test for significant association between seroprevalence and increase of the age of the dogs, with 5% as a significant level (STAT X act-3 for Windows).

Antibodies to *N. caninum* were found in 29 (21.6%) out of 134 dogs, and seropositive dogs were found on 14 (63.6%) out of 22 dairy cattle farms. The IFAT titers were 1:50 (3 dogs), 1:100 (7 dogs), 1:200 (7 dogs), 1:400 (6 dogs), and $\geq 1:800$ (6 dogs) (Table I). Seropositive ($\geq 1:50$) dogs were found on 14 (63.6%) of the 22 farms. The 18.7% seroprevalence of *N. caninum* in male dogs (16 out of 87) was not statistically significant ($P < 0.05$) from the 27.6% (13 out of 47) seroprevalence in female dogs.

Seroprevalence increased with age ($P < 0.05$) (Table I). Recent studies have indicated that dogs can excrete *N. caninum* oocysts after in-

TABLE 1. Prevalence of *Neospora caninum* antibodies in dogs of different age groups from dairy farms of Parana, Brazil.

Age group (yr)	No. of dogs	No. of seropositive dogs ($\geq 1:50$)	% Positive	Antibody titers				
				50	100	200	400	800
<1	33	3	9.0	0	2	1	0	0
≥ 1 to ≤ 2	29	5	17.2	1	1	2	0	1
>2 to ≤ 4	34	10	29.4	1	2	1	2	4
≥ 4 to 12	38	11	28.9	1	2	3	4	1

gesting placentas of naturally infected cattle (Dijkstra, Eysker et al. 2001), and tissue cysts have been reported in extraneural tissues of animals infected with *N. caninum* (Peters et al., 2001). Thus, there are many opportunities for farm dogs to ingest *N. caninum*-infected tissue.

Although there have been many serologic surveys of *N. caninum* in urban dogs (reviewed in Lindsay and Dubey, 2000), only 3 reported prevalence in rural dogs. Sawada et al. (1998) reported *N. caninum* antibodies in 31% of 48 dogs from dairy farms and in 7% of 198 dogs from urban areas in Japan. Wouda et al. (1999) reported a higher prevalence in farm dogs (23.6% of 152) when compared with urban dogs (5.5% of 344) from the Netherlands. Basso, Venturini L, Venturini MC, Moore et al. (2001) reported IFAT antibodies in 48% of 125 dogs from dairy farms, in 54.2% of 35 dogs from beef-cattle farms, and in 26.2% of 160 dogs from urban areas in Argentina.

Increasing rates of prevalence of *N. caninum* antibodies in older dogs in the present study, and those reported previously, suggests postnatal exposure to this parasite. Cattle are economically important to Brazil's economy. Whether dogs on cattle farms are the source of some of the *N. caninum* infections in cattle or whether both cattle and dogs are infected from a common source requires further investigation.

This project was supported by FAPESP (99/01208-5), which also provide a scholarship to S.L.P.deS. (99/01207-G). S.M.G. is the recipient of a scholarship from CNPq.

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J. Parasitol., 88(2), 2002, pp. 409–411
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Arthropod and Helminth Parasites of the Wild Guinea Pig, *Cavia aperea*, From the Andes and the Cordillera in Peru, South America

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ABSTRACT: As part of an ongoing research project concerning the diversity and distribution of parasites of Caviidae in South America, 143 wild guinea pigs (*Cavia aperea*) were collected from 3 localities in the Andean Highlands of Peru. Samples were collected between November 1996 and May 1999 and included representatives of arthropods, nematodes, and trematodes. Seven species of arthropods: *Leptopsylla segnis* (27.3%), *Tiamastus cavicola* (6.9%), *Gliricola porcelli* (55.2%), *Hoplopleura alata* (6.9%), *Polyplax spinulosa* (12.6%), *Myobia musculi* (1.4%), and *Eutrombicula bryanti* (49.6%); 4 species of nematodes: *Capillaria hepatica* (6.9%), *Graphidioides mazzai* (18.8%), *Trichuris gracilis* (3.5%), and *Paraspirudera uncinata* (37%); and a single trematode, *Fasciola hepatica* (4.2%), were identified.

The wild guinea pig, *Cavia aperea* Erxleben, 1777, is regarded as the ancestor to the domesticated guinea pig *C. porcellus* Linnaeus, 1758, which is widely used as a source of meat for local Andean communities (Mueller-Haye, 1984). The species belongs to the Caviidae (Order: Rodentia; Suborder: Hystricognathi), whose taxonomy remains a point of discussion. *Cavia aperea* occurs in Peru in the Sierra of the Andean Highlands and the Cordillera, stretching from the Departments of Cuzco and Ayacucho in the south to the Department of Cajamarca in the north. Although it has historically been an abundant species throughout its range, population densities have declined precipitously in recent years because of increased human activities. Few data exist concerning the parasites of the wild guinea pig. Macchiavello (1948) and Johnson (1957) reported some fleas (Siphonaptera) of *C. aperea*, i.e., *Tiamastus cavicola* Weyenberg, 1881 (Argentina, Bolivia, Peru, Chile) and *Hectopsylla suarezi* Fox, 1929 (Peru). Johnson (1957) and Hastriter and Peterson (1997) reported *Polygenis tripus* Jordan, 1933 from Argentina, *P. bohlsi jordani* Costa Lima, 1937, and *Adoratopsylla (Tritopsylla) intermedia intermedia* Wagner, 1901 from Brazil. Ferris (1951) and Johnson (1972) found the biting louse *Pterophthirus immitans* Werneck, 1924 on *C. aperea* (type host) in Brazil, Argentina, Venezuela, and

Uruguay. Durden and Musser (1994) mention *P. alata* Ferris, 1921 from *C. aperea* in Argentina. Hopkins and Clay (1952) reported *Gliricola distinctus* Ewing, 1924, *G. lindolphoi* Werneck, 1934, *G. braziliensis* Werneck, 1934, and *Trimenopon hispidum* Burmeister, 1838 from *C. aperea* in Brazil. There are no reports of Acari in wild guinea pigs in South America. Ramon Zaldivar (1991) found *Ornithonyssus bacoti* Hirst, 1913 on *C. porcellus* in Peru. Nematodes that were mentioned by Yamaguti (1961) included *Paraspirudera uncinata* (Porter, 1934) in *C. porcellus*, *C. aperea*, *Ctenomys* spp., and *Agouti paca* in South America; *Trichuris gracilis* Rudolphi, 1819 was mentioned from *C. aguti* (probably *A. paca*) in Brazil, *Capillaria hepatica* Travassos, 1915 from the genus *Cavia* in Brazil.

Cavia aperea individuals (143) were examined from 3 areas in Peru. Five species of endo- and 7 species of ectometazoan parasites were identified (Table I). Of these, 4 new host and 6 new locality records are reported.

The guinea pigs were trapped alive in the following areas: La Raya, 64 individuals; Lago Junin, 40 individuals; and El Paramo, 39 individuals. All areas are isolated from each other. La Raya is situated at an elevation of 4,000 m in the Sierra Alta of Department of Cuzco, near the village of Marangani; the Lago Junin (lake Junin) lies in the Department of Junin, at an elevation of 4,100 m; and El Paramo is an Andean paramo ecosystem situated in the Cordillera Blanca of the Department Cajamarca, at an elevation of 4,300 m. After encircling the guinea pigs by nets, they were then captured by hand. The ectoparasites were brushed off into plastic beakers and preserved in 70% ethanol; later they were transferred to 99.6% ethanol and stored at 4 C. All inner organs and cavities were examined for parasites. Stomach contents and small and large intestines were mixed with water and passed separately through a 100-mesh screen. Screenings were examined in a petri dish, using a magnifying glass (×10). Nematodes and the trematode were fixed in buffered formalin. Identification took place in the Parasitological Laboratories of the Veterinary Faculty of the University of Leipzig,

TABLE I. Prevalence (%) and mean intensity (\bar{x}) of parasites of the wild guinea pig (*Cavia aperea*) from three localities in Peru.

	La Raya p*(%)/ \bar{x}	El Paramo p*(%)/ \bar{x}	Junin p*(%)/ \bar{x}	Location in host	Voucher nr.
Siphonaptera					
<i>Leptopsylla segnis</i> †	—	100/0–4	—	Fur	Lept/Jun1
<i>Tiamastus cavicola</i>	6.2/0–18	15.4/0–16	—	Fur	Tia/LR1
Mallophaga					
<i>Gliricola porcelli</i>	—	100/0–218	100/0–308	Fur	
Anoplura					
<i>Pterophtirus alata</i> †	15.6/0–16	—	—	Fur	Ptal/LR1-3
<i>Polyplax spinulosa</i> †‡	9.4/0–29	—	30/0–5	Fur	Posp/LR1-3
Acari					
<i>Eutrombicula bruyanti</i> †‡	92.2/0–36	—	30/0–41	Skin	Eutr/LR1-4
<i>Myobia musculi</i> ‡	—	—	5/0–4	Fur	Mym/EP1-5
Nemata					
<i>Graphidioides mazzai</i> †	28.1/0–7	—	22.5/0–8	Small intestine	
<i>Capillaria hepatica</i>	9.4/0–4	10.3/0–2	—	Liver	Cap/Caj1-5
<i>Paraspirodera uncinata</i>	39.1/0–4	28.2/0–7	42.5/0–6	Large intestine, cecum	
<i>Trichuris gracilis</i> †	—	7.7/0–9	5/0–4	Large intestine	Trg/Jun1
Trematoda					
<i>Fasciola hepatica</i> ‡	—	—	17.6/0–2	Liver	FH/Jun1-2

* Prevalence.

† New locality record.

‡ New host record.

the Universidad de Cajamarca, and the Veterinary Faculty (UNMSM) in Lima. Techniques for dehydrating, clearing, mounting, and specification were used according to established standards. Voucher specimens of parasites and hosts have been placed at the Institute of Parasitology at the Veterinary Faculty, University of Leipzig, Germany (Table I). Specimens of *P. uncinata* and *Graphidioides mazzai* are deposited with the Facultad de Medicina Veterinaria of the UNMSM in Lima, Salamanca, Peru.

Siphonaptera (fleas) were represented by the genera *Leptopsylla* and *Rhopalopsyllus*. Species of the genus *Leptopsylla* normally occur in the Palaearctic and Ethiopian regions; one species, *Leptopsylla segnis* Schönherr, 1811, has been carried with its principal synanthrope host (*Mus musculus*) and is now cosmopolitan (Hopkins and Rothschild, 1971). In Peru, *L. segnis* has been reported from several ecological associates of *C. aperea* such as *Rattus* spp., *Akodon* spp., and *M. musculus* (Johnson, 1957), and on *C. porcellus* (Macchiavello, 1948). This is the first record of *L. segnis* on wild guinea pigs. The guinea pig flea *T. cavicola* is indigenous in South America (neotropical region). Its geographical range is apparently broad, occurring in Argentina, Peru, Chile, and Bolivia (Johnson, 1957). This flea has been reported from *Akodon* spp., *Kerodon* spp., *Ctenomys* spp., and *Oligoryzomys* spp. (Macchiavello, 1948; Hopkins and Rothschild, 1962; Johnson, 1957). *Tiamastus cavicola* has also been collected from 123 *C. porcellus* individuals examined in Moquegua, Peru (Valcarcel, 1999). The species occurred in the La Raya region on 4 (6.9%), and in the El Paramo region on 6 (15.4%) of the guinea pigs we collected.

The only species of chewing lice collected during this study was *G. porcelli*. Thirty-nine of the guinea pigs of the El Paramo region and 40 of the Lake Junin area were infested with this louse, showing a high infestation rate.

Johnson (1972), in her study of sucking lice (order: Anoplura), reports *Hoplopleura alata* (now *Pterophtirus*) on *Microcavia australis* (type host) in Argentina. Durden and Musser (1994) report it to be abundant on *C. aperea* in Argentina. *Pterophtirus alata* is the only representative of the genus *Pterophtirus* found on *C. aperea* in the Department of Cuzco, Peru, representing a new locality record. Another species of sucking lice on the wild guinea pigs was *Polyplax spinulosa*

Burmeister, 1839, which was recovered from 12.6% of all studied individuals. The species is cosmopolitan, mostly occurring on rodents. It has been reported from Venezuela and Brazil on *Rattus rattus*, *Proechimys semispinosus*, and *Oryzomys minutus* (Johnson, 1972; Yoshizawa, 1996). The occurrence on *C. aperea* in the La Raya and Junin populations represents a new host and locality record. A maximum of 29 specimens from a single individual from La Raya suggests that the wild guinea pig is a common host, although the intensity of infestation was much lower in Junin.

Acari were represented by the genera *Trombicula* and *Myobia*. Thor and Willmann (1947) list in their revision of the Trombiculidae *Eutrombicula bruyanti* Oudemans, 1910 from Brazil (type host: *Didelphys opossum*). The prevalence of the *E. bruyanti* larvae, found on the ears and around the eyes of the guinea pigs, was very high in the La Raya population (92.2%) and reached 30% in the Junin population. The presence of *E. bruyanti* on *C. aperea* in these 2 areas represents a new host and geographical record. The species *Myobia musculi* Schrank, 1781, which occurs throughout the world, is commonly found on *Rattus* spp. and *M. musculi* (Baker et al., 1956). The finding of *M. musculi* also represents a new host record, although the low infestation level suggests a rare ecological associate with *C. aperea*.

Nematodes of the genera *Graphidioides*, *Capillaria*, *Paraspirodera*, and *Trichuris* were found during this study. *Graphidioides mazzai* Lent and Freitas, 1935 occurred in *C. aperea* from the La Raya and Junin populations, a new host and locality record. Several species of the genus *Graphidioides* are reported from the small intestine of some caviids in Argentina and Brazil (Yamaguti, 1961); *G. mazzai* was mentioned from *C. porcellus* in Puno, Peru (Ramon Zaldivar, 1991) and *Galea leucob-lephara* in Argentina (Lent, 1935).

Capillaria hepatica Travassos, 1915 was reported from numerous mammalian hosts throughout the world, including several ecological associates of *Cavia* spp., such as *Sigmodon* spp., *Akodon* spp., and man (Turhan et al., 1999). Olortegui (1961) and Gonzales (1970) found this species in the liver of 11 *C. porcellus* and 1 *C. aperea* in Cajamarca, Peru. Only 10 of the guinea pigs (6.9%) examined in this study were infested with this parasite. These did not occur around lake Junin. The typical guinea pig heterakid nematode *P. uncinata* was found in all 3

studied areas. The species *P. uruguayana*, from *C. aperea* in Uruguay (Freitas, 1956), can be regarded as *P. uncinata*. In total, 37% of *C. aperea* examined in this study were infested with *P. uncinata*. Olortegui (1961) and Gonzales (1970) reported higher levels of infested guinea pigs from the Cajamarca area (58%) and 72% from the Department of Cuzco, respectively. The nematode *T. gracilis* occurred in the large intestine of 3.5% of the guinea pigs examined. It was not detected from the La Raya region, although Gonzales (1970) reported a 23% prevalence among *C. aperea* from this area.

A single species of trematode was collected, *Fasciola hepatica* Linnaeus, 1758. This parasite was introduced into South America with sheep. Because of increased herding, the parasite could establish in new ecological areas. Yamaguti (1961) lists several herbivorous host species, whereas Vilchez (1988) found a prevalence of 40.5% in domesticated guinea pigs (*C. porcellus*) from the Department of Cajamarca. No *F. hepatica* was found in the wild guinea pigs of this department (El Parano), but the Junin population showed a 15.4% prevalence (Table I). The maximum of 2 specimens per infected liver corresponds with the findings of Vilchez (1988) in *C. porcellus*. In total, no cestodes have been found, although Gonzales (1970) lists *Monoecocestus* sp. (28%) from *C. aperea* of the Cuzco area. No parasites were noted in the corporal cavities of the guinea pigs that were examined.

I am grateful to Felipe San Martin, Norma Noe Mocetti, and Jane Wheeler for supporting me and making contact with the research stations of IVITA (Instituto Veterinario de Investigaciones Tropicales y de Altura).

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Seroepidemiology of *Toxoplasma gondii* Infection Among Two Mountain Aboriginal Populations and Southeast Asian Laborers in Taiwan

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ABSTRACT: A seroepidemiological survey of *Toxoplasma gondii* infection among Atayal and Paiwan mountain aborigines and Southeast Asian laborers in Taiwan was assessed from February 1998 to July 2000 using a latex agglutination test. To determine risk factors for *T. gondii* infection among Taiwan aborigines, the consumption of raw meat and valley water were given particular attention in a self-administered questionnaire. The overall seroprevalence of *T. gondii* infection was 19.4% for Atayal, 26.7% for Paiwan, 42.9% for Indonesian, 14.7% for Thai, and 11.3% for Filipinos. No significant gender difference in seroprevalence was found among Atayals, Paiwans, Indonesians, and Filipinos ($P > 0.05$). In the Thai group, however, males had a higher seroprevalence than females ($P < 0.001$).

Results of the multiple logistic regression analysis indicate a higher odds ratios (OR) with age in both aboriginal groups. In contrast, the OR was lower among older Indonesians and Thais. Those Atayals and Paiwans with a history of eating raw meat seemed more susceptible to *T. gondii* infection than those who had never consumed raw meat. Ethnically, a significant difference in seroprevalence was observed between Indonesians and Paiwans, Atayals, Thais, and Filipinos ($P < 0.001$).

Infection by the protozoan parasite *Toxoplasma gondii* is widely prevalent in animals and humans worldwide (Dubey and Beattie, 1988).

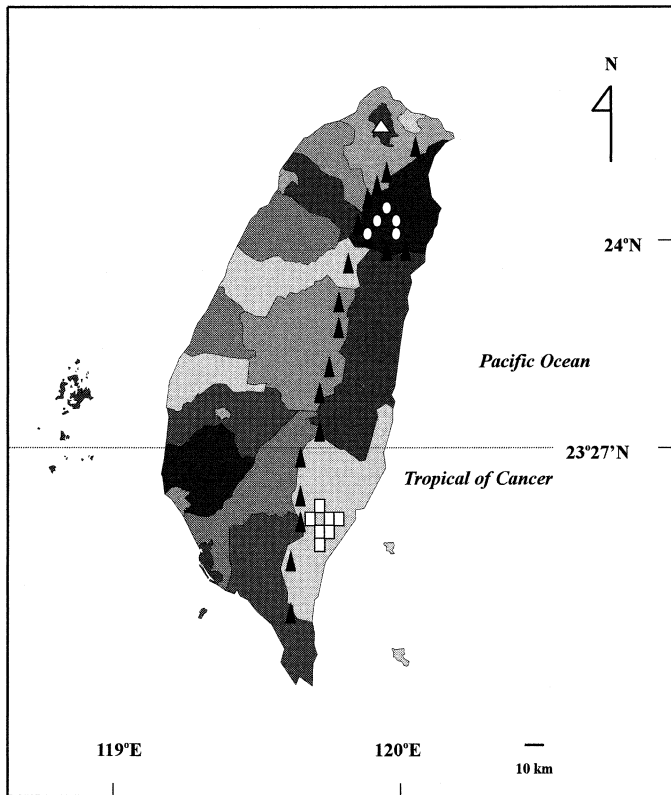


FIGURE 1. Map of Taiwan showing selected study areas: (○) for Atayal mountain aboriginal villages, (□) for Paiwan mountain aboriginal villages, and (△) for Taipei City in Taiwan.

There are only a few reports concerning the seroprevalence of *T. gondii* infection in Taiwanese people (Tsai and Cross, 1972; Durfee et al., 1975; Liu et al., 1975; Lin et al., 1998). In general, reports concerning the seroprevalence of *T. gondii* infection in aborigines are rare (Wallace, 1976; Cross and Hsu, 1989; Fan, Su, Chung, Tsai, Chiou et al., 1998).

In Taiwan, there are 9 different aboriginal populations. The Atayal aborigines are the second-largest population, living mostly at elevations of 500–600 m in the middle and northeastern parts of the country.

Paiwan aborigines belong to the third-largest group, with most living at elevations of 400–500 m in southeastern Taiwan. These aboriginal populations differ in their traditional culture and food habits; both still have the habit of hunting wild animals and eating raw meat (Xiun, 1996). It is believed that the majority of the ancestors of the aborigines migrated to Taiwan from Southeast Asia 4,000 yr ago and that they had similar traditional cultures and food habits as others in Southeast Asian countries (Bellowood, 1991).

Beginning in 1992, more than 320,000 laborers, mostly from Southeast Asian countries, have been employed in various Taiwanese industries, mostly in labor-intensive sectors. Of this number, 98,161 are from the Philippines, 142,665 are from Thailand, and 77,830 are from Indonesia. The immune status of latent *T. gondii* infection among the imported foreign laborers in Taiwan is unknown. Therefore, between February 1998 and July 2000, an effort was made to conduct a seroepidemiological survey of Taiwan aborigines and Southeast Asian laborers for *T. gondii* antibodies using the latex agglutination test.

Five Atayal (Datung District in Ilan County) and 7 Paiwan (Kingfong District in Taitung County) aboriginal mountain villages located in eastern Taiwan were included in the present study (Fig. 1). Additionally, a self-administered questionnaire for aborigines, which requested various personal details, was included to determine the risk factors for *T. gondii* infection, i.e., eating raw meat (symbol D in Table I) and consuming valley water (symbol E in Table I). Laborers from Thailand, Indonesia, and the Philippines working in northern Taiwan were also included in the study. Serum samples (2,362) were obtained by venipuncture, of which 1,039 serum samples, including 428 from Atayal males (212) and females (216) and 611 from Paiwan males (269) and females (342), were randomly collected from apparently healthy individuals. The mean age was similar for both sexes, ranging between 6 and 69 yr old in both aboriginal populations. (Informed consent was obtained from each subject prior to that person participating in the study.) Serum samples (1,323), including 266 samples from 42 males and 224 females from Indonesia, 409 samples from 317 males and 92 females from Thailand, and 648 samples from 115 males and 533 females from the Philippines, was randomly collected from foreign laborers who appeared at the Center for Foreign Laborer Health for routine examination at Taipei Medical University Hospital (a district hospital) in Taipei City in north Taiwan. The mean ages were similar in both sexes and ranged between 20 and 56 yr old for all laborers from these 3 countries.

All sera were screened by the *T. gondii* latex agglutination test (LA) using commercial reagents (TOXO Test-MT, Eiken Co., Ltd. Tokyo, Japan). The test was performed according to the manufacturer's instructions. Sera found positive at titers from 1:32 were regarded as positive.

TABLE I. Seroprevalence of *Toxoplasma* antibody among Atayal and Paiwan mountain aborigines and Southeast Asian laborers in Taiwan from 1998 to 2000, according to ethnicity, sex, age, and risk factor distribution.

Variable	Group	Taiwan aborigines				Southeast Asian laborers						
		Atayal		Paiwan		Indonesian		Thai		Filipino		
		Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	
Sex	Male	212	40 (18.9)	269	75 (27.9)	42	21 (50.0)	317	57 (18.0)	115	17 (18.1)	
	Female	216	43 (19.9)	342	88 (25.7)	224	93 (42.0)	92	3 (3.3)	533	56 (10.5)	
Age group*	A	299	47 (15.7)	198	43 (22.0)	133	60 (45.1)	117	23 (42.2)	310	36 (11.6)	
	B	72	15 (20.8)	183	43 (23.5)	106	45 (42.5)	190	27 (14.2)	277	30 (10.9)	
	C	57	21 (36.8)	230	77 (33.5)	27	9 (33.3)	102	10 (9.8)	61	7 (11.5)	
Risk factor†	D	No	113	12 (10.6)	193	36 (19.0)	NA	NA	NA	NA	NA	NA
		Yes	315	71 (22.5)	418	127 (30.0)	NA	NA	NA	NA	NA	NA
	E	No	327	62 (19.0)	485	127 (26.0)	NA	NA	NA	NA	NA	NA
		Yes	101	21 (20.8)	126	36 (29.0)	NA	NA	NA	NA	NA	NA
Mean		428	83 (19.4)	611	163 (26.7)	266	114 (42.9)	409	60 (14.7)	648	73 (11.3)	

* A, ≤14 or 20–29 yr old; B, 15–49 or 30–39 yr old; C, ≥50 or ≥40 yr old.

† D, eating raw meat; E, consuming valley water; NA, no data available.

The LA test was applied because of its simplicity and qualitative agreement with the dye test (Balfour et al., 1982).

Three age groups were arbitrarily established—A, ≤ 14 or 20–29 yr old; B, 15–49 or 30–39 yr old; and C, ≥ 50 or ≥ 40 yr old—for Taiwan aborigines and Southeast Asian laborers, respectively. The increasing trend of age-specific seropositive rates was examined for statistical significance by the chi-square test (SAS Institute, Inc., Cary, North Carolina). Multivariate-adjusted odds ratios (OR) with their 95% confidence intervals (CI) were estimated through multiple logistic regression analysis.

Antibodies to *T. gondii* were found in 493 (20.9%) of 2,362 serum samples, with seropositivity of 19.4% (83/428) for Atayals, 26.7% (163/611) for Paiwans, 42.9% (114/266) for Indonesians, 14.7% (60/409) for Thais, and 11.3% (73/648) for Filipinos (Table I). Atayal aborigines consume various types of meat, including pork, and hunt wild animals and eat raw meat more frequently than do Paiwan aborigines (Fan et al., 1992). However, no significant difference in seropositivity was observed between Atayal and Paiwan aborigines in eastern Taiwan in the present study, whereas the seroprevalence either in Atayals (19.4%) or Paiwans (26.7%) was higher than that of the Ames (10%) and Yami aboriginal people (18%) surveyed in different parts of Taiwan (Tsai and Cross, 1972; Cross and Hsu, 1989). The seroprevalence of *T. gondii* infection varies ethnically, which may be ascribed to higher exposure to *T. gondii* tissue cysts through the eating of raw or undercooked meat through hunting or food preparation (Wallace, 1976).

The multiple logistic regression analysis revealed that the older the age, the higher the OR was in both groups of aboriginal people (age group ≥ 50 yr old vs. ≤ 14 yr old, OR = 2.6; 95% CI: 1.5, 4.6; $P < 0.01$ [Atayals] vs. OR = 1.8; 95% CI: 1.2, 2.8; $P < 0.01$ [Paiwans]). The OR was, conversely, lower among older Indonesians and Thais (OR = 0.6; 95% CI: 0.3, 1.4; $P > 0.05$ [Indonesians] vs. OR = 0.4; 95% CI: 0.2, 0.9; $P < 0.05$ [Thais]). Seroprevalence generally increases with age, as seen in studies conducted in other countries (Dubey and Beattie, 1988).

A previous study indicated that the seroprevalence of the *T. gondii* antibody among Atayal aboriginal children in mountains of northeastern Taiwan was about 18.6% (Fan, Su, Chung, Tsai, Chiou et al., 1998). Interestingly, most of these seropositive children indicated that they had eaten raw meat and consumed valley water, based on the self-administered questionnaire investigation (C. K. Fan, unpubl. obs.). Thus, a history of eating raw meat, drinking valley water, or both might be a risk factor for the *T. gondii* infection among aboriginal peoples. However, the seropositivity of *T. gondii* antibodies with respect to a history of eating raw meat and drinking valley water revealed that the prevalence was 22.5% (71/315) and 20.8% (21/101) for Atayal aborigines, respectively. On the other hand, the prevalence was 30.0% (127/418) and 29.0% (36/126) for Paiwan aborigines, respectively (Table I). Because of language differences, no questionnaire data were available for foreign laborers. The multiple logistic regression analysis revealed no significant association between seropositivity and a history of drinking valley water in either Atayal or Paiwan aboriginal people in this study ($P > 0.05$). Perhaps the valley water was not contaminated with *T. gondii* oocysts, or it might not be an important risk factor in the transmission of *T. gondii* in the 2 aboriginal districts. However, further investigation of the contamination of valley water with *T. gondii* oocysts is required. Nevertheless, a poor water supply system and inadequate environmental hygiene in aboriginal districts might play contributing roles in the acquisition of *T. gondii* infection (Fan, Su, Chung, Tsai, Chiou, 1998). In 1995, contamination of drinking water with *T. gondii* oocysts in Canada was reported. It was confirmed that domestic cats or cougar feces had contaminated a surface water reservoir with *T. gondii* oocysts (Aramini et al., 1999).

In our previous study, there was no evidence to suggest that ingestion of oocysts was an important means of transmission of *T. gondii* in aboriginal people because the prevalence of *T. gondii* in local cats was low (Tsai et al., 1997). In fact, dogs, not cats, are popular pets in these 2 communities. Also, most of the Atayal and Paiwan aborigines in our study live in mountainous areas working as laborers or farmers, and they still hunt wild pigs and goats with the help of dogs. However, there is no information on feral cats in these areas. It is believed that dogs having the same source of food as their masters may have contributed to the similarly high seroprevalence of anti-*Toxoplasma* antibodies (Fan, Su, Chung, Tsai, Lu, and Chao, 1998).

On the contrary, those Atayals and Paiwans who had a history of eating raw meat seemed more susceptible to *T. gondii* infection than did those who had never eaten raw meat (OR = 1.9; 95% CI: 1.2, 3.5; $P < 0.05$ for Atayals and OR = 1.9; 95% CI: 1.3, 2.9; $P < 0.01$ for Paiwans). Not surprisingly, a previous report indicated that *Taenia saginata asiatica* (Taiwan taenia) infection through eating raw wild pig or goat, especially the livers, was common in many different aborigines in Taiwan (Fan et al., 1992). Both Atayal and Paiwan aborigines also have the habit of eating raw meat from wild pigs, rats, and goats. This is postulated as the reason for the high prevalence of sera anti-*T. gondii* antibodies in this study.

The reason for the high seroprevalence of *T. gondii* infection among Indonesian laborers is unclear. However, Gandahusada (1991) indicated that consumption of goat meat or "sate" (a traditional Indonesian dish comprised of a grilled skewer of goat, beef, or chicken, not always fully cooked) was strongly related to the high prevalence of *T. gondii* antibodies in Indonesia. Moreover, Chomel et al. (1993) also indicated that eating raw pork in the form of "lowar" in Bali Indonesia is very similar to "lahb" or "nahm" found in Thailand. Such food items may play an important role in the acquisition of *T. gondii* infection among Balinese people. Nevertheless, Gandahusada (1991) also indicated that higher rates of *T. gondii* prevalence were found in areas of Indonesia with higher cat populations.

Additionally, a positive correlation has been demonstrated between the duration of latent *T. gondii* infection and the intensity of the decrease in superego strength, and a decrease in the strength of an individual's superego (the willingness to accept group moral standards), suggesting that this alteration in behavior was induced by *T. gondii* infection (Flegr et al., 1996). It may be relevant to examine not only whether instability of *T. gondii*-infected Taiwan aborigines and Southeast Asian laborers leads to an increase in social problems, but also whether they are more likely to develop toxoplasmosis upon acquisition of the human immunodeficient virus (HIV) infection.

The authors are grateful to Taipei Medical University, for its support of this investigation (grant TMC 87-Y05-A104). The authors thank H. S. Lee, C. W. Liao, and D. C. Kao for their valuable technical assistance and collection of human sera. Furthermore, we would like to express our appreciation to J. P. Dubey for his helpful and valuable suggestions on this manuscript.

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J. Parasitol., 88(2), 2002, pp. 414–415
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Congenital Infection with *Schistosoma japonicum* but not with *Schistosoma bovis* in Sheep

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ABSTRACT: The present study investigated whether *Schistosoma japonicum* or *Schistosoma bovis* could establish prenatally in lambs. Three ewes were exposed to *S. japonicum* by intramuscular injection of cercariae, and 3 ewes were exposed to *S. bovis* cercariae using the leg-emerging technique approximately 2 mo before delivery, and 1 age-matched pregnant ewe served as an uninfected control. The study lasted 18–20 wk after infection, which was 8–9 wk after delivery. All 6 exposed ewes became infected with either *S. bovis* or *S. japonicum*. Eight lambs were borne by the 7 ewes, of which 1 (*S. bovis* exposed) was dead and 1 (*S. japonicum* exposed) died at delivery. Of the 3 *S. japonicum*-exposed lambs, 2 were found infected. Four lambs born of *S. bovis*-exposed ewes were negative. Despite having no worms, these 4 *S. bovis*-exposed lambs as well as the 1 negative *S. japonicum*-exposed lamb had, in contrast to the nonexposed control lamb, few, but distinct, liver granulomas dominated by eosinophils and giant cells with large central necrotic areas but with no remnants of eggs or worms. Hence, congenital infection was demonstrated in *S. japonicum*-infected lambs, but not in *S. bovis*-infected ones.

Schistosomes are generally considered to infect their host percutaneously, but there are reported cases of congenital transmission of *Schistosoma japonicum* in humans, dogs, cattle, goats, and rodents (Wang, 1958; Okabe, 1961). Initial studies by our group have demonstrated that congenital transmission of *S. japonicum* is also possible during mid- to late pregnancy in pigs (Willingham et al., 1999) and rabbits (Qian et al., 2000), but not mice (Bendixen et al., 1999). Neither prenatal infection of lambs with *S. japonicum* has been reported nor prenatal infection with *Schistosoma bovis*. Hence, the objective of this pilot study was to assess if prenatal infections with either *S. japonicum* or *S. bovis* could establish in lambs.

Three Texel ewes, 2–3 yr of age and 89–100 days pregnant, were infected with 1,000 *S. bovis* cercariae (Tanzanian strain) using the leg-emerging technique, where the sheep's front leg was held for 30 min in a cylinder containing cercariae in pond water (Van Wyk et al., 1975); 3 similar ewes were infected with 1,000 *S. japonicum* cercariae using the intramuscular injection technique (Willingham et al., 1996). One ewe and its lamb served as uninfected controls. Fecal egg examinations were carried out using the method described by Willingham et al. (1998) for *S. japonicum* eggs and a modified Bell's filtration technique (Kassuku, 1985) for *S. bovis* eggs. The ewes were killed

18–20 wk postinfection, and the lambs were killed 8–9 wk after delivery. Two of the 8 lambs born (1 exposed to *S. bovis* and 1 to *S. japonicum*) were dead or died at delivery. From both dead lambs, liver samples were taken for tissue egg counts. All sheep were killed by an overdose of pentobarbital given intravenously and were perfused to collect worms as described by Johansen et al. (1996). Tissue egg counts were determined on samples from the liver and cecum, according to the method described by Johansen et al. (1996). Gross pathological changes were recorded, and samples were collected at predetermined sites for histopathology. The samples were fixed in 10% buffered neutral formalin, and, after conventional processing and paraffin embedding, 2 sections were prepared and stained with hematoxylin and eosin. The liver sections were additionally stained with Van Giesson to facilitate fibrosis scoring. The granulomas were classified according to the predominant cell type, and granulomas with more than 2 eggs or remnants of more than 1 egg were classified as multiple egg granulomas. Tissue eosinophilia and portal and septal fibrosis were scored as absent, mild, moderate, or marked.

The ewes delivered between days 138 and 147 (normal gestation period = 151 days), and they were all, except the control ewe, excreting either *S. bovis* or *S. japonicum* eggs in feces at the time of delivery. No other parasite eggs were observed. Worm establishment in the ewes was 22–24% for *S. japonicum* (mean number of worms \pm SD = 228 \pm 11.5) and 9–15% for *S. bovis* (109 \pm 32.7). Egg counts per gram (epg) tissue for *S. japonicum*-infected ewes were 1,929 \pm 1,294 epg in the liver and 620 \pm 327 epg in the cecum. For *S. bovis*-infected ewes, the egg counts were 48 \pm 27 epg in the liver and 3,353 \pm 513 epg in the cecum. The gross pathology of ewes infected with *S. bovis* or *S. japonicum* revealed slight hepatomegaly, with multiple small, 3–4 mm diameter, gray-white nodules disseminated in the liver; some were confluent, creating larger, irregular nodules. Portal fibrosis was focal and mild to moderate, whereas septal fibrosis was not demonstrated. Two of the *S. japonicum*-infected ewes had focal, fibrotic, adhesive perihepatitis. The histopathological examination revealed multiple granulomas, exclusively in the portal area, dominated by epithelioid or giant cells and, centrally, containing degenerated eggs or detritus. Very few intact eggs or miracidia were found. The granulomas with central necrosis and detritus were often large and aggregated. Hoeffli reactions were observed only in *S. bovis*-infected ewes. Intestinal lesions in the *S. japonicum*-infected ewes were mainly located in the mucosa of the distal part

of the descending colon. Here, several multiple egg granulomas were found. In contrast, in *S. bovis* infections many granulomas were found in both the mucosa and the submucosa and mainly located in the small intestines. Additionally, several calcified eggs were observed. Mild to moderate infiltration with eosinophils of the lamina propria was seen throughout the intestines in all infected ewes.

No parasitological evidence was obtained for congenital infection in the 4 lambs borne by *S. bovis*-infected ewes. Thus, worms were not recovered at perfusion, and neither tissue nor fecal eggs were demonstrated. In contrast, congenital infections were demonstrated in 2 of the 3 lambs borne by *S. japonicum*-infected ewes. Liver tissue egg counts were 13 egg in the *S. japonicum*-exposed lamb that died at delivery. In the other *S. japonicum*-exposed lamb, 12 male and 6 female *S. japonicum* worms were recovered by perfusion; 220 egg were found in the liver tissue and 433 egg in the cecum; and fecal egg counts dropped from 10 egg in week 4 to 4 egg in week 6 and reached zero at the time of necropsy. The liver and intestinal gross- and histopathology in the infected lamb paralleled that in the ewes, although milder, and hepatomegaly was not observed. In spite of the lack of parasitological evidence of congenital infection in 1 lamb borne by a *S. japonicum*-infected ewe and in 3 lambs borne by *S. bovis*-infected ewes, the livers of these lambs had a few small, 3–4 mm, gray-white nodules. The granulomas were of the giant cell type, with large central necrotic areas, but without remnants of eggs or worms. Portal fibrosis was focal and mild, and no septal fibrosis was seen. No intestinal lesions of significance were found in these lambs. In the control ewe and her lamb, no pathological lesions were found either in the livers or in the intestines.

The present study showed that congenital infection can occur in ewes infected with *S. japonicum* when infected in mid- to late pregnancy, resulting in patent infections in the lambs at delivery. In contrast, patent infections were not found in prenatally *S. bovis*-exposed lambs, and the etiology of the histopathological findings in the livers could not be determined. These results thus extend earlier findings from other mammalian hosts that congenital infection with *S. japonicum* may occur (Wang, 1958; Okabe, 1961; Willingham et al., 1999; Qian et al., 2000; Johansen et al., 2001) and support the hypothesis that this may not be common for other schistosome infections in either man or domestic stock. Whether the parasite *S. japonicum* has adapted special abilities for congenital transmission because of its wide range of definitive hosts can only be speculated on at this stage. Clearly, further studies are needed to elucidate this phenomenon thoroughly.

We are thankful to late professor Peter Nansen for initiating this project. Charlotte R. Bergholdt, Niels P. Hansen, and Niels Midtgaard, from the Danish Centre for Experimental Parasitology, are thanked for their technical assistance. The Danish National Research Foundation is acknowledged for financial support.

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J. Parasitol., 88(2), 2002, pp. 415–417
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Prevalence of Antibodies to *Neospora caninum* in White-Tailed Deer, *Odocoileus virginianus*, From the Southeastern United States

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ABSTRACT: Serum samples from 305 white-tailed deer (*Odocoileus virginianus*) from 14 states in the southeastern United States were examined for antibodies to *Neospora caninum* using a direct agglutination test. Positive agglutination titers were found in 145 (48%) of the white-tailed deer examined: 21 (7%) had titers of 1:25, 92 (30%) had titers of 1:50, and 32 (10%) had titers of \geq 1:500. These findings that antibodies to *N. caninum* are common in white-tailed deer support the con-

cept that a sylvatic cycle might exist for this economically important parasite of domestic cattle.

Neospora caninum is recognized as a cause of neonatal neuromuscular disease in dogs and abortion in cattle worldwide (reviewed by Dubey and Lindsay, 1996; Lindsay and Dubey, 2000). Dogs are a de-

finitive host and excrete coccidial oocysts in their feces after ingesting *N. caninum* tissue cysts (McAllister et al., 1998; Lindsay, Dubey, and Duncan, 1999; Lindsay, Upton, and Dubey, 1999). However, little is known about the prevalence of the parasite in wildlife populations. *Neospora caninum* antibodies have been found in coyotes (Lindsay et al., 1996), dingos (Barber et al., 1997), and red foxes (Barber et al., 1997; Buxton et al., 1997; Simpson et al., 1997), suggesting a role for wild canids in the epidemiology of neosporosis. Dubey et al. (1999) found that 162 (41%) of 400 white-tailed deer (*Odocoileus virginianus*) from northeastern Illinois had agglutinating antibodies to *N. caninum*. A fatal case of neosporosis has been found in a 2-mo-old black-tailed deer fawn (*O. hemionus columbianus*) from California (Woods et al., 1994). These reports suggest that a wild canid-wild ruminant cycle might exist for *N. caninum*.

The present study was done to determine the prevalence of antibodies to *N. caninum* in white-tailed deer from the southeastern United States. Samples were collected from 305 white-tailed deer by members of the Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia. Samples were sent to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, for serological testing for agglutinating antibodies to *N. caninum*.

Tachyzoites of the NC-1 strain of *N. caninum* were used as antigen for the *Neospora* agglutination test (NAT). The general procedure described by Packham et al. (1998) was used with slight modifications. Briefly, tachyzoites were collected from infected African green monkey (*Cercopithecus aethiops*) kidney cells (CV-1 cells, ATCC CCL-70, American Type Culture Collection, Manassas, Virginia) and purified from host cells by passage through a 27-ga needle attached to a 10-ml syringe and filtration through a 3- μ m filter. The purified tachyzoites were fixed in 2 ml of 37% formaldehyde solution, which was then diluted to 6% with phosphate-buffered saline (PBS) and stored at 4 C. Prior to use in the NAT, the tachyzoites were washed twice in PBS and resuspended in alkaline buffer containing eosin (Packham et al., 1998) at 4×10^7 tachyzoites/ml and 0.2 M 2-mercaptoethanol. The eosin aided in the visualization of the agglutination reaction, and the mercaptoethanol was added to destroy IgM antibodies and prevent nonspecific agglutination caused by IgM molecules. The direct agglutination test was conducted in 96-well, round-bottom plates. Test sera were diluted with PBS, and 25 μ l of serial test dilutions was added to 75 μ l antigen solution and mixed thoroughly by pipetting up and down several times. Positive serum from *N. caninum*-infected mice and negative control sera from mice not infected were run on each plate. Sera from mice infected with *Toxoplasma gondii* and *Sarcocystis neurona* were also used on each plate as controls. The plates were covered and incubated

overnight at 37 C in a CO₂ incubator. The reaction was read the next morning. Diffuse white opacity across the entire diameter of the well was considered a positive agglutination reaction. A central discrete opaque dot or button was considered a negative reaction.

Serum samples from 145 (48%) of the 305 white-tailed deer examined were positive (Table I). Positive white-tailed deer samples were present in all 14 states. Of the positive white-tailed deer, 21 (7%) had titers of 1:25, 92 (30%) had titers of 1:50, and 32 (10%) had titers of $\geq 1:500$.

The results of the present study indicate that 48% of white-tailed deer from the southeastern United States have antibodies to *N. caninum*, similar to the 41% of white-tailed deer from northeastern Illinois reported by Dubey et al. (1999). These studies indicate that exposure to *N. caninum* by white-tailed deer is common. Because white-tailed deer are ruminants, it is likely that they become infected by ingesting *N. caninum* oocysts while feeding or drinking. The effect of *N. caninum* on white-tailed deer populations is not known. The finding of neonatal neosporosis in a black-tailed deer (Woods et al., 1994) indicates the potential for disease in white-tailed deer as well.

Dogs are the only known definitive host for *N. caninum* (McAllister et al., 1998; Lindsay, Dubey, and Duncan, 1999; Lindsay, Upton, and Dubey, 1999). Antibodies to *N. caninum* have been found in wild canids, but patent infections or clinical disease has not been observed (Lindsay and Dubey, 2000). Domestic dogs excrete relatively few *N. caninum* oocysts in their feces (Lindsay, Dubey, and Duncan, 1999; Lindsay et al., 2001), indicating that they are a poor definitive host. The role of wild canids needs to be studied further to determine their importance in the transmission of *N. caninum*.

We thank Kay Carlson, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, for technical assistance. The contribution of D.S.L. was supported in part by a Clinical Research grant from Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech.

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TABLE I. Prevalence of agglutinating antibodies to *Neospora caninum* in white-tailed deer from the southeastern United States.

State	Total*	Positive agglutination titer†			
		<1:25	1:25	1:50	1:500
Alabama	10/5 (50)	5 (50)	1 (10)	4 (40)	0
Arkansas	14/10 (71)	4 (29)	4 (29)	5 (36)	1 (7)
Florida	21/6 (29)	15 (71)	2 (10)	4 (19)	0
Georgia	61/20 (33)	41 (67)	1 (2)	17 (28)	2 (3)
Kentucky	24/6 (25)	18 (75)	1 (4)	3 (13)	2 (8)
Louisiana	20/6 (30)	14 (70)	0	3 (15)	3 (15)
Maryland	10/10 (100)	0	4 (40)	4 (40)	2 (20)
Mississippi	15/6 (40)	9 (60)	0	6 (40)	0
Missouri	22/12 (55)	10 (45)	3 (14)	7 (32)	2 (9)
North Carolina	15/5 (33)	10 (67)	0	4 (27)	1 (7)
South Carolina	27/15 (56)	12 (44)	2 (7)	12 (44)	1 (4)
Tennessee	20/11 (55)	9 (45)	2 (10)	5 (25)	4 (20)
Virginia	15/7 (47)	8 (53)	1 (7)	4 (27)	2 (13)
West Virginia	31/26 (84)	5 (16)	0	14 (45)	12 (39)
Total, all animals	305/145 (48)	160 (52)	21 (7)	92 (30)	32 (10)

* Total number sampled/number positive (percent positive from that state).

† Number positive at that titer (percent of total for that state).

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J. Parasitol., 88(2), 2002, pp. 417–418
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Identical ITS-1 and ITS-2 Sequences Suggest *Spiculopteragia asymmetrica* and *Spiculopteragia quadrispiculata* (Nematoda: Trichostrongylidae) Constitute Morphologically Distinct Variants of a Single Species

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ABSTRACT: Sequences of ITS-1 and ITS-2 rDNA for adult males of *Spiculopteragia asymmetrica* and *Spiculopteragia quadrispiculata* in red deer (*Cervus elaphus*) were determined. They were found to be identical, suggesting that *S. asymmetrica* and *S. quadrispiculata* represent a single species and do not refute the concept of dimorphic species in the *Spiculopteragia*.

Since the hypothesis for polymorphism among male nematodes within species of the Ostertagiinae was proposed (Daskalov, 1974; Drózdź, 1974, 1995; Lancaster and Hong, 1981), numerous studies have been conducted to examine that assumption. Polymorphism has been demonstrated in *Marshallagia*, *Teladorsagia*, and *Ostertagia* in the following species: *M. marshalli*/*M. occidentalis*, *T. circumcincta*/*T. trifurcata*/*T. davtiani*, *T. boreoarcticus* forma major/*T. boreoarcticus* f. minor, *O. ostertagi*/*O. lyrata*, *O. mossi*/*O. dikmansii*, *O. leptospicularis*/*O. kolchida*, and *O. gruehneri*/*O. arctica*. These conclusions were based on morphological studies (Lichtenfels and Hoberg, 1988; Lichtenfels et al., 1990; Hoberg et al., 1993, 1999; Lichtenfels and Hoberg, 1993; Drózdź, 1995), cross-breeding experiments (Lancaster et al., 1983; Suárez and Cabaret, 1992), allozyme electrophoresis (Andrews and Beveridge, 1990; Gasnier et al., 1993), and comparisons of DNA sequences (Stevenson et al., 1996; Zarlenga et al., 1998; Hoberg et al., 1999; Dallas et al., 2000). It is considered that continued documentation of polymorphism within Ostertagiinae, as well as standardization of taxonomy for major and minor morphotypes, is of interest for biological and epidemiological studies (Hoberg et al., 1999).

Within *Spiculopteragia*, the sole basis for suspecting polymorphism has been the co-occurrence of 2 morphotypes of male nematodes in the same host (Drózdź, 1995). For example, *Spiculopteragia asymmetrica* and *S. quadrispiculata* are commonly found in the abomasum of cervids. They were considered separate species based on morphological characters of male nematodes (Drózdź, 1965). To date, no genetic studies have been performed to investigate polymorphism in any species of *Spiculopteragia*. In this study, the hypothesis that *S. asymmetrica* and *S. quadrispiculata* could be differentiated genetically was tested. For

this purpose, the DNA sequences of the first internal transcribed spacer (ITS-1) and second internal transcribed spacer (ITS-2) of rDNA were determined and compared in *S. asymmetrica* and *S. quadrispiculata*.

Abomasa were removed from red deer at Extremadura (Spain), and adult nematodes were preserved in 70% ethanol. The caudal extremity was cut from each male specimen, and identity of the specimens was determined by examination of structural characters of the bursa and spicules (e.g., Drózdź, 1965). Genomic DNA was isolated from 5 individual worms corresponding morphologically to *S. asymmetrica* and from 5 representing *S. quadrispiculata* using DNeasy[®] (Qiagen, Valencia, California). The ITS-1 was subsequently amplified using primers 241 (5'AAAGGAATTCAAGTCGTAACAAGGTTTCCGTAGG 3') and 242 (5'ATTGGATCCAACAACCCTGAACGACAGCTAC 3') (Zarlenga et al., 1998) and the ITS-2 with primers NC1 (5'ACGTCGTGTTTCAGGGTTGTT 3') and NC2 (5'TTAGTTTCTTTTCTCCGCT 3') (Gasser et al., 1993). The polymerase chain reaction (PCR) was performed in a 50- μ l reaction volume containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM MgCl₂, 2.5 U of Taq DNA polymerase, 40 μ M of each deoxynucleotide triphosphate (Gibco BRL[®], Foster City, California). The PCR program was as follows: 35 cycles of 94 C for 1 min, 55 C for 1 min, and 72 C for 2 min, followed by a final extension at 72 C for 7 min. Negative (no-DNA) and positive controls (*Haemonchus contortus* DNA) were included in each set of reactions. PCR products were detected on ethidium bromide-stained 1.5% TAE (0.04 M Tris-acetate, 1 μ M EDTA) agarose gels. PCR products were purified using Qiaquick[®] spin columns (Qiagen) then sequenced using the same primers as for PCR in 10- μ l reactions using BigDye chemistries and a 377 automated sequencer (PE Biosystems, Rockland, Maine). Each of the 10 individuals were bidirectionally sequenced at the ITS-1 and ITS-2 loci. Sequence chromatograms from each strand were aligned and inspected using Sequencer version 3.1 (Gencodes Corp., Ann Arbor, Michigan).

The PCR products represented single fragments of ~500 bp ITS-1 and ~300 bp ITS-2, comparable to those found in other members of the subfamily (Stevenson et al., 1996; Dallas et al., 2000). The sequence

corresponding to the ITS-1 and ITS-2 locus for each of these individuals has been deposited in GenBank[®] (GenBank accession nos. AF480615–AF480618, respectively). No fixed differences between *S. asymmetrica* and *S. quadrispiculata* were detected in the ITS-1 or ITS-2 sequence. Instead, all but 1 nucleotide position were invariant. For this sole exception, individual worms exhibited both C and T in position 50 of the ITS-2 sequence. Such dimorphism was not restricted to members of either putative taxon, or was it restricted to either sequencing direction.

The absence of differences in the ITS-1 and ITS-2 rDNA sequences has previously been interpreted as evidence that morphological polymorphism exists among males belonging to single species of *Teladorsagia* and *Ostertagia* (see Stevenson et al., 1996; Zarlenga et al., 1998; Dallas et al., 2000). Additionally, the occurrence of polymorphism within *Teladorsagia* spp. has been assessed based on sequences of mitochondrial DNA (Hoberg et al., 1999). Clearly the possibility of *S. asymmetrica* and *S. quadrispiculata* representing 2 different species cannot be ruled out because variations at other loci not examined could refute the hypothesis that both taxa comprise a single reproductive and evolutionary lineage. However, results obtained are consistent with the concept of polymorphism observed within species among related genera, including *Ostertagia* and *Teladorsagia* (Zarlenga et al., 1998; Hoberg et al., 1999). Their close evolutionary relationship is further suggested by the segregation of the same nucleotide polymorphism in individuals corresponding to each morphological type. The absence of any fixed genetic difference in ITS-1 and ITS-2 between *S. asymmetrica* and *S. quadrispiculata* comprises the first genetic evidence for morphological polymorphism among individuals of species in the genus *Spiculopteragia*.

Mónica Santín-Durán was supported by a predoctoral fellowship from Comunidad de Madrid. The study was conducted while M.S.-D. was a visiting scientist at the Biosystematics Unit, Parasite Biology, Epidemiology and Systematics Laboratory, ARS, and was assisted by Mayee Wong.

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Prevalence of *Toxoplasma gondii* Antibodies in Sera of Domestic Cats From Guarulhos and São Paulo, Brazil

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ABSTRACT: Antibodies to *Toxoplasma gondii* were determined in serum samples of 502 domestic cats from Brazil by the modified agglutination test (MAT), using formalin-fixed whole tachyzoites and mercaptoethanol. Antibodies (MAT \geq 1:20) were found in 132 (26.3%) of 502 cats. With respect to origin, antibodies were found in 26.7% of 430 stray cats from São Paulo, 10% of 40 stray cats from Guarulhos, and 40.6% of 32 cats from a cat breeder in São Paulo. Antibody titers were: 1:20 in 10 cats, 1:25 in 40 cats, 1:50 in 73 cats, and \geq 1:500 in 9 cats. Exposure rates of *T. gondii* in cats from São Paulo, Brazil are similar to that in domestic cats in North America.

Infections by *Toxoplasma gondii* have been reported in numerous species of warm-blooded animals worldwide (Dubey and Beattie, 1988). Cats are pivotal in the transmission of *T. gondii* to humans and other animals because they are the only hosts that can excrete the environmentally resistant oocysts in feces (Dubey and Beattie, 1988). Because oocysts are rarely found in feces of cats, serologic prevalence data in cats are important for the determination of epidemiologic significance of *T. gondii* infection (Dubey, Weigel et al., 1995).

Antibodies to *T. gondii* have been reported in domestic cats from Brazil, in São Paulo city, São Paulo (Sogorb et al., 1972; Santos et al., 1983; Lucas et al., 1999), Botucatu, São Paulo (Salata et al., 1985), Jaguapitã, Paraná (Garcia et al., 1999), and Manaus, Amazonas (Ferraroni et al., 1980). The number of cats examined ranged from 9 to 248 and the prevalence of antibodies to *T. gondii* ranged from 0% to 81.0%. Most of these surveys were done approximately 20 yr ago. The objective of the present paper is to document the prevalence of *T. gondii* antibodies in cats from São Paulo and Guarulhos, São Paulo State, Brazil.

Sera were collected during 1993 to 2000 from a total of 502 domestic cats (*Felis catus*) from São Paulo and Guarulhos cities, São Paulo State in Brazil. A total of 470 stray cats was captured by Center for Zoonosis Control of São Paulo and Guarulhos. Thirty-two cats were from a breeder in São Paulo; 26 of these 32 cats were born in house, and 6 were feral.

Cats were physically or chemically restrained using ketamine and xylazine. Blood samples were collected from each animal, and sera were stored at -20°C until serologic analysis.

Sera were analyzed for antibodies to *T. gondii* at the Parasite Biology, Epidemiology, and Systematics Laboratory of the U.S. Department of Agriculture, Beltsville, Maryland by the modified agglutination test (MAT) using formalin-fixed whole tachyzoites and 2-mercaptoethanol as previously described (Dubey and Desmots, 1987). Sera were thawed and frozen several times for testing. Sera were initially screened in 1:25, 1:50, and 1:500 dilutions. Sera with doubtful result at 1:25 dilution were reexamined in 1:10, 1:20, and 1:40 dilutions. A titer of \geq 1:20 was considered indicative of past *T. gondii* infection (Dubey and Thulliez, 1989; Dubey, Lappin, and Thulliez, 1995a, 1995b). Controls were included in each test.

Antibodies (MAT \geq 1:20) to *T. gondii* were found in 132 (26.3%) of 502 cats. With respect to origin, antibodies were found in 26.7% of 430 stray cats from São Paulo, 10% of 40 stray cats from Guarulhos, and 40.6% of 32 cats from a breeder in São Paulo. Antibody titers were: 1:20 in 10 cats, 1:25 in 40 cats, 1:50 in 73 cats, and \geq 1:500 in 9 cats (Table I). Seven of 58 (12.1%) kittens ($<$ 12 wk), 10 of 71 (14.1%) young cats ($<$ 6 mo), and 14 of 61 (22.9%) adult cats were seropositive; age was not recorded for 313 cats. Antibodies in 7 kittens were low (3,

1:20; 4, 1:25) and might have been acquired colostrally. Colostrally acquired *T. gondii* antibodies disappear in kittens by 16 wk of age (Dubey, 1973; Dubey, Lappin, and Thulliez, 1995b).

The seroprevalence of *T. gondii* antibodies found in the present study was different from those reported previously from Brazil (Table II). It is not possible to compare results of the present study with other surveys in cats from Brazil, because of the sample size, period of survey, and the type of serologic test used. The present study used MAT, which is considered the most specific and sensitive for determining *T. gondii* antibodies in cats (Dubey and Thulliez, 1989; Dubey, Lappin, and Thulliez, 1995a, 1995b).

The seroprevalence of *T. gondii* in humans varies depending on the region, age of the people, and the economic status of the population. For example, in a recent study, Bahia-Oliveira et al. (2001) found that *T. gondii* prevalence in 6- to 10-yr-old children in Campos dos Goytacazes, Rio de Janeiro varied from 70% to 0%, and it was correlated with hygienic and social conditions. The group with the highest prevalence was very poor economically and could not afford to eat meat. The ingestion of food and water contaminated directly with oocysts was considered to be the main source of infection. Souza et al. (1987) also reported that 68.4% of 6- to 8-yr-old children in Rio de Janeiro, Brazil had antibodies to *T. gondii*. Thus, in certain areas of Brazil there is a wide contamination of environment with *T. gondii* oocysts.

Results of this present study indicate that 26.3% of cats from São Paulo and Guarulhos, Brazil were exposed to infection to *T. gondii*. Cats generally become infected with *T. gondii* by ingestion of tissue cysts from uncooked or undercooked infected meat (Dubey and Beattie, 1988). Freezing of meat kills tissue cysts of *T. gondii* (Kotula et al., 1991). Therefore, all meat should be frozen before feeding to cats. The higher seroprevalence of *T. gondii* in cats in the breeder facility than in other cats may be because the owner probably fed uncooked meat to the cats.

We are grateful to Danilli Del Guercio (breeder), Renato Caravieri,

TABLE I. Antibodies to *Toxoplasma gondii* by the modified agglutination test (MAT) in cats from areas or type in São Paulo, Brazil.

Area or type	No. of cats examined	No. positives with MAT (\geq 1:20)	Per cent positive (%)	No. of cats with MAT titers of:		
				1:20 or 1:25	1:50	\geq 1:500
São Paulo	430	115	26.7	38 (6* + 32†)	68	9
Guarulhos	40	4	10.0	2 (1* + 1†)	2	0
Breeder	32	13	40.6	10 (3* + 7†)	3	0
Total	502	132	26.3	50 (10* + 40†)	73	9

* = Titer of 1:20.

† = Titer of 1:25.

TABLE II. Prevalence of antibodies of *Toxoplasma gondii* in sera of domestic cats from Brazil.

Locality*	Reference	Test†	No. of cats		% positive of cats with titers of:									
					16	20	25	50	64	128	256	≥500	1024	>1024
São Paulo—SP	Sogorb et al. (1972)	DT	130	50.8									5	5.4
Manaus—AM	Ferraroni et al. (1980)	IHA	32	81.0						81				
São Paulo—SP	Santos et al. (1983)	IHA	100	59.0					1		3		19	36
Botucatu—SP	Salata et al. (1985)	IFA	9	0	4.8									
São Paulo—SP	Lucas et al. (1999)	IFA	248	17.7	7.4				5.7		4.8		1.2	1.2
Jaguapitã—PR	Garcia et al. (1999)	IFA	163	73.0					10.4		16		12.3	27
São Paulo, and Guarulhos—SP	Present study	MAT	502	26.3		2	8	14.5					1.8	

* SP = São Paulo, AM = Amazonas, PR = Paraná.

† DT = dye test, IHA = indirect hemagglutination, IFA = indirect fluorescent antibody, MAT = modified agglutination test.

‡ = Percentage of seropositive cats.

Vanessa Muradian, Maria Fernanda V. Marvulo, and Sandra Nishi for their assistance and support in collection of the samples.

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J. Parasitol., 88(2), 2002, pp. 420–422
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Distribution and Prevalence of *Echinococcus multilocularis* in Wild Predators in Nebraska, Kansas, and Wyoming

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ABSTRACT: To further determine the distribution and prevalence of *Echinococcus multilocularis* in the central United States, 245 wild canids (125 red foxes, 120 coyotes) and 33 bobcats were collected from Nebraska, Kansas, and Wyoming and examined for this parasite. Ani-

mals examined included 11 red foxes from the western panhandle of Nebraska; 5 red foxes and 30 coyotes from southern Nebraska; 56 red foxes and 1 coyote from northeastern Nebraska; 20 red foxes, 63 coyotes, and 13 bobcats from northern Kansas; 2 red foxes, 26 coyotes,

and 20 bobcats from southern Kansas; and 31 red foxes from east-central Wyoming. Of these, 27 of 72 (37.5%) red foxes from Nebraska were positive, including 2 of 11 (18.2%) from the western panhandle and 25 of 56 (44.6%) from the northeastern part of the state. Mean intensity of infection was 282 worms (range, 1–5,150). New distribution records were established for *E. multilocularis* in western Nebraska as well as for several northeastern counties. These findings support previous estimates that the southernmost front of the parasite's range extends along the southern border of Wyoming, eastward through central Nebraska and central Illinois into Indiana and Ohio.

In North America, *Echinococcus multilocularis* occurs in 2 endemic areas: 1 encompassing the tundra zone of Alaska and Canada and the other the central part of the continent (Rausch, 1985, 1995). In the latter area, the parasite has been reported from 3 Canadian provinces and 11 contiguous states and has been found as far east as Ohio (Storandt and Kazacos, 1993). In the central region, the primary definitive hosts are red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*). Meadow voles (*Microtus pennsylvanicus*) and deer mice (*Peromyscus maniculatus*) are the main intermediate hosts (Hildreth et al., 1991). Because of the abundance of suitable hosts in areas surrounding the known endemic region, further spread of *E. multilocularis* is anticipated (Rausch, 1995). To further determine the distribution and prevalence of *E. multilocularis* in the central United States, wild canids and bobcats (*Lynx rufus*) from Nebraska, Kansas, and Wyoming were examined for this parasite.

During 1991–1992 (in Kansas) and 1994–1996 (Nebraska and Wyoming), 245 wild canids (125 red foxes, 120 coyotes) and 33 bobcats were collected and examined for *E. multilocularis*. Most were collected from participating fur buyers and trappers during the fall and winter trapping seasons, and some were supplied by USDA-APHIS Wildlife Services personnel. The small intestine was ligated, removed, double-bagged, frozen, and later processed and examined for *E. multilocularis* at Purdue University as previously described (Storandt and Kazacos, 1993). Representative specimens have been deposited in the U.S. National Parasite Collection (USNPC 091271.00).

Animals examined included 11 red foxes from the western panhandle of Nebraska; 5 red foxes and 30 coyotes from southern Nebraska; 56 red foxes and 1 coyote from northeastern Nebraska; 20 red foxes, 63 coyotes, and 13 bobcats from northern Kansas; 2 red foxes, 26 coyotes, and 20 bobcats from southern Kansas; and 31 red foxes from east-central Wyoming. Of these, 27 of 72 (37.5%) red foxes from Nebraska were positive, including 2 of 11 (18.2%) from the western panhandle and 25 of 56 (44.6%) from the northeastern part of the state (Table I; Fig. 1). Mean intensity of infection was 282 worms (range, 1–5,150).

In Nebraska, *E. multilocularis* was collected from red foxes taken in the counties listed in Table I. No positive animals were collected from the following counties in Nebraska, Kansas, or Wyoming (number of animals examined in parentheses; F = red fox, C = coyote, B = bobcat)—Nebraska: Chase (1C), Clay (1C), Colfax (2F), Dawson (4F, 9C), Dodge (1C), Dundy (3C), Fillmore (1C), Franklin (3C), Frontier (1F), Furnas (2C), Hitchcock (2C), Lincoln (1C), Morrill (1F), Nuckolls (2C), Red Willow (2C), Webster (3C); Kansas: Chase (1F, 12C, 7B), Clay (1F, 2C), Coffey (11C, 10B), Ellis (1F), Graham (10C, 1B), Harper (1C),

TABLE I. *Echinococcus multilocularis* infection in red foxes in Nebraska (1994–1996).

County	Number examined	Infected	
		Number	%
Burt	5	2	40.0
Box Butte	4	1	25.0
Cuming	7	4	57.1
Dodge	16	10	62.5
Platte	12	3	25.0
Saunders	7	2	28.6
Scotts Bluff	6	1	16.7
Thurston	6	3	50.0
Unknown (northeast)	1	1	100.0

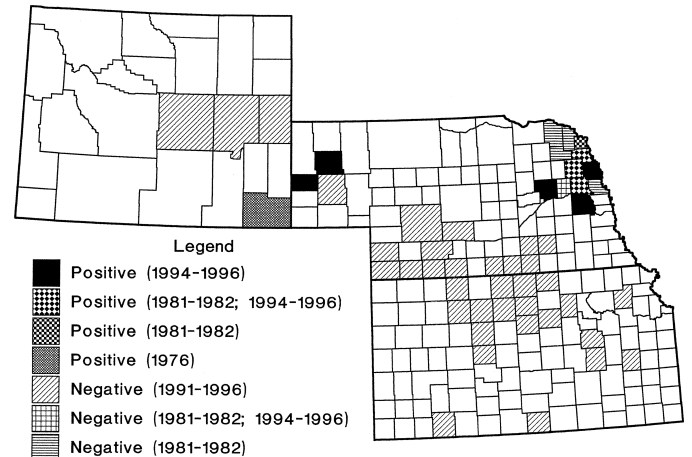


FIGURE 1. Distribution of *Echinococcus multilocularis* in wild canids in Nebraska, Kansas, and Wyoming, including data for Nebraska from Ballard and Vande Vusse (1983), and for a rodent infection in Wyoming from Kritsky et al. (1977).

Jackson (5F, 12C, 1B), Jewell (2C), Lincoln (9C, 3B), Meade (1F, 2C, 1B), Mitchell (1F, 3C, 3B), Morris (2B), Norton (10C, 1B), Osborne (1F, 9C), Ottawa (4F), Republic (1F, 4B), Rooks (1C), Rush (4C), Smith (6F, 1C); Wyoming: Converse (2F), Natrona (13F), Niobrara (5F), and unknown of latter 3 counties (11F).

Since it was first discovered in the north-central United States in the mid-1960s (Leiby and Olsen, 1964), *Echinococcus multilocularis* has steadily expanded its range to include all or part of 11 contiguous states. The parasite was found in wild canids or rodents in South Dakota, Iowa, Minnesota, and Montana in 1965–1969 (Carney and Leiby, 1968; Leiby et al., 1970; Rausch and Richards, 1971); in a wild woodrat in southeastern Wyoming in 1976 (Kritsky et al., 1977); and in wild canids in northeastern Nebraska and northern Illinois in 1981–1982 (Ballard and Vande Vusse, 1983), in Wisconsin in 1982–1983 (Ballard, 1984), and in northern Indiana, northwestern Ohio, and east-central Illinois in 1990–1991 (Storandt and Kazacos, 1993). Annual surveys conducted in eastern South Dakota in 1987–1991 found a high prevalence of infection in red foxes in that area (64.0–88.9%, mean 74.5%; Hildreth et al., 2000). In the early 1990s, it was estimated that the southernmost front of the parasite's range extended along the southern border of Wyoming, eastward through central Nebraska and central Illinois into Indiana and Ohio (Hildreth et al., 1991; Storandt and Kazacos, 1993). Results of the present study further support this assertion because *E. multilocularis* was not found south of a line through central Nebraska (Fig. 1). The parasite was not detected in 35 wild canids from southern Nebraska or 111 wild canids and 33 bobcats from Kansas (Fig. 1).

Echinococcus multilocularis has a much lower prevalence in wild canids in western Nebraska and Wyoming compared to northeastern Nebraska, in that only 2 of 42 (4.8%) red foxes from the west were infected versus 25 of 56 (44.6%) red foxes from the northeast. Presumably, this same pattern would hold true for coyotes but could not be assessed because only a single coyote was examined from the northeastern counties and none from the west. In other studies, coyotes have been found to be as susceptible to infection with *E. multilocularis* as red foxes and, in several cases, to have a higher prevalence and intensity of infection. For example, in Indiana, 22.5% of 71 red foxes and 18.6% of 70 coyotes were infected, and in Illinois, 35.3% of 17 coyotes carried the parasite. Mean intensity of infection for red foxes was 372 worms (range, 2–3,640) versus 6,579 worms (range, 1–52,000) for coyotes (Storandt and Kazacos, 1993).

Coyotes typically have a more diverse diet than red foxes, eating rabbits, hares, and other larger mammals, in addition to rodents. However, even though they consume fewer rodents than do red foxes (Voigt and Berg, 1987), they eat enough rodents to contact the larval cestode and become infected with *E. multilocularis*. It is well known that a very low prevalence of larval infection in rodents in an area is sufficient to maintain moderate to high levels of infection in wild canids (Rausch,

1995). It is likely that infected coyotes are more important in the spread of this parasite because they typically have larger home ranges and can travel much longer distances than red foxes (Voigt and Berg, 1987). Suitable intermediate hosts for *E. multilocularis* occur in all of the areas examined in the present study. Therefore, the absence of *E. multilocularis* in wild canids in southern Nebraska, Kansas, and east-central Wyoming either reflects the absence of the parasite in these areas (southern Nebraska, Kansas) or a sufficiently low prevalence (<1–2% in canids) so that it was not detected based on the number of hosts examined. Continued vigilance is necessary to monitor the distribution, prevalence, and spread of this important parasite in different areas of central North America.

We thank the fur buyers of Nebraska and Kansas, particularly S&D Furs, Tate Hide and Fur Co., Petzka Furs, and Bo Viter; Nebraska field personnel of USDA-APHIS Wildlife Services; and Lloyd Fox of the Kansas Department of Wildlife and Parks, who provided animals for this investigation. We also thank David E. Williams, State Director of USDA-APHIS Wildlife Services, and Chuck McCullough of the Nebraska Game and Parks Commission, for facilitating this study and Ralph Gann and Susan Reynolds of the Indiana Agricultural Statistics Service for help in preparing Figure 1.

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J. Parasitol., 88(2), 2002, pp. 422–425
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Infection of *Aotus* and *Saimiri* Monkeys with *Plasmodium gonderi*

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ABSTRACT: Attempts were made to infect 4 species of New World monkeys (*Saimiri boliviensis*, *Aotus nancymai*, *A. vociferans*, *A. azarae boliviensis*) with *Plasmodium gonderi*, a malaria parasite of African monkeys. Sporozoites were obtained from *Anopheles dirus* or *A. stephensi* mosquitoes that fed on an infected rhesus monkey (*Macaca mulatta*). Inoculation of sporozoites was by injection of dissected sporozoites by either the intravenous or intrahepatic routes, or by mosquito bite. Liver biopsies done 7 or 8 days after sporozoite inoculation showed that hepatocytes of all 4 species of these New World monkeys supported exoerythrocytic stages of *P. gonderi*, but daily blood film examination during a 60-day observation period failed to detect blood stages of the parasite.

Plasmodium gonderi (Sinton and Mulligan, 1933) is a tertian malaria parasite of monkeys from Africa (*Cercocebus*, *Cercopithecus*, and *Mandrillus*) (Garnham et al., 1958; Coatney et al., 1971; Poirriez et al., 1995) that has been adapted to the rhesus monkey *Macaca mulatta*, as an experimental host. Infection can be induced by either blood passage or sporozoite transmission. Because the rhesus monkey is expensive, finding other hosts for Old World nonhuman primate malaria might be of value. Attempts have been made to transmit plasmodia that grow well in Old World monkeys, such as the rhesus, to New World monkeys, such as *Aotus* or *Saimiri*, by blood or sporozoite inoculation. Successful transmissions of such plasmodia into *Aotus* or *Saimiri* monkeys by sporozoite inoculation include *P. fragile* (Collins et al., 1990), *P. knowlesi*

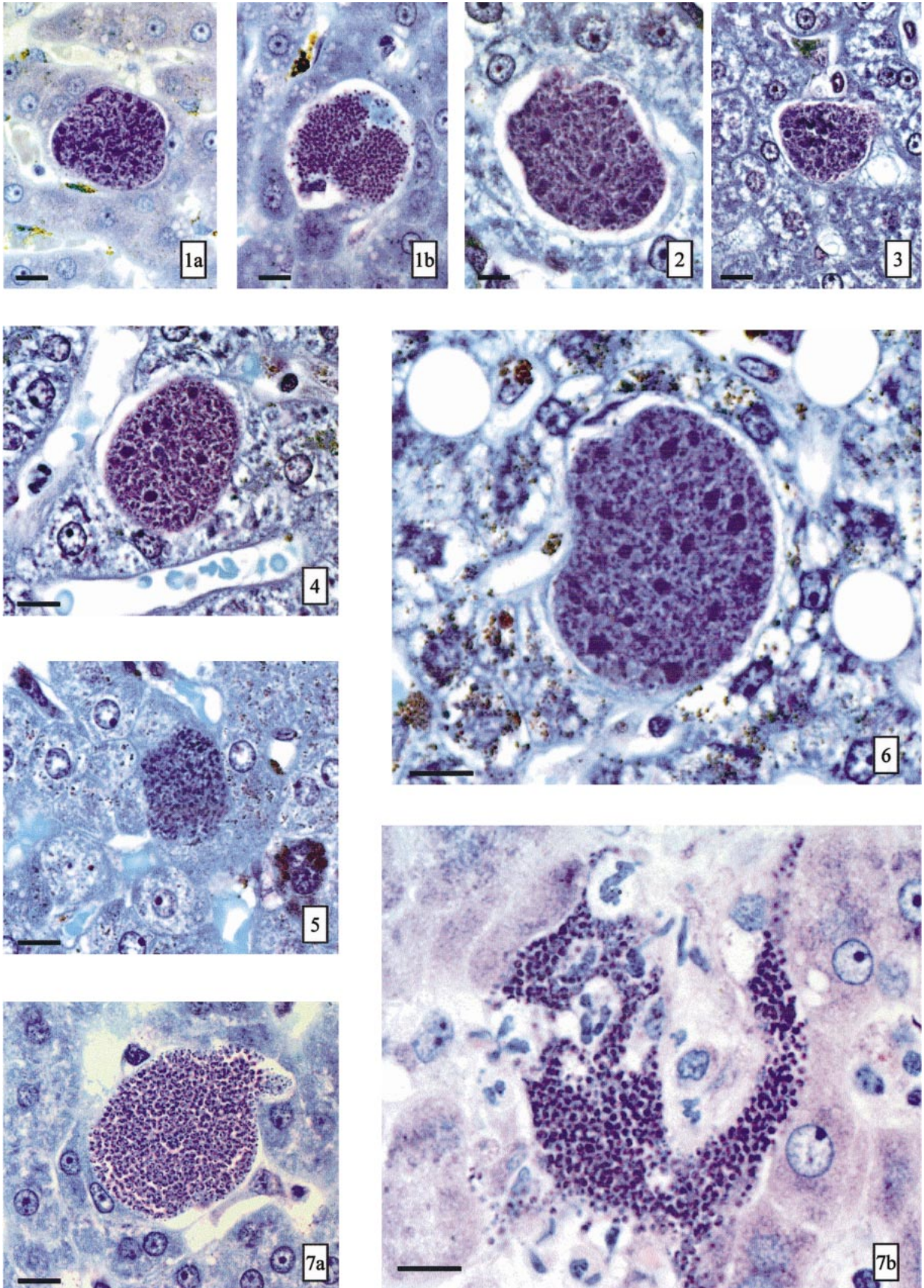
(Sullivan et al., 1996), *P. cynomolgi* (Collins et al., 1985; Collins et al., 1999), and *P. inui* (Collins et al., 1988). It has also been shown that sporozoite inoculation of *P. fieldi* (Sullivan et al., 1998) and of the human malaria parasite, *P. ovale* (Millet et al., 1994), into New World monkeys will result in the formation of exoerythrocytic (EE) stages in the liver, but not in the appearance of blood-stage parasites.

Reported here are the results of a study to determine: (1) if sporozoite inoculation of *P. gonderi* into *Aotus* and *Saimiri* monkeys would result in the formation of EE bodies in the liver or the appearance of erythrocytic stages of the parasite, and (2) if one particular route of sporozoite inoculation (intravenous [IV], intrahepatic [IH], or mosquito bite) is more successful at producing infection than another.

The strain of *P. gonderi* used in this study was originally from a drill (*Mandrillus leucophaeus*) from Liberia and has been maintained either in rhesus monkeys or frozen over liquid nitrogen.

Ten monkeys were used in this study: 5 *Saimiri boliviensis*, 2 *Aotus nancymai*, 1 *A. vociferans*, 1 *A. azarae boliviensis*, and 1 rhesus (*M. mulatta*). Monkeys were obtained commercially or were born in the laboratory, and were maintained on a diet consisting of monkey chow, fruits, and vegetables considered adequate for good health. All had been previously infected with other *Plasmodium* species, and all but the rhesus monkey were splenectomized before sporozoite inoculation. The rhesus monkey was splenectomized after sporozoite inoculation, at the time of liver biopsy.

Anopheles stephensi and *A. dirus* mosquitoes were reared in the Cen-



FIGURES 1–7. Exoerythrocytic (EE) stages of *Plasmodium gonderi* in liver sections from *Aotus*, *Saimiri*, and *Macaca mulatta* monkeys. **1a**, **b**. EE bodies from liver of SI-682 at day 7. **2**. EE body from liver of SI-804 at day 7. **3**. EE body from liver of R-8904 at day 7. **4**. EE body from liver of AI-1736 at day 7. **5**. EE body of WR-216 at day 8. **6**. EE body of AI-2152 at day 8. **7a**, **b**. EE bodies from liver of SI-674 at day 8. Scale = 10 μ m.

TABLE I. Inoculations of *Saimiri*, *Aotus*, and *Macaca mulatta* monkeys with sporozoites of *Plasmodium gonderi*.

Animal species	Animal ID	No. sporozoites inoculated/route*	Vector (<i>Anopheles</i> sp.)	Liver biopsy (days after inoculation)	Previous malaria†	EE bodies seen	Blood parasites seen
<i>Saimiri boliviensis</i>	SI-425	160,000/IH	<i>dirus</i>	7	Pf, Pv	Yes	No
	SI-674	115,000/IH	<i>dirus</i>	8	Pf, Pv	Yes	No
	SI-682	245,000/IV	<i>dirus</i>	7	Pf, Pv	Yes	No
	SI-704	75,000/IV	<i>stephensi</i>	7 and 8‡	Pf, Pv	Yes	No
	SI-804	bites: 180+	<i>dirus</i>	7	Pv	Yes	No
<i>Aotus nancymai</i>	AI-1735	120,000/IH	<i>dirus</i>	7	Pv	No	No
	AI-1736	620,000/IV	<i>dirus</i>	7	Pv	Yes	No
<i>A. vociferans</i>	AI-2152	200,000/IH	<i>dirus</i>	8	Pf, Pv, Pm	Yes	No
<i>A. azarae boliviensis</i>	WR-216	470,000/IV	<i>dirus</i>	8	Pf, Pv	Yes	No
<i>Macaca mulatta</i>	R-8904	165,000/IH bites: 452+	<i>dirus</i>	7	cyn, kno	Yes	Yes

* IH = intrahepatic; IV = intravenous.

† Pf = *Plasmodium falciparum*; Pv = *P. vivax*; Pm = *P. malariae*; cyn = *P. cynomolgi*; kno = *P. knowlesi*.

‡ SI-704 was inoculated 2 days in succession.

ters for Disease Control and Prevention insectary and were infected by feeding caged mosquitoes on an anesthetized rhesus monkey infected with *P. gonderi*. After feeding, these mosquitoes were maintained on a sugar solution in an incubator at 25 C. The 10 monkeys were then exposed to infection either by mosquito bite, or by the inoculation of sporozoites, or both, and harvested from the salivary glands of the mosquitoes via either the IV or IH route (Table I). One monkey, SI-704, was inoculated with sporozoites 2 days in succession. In the case of mosquito bites, the intensity of the sporozoite infection of the salivary glands was rated from 1+ to 4+ (Sullivan et al., 1996). For inoculation of sporozoites, the glands were dissected in 50% fetal bovine serum/saline, and the number of sporozoites counted using a Neubauer counting chamber. Liver biopsies were performed at laparotomy either 7 or 8 days after inoculation, the liver tissue sectioned at 3–5 µm (50–200 sections per liver), stained with Giemsa stain, and examined for EE bodies. Blood films were made daily by the method of Earle and Perez (1932) and examined daily for 60 days after inoculation to detect erythrocytic parasites.

EE bodies were observed in liver sections from 9 of the 10 animals inoculated (all except AI-1735), but during the 60-day observation period, blood stages of *P. gonderi* were seen only in the rhesus monkey, R-8904. Liver stages were abundant in sections from SI-682 (inoculated IV), with some sections containing multiple EE bodies. Fewer were observed in liver sections from SI-674 (IH) and AI-1736 (IV), and were infrequently seen in the other infected animals, including the rhesus monkey, R-8904. The morphology of the liver stages in the 3 types of monkeys differed mainly in the varying stages of maturity. Liver sections taken on day 7 from SI-682 showed EE bodies that were either intact or beginning to differentiate (Figs. 1a, b). EE bodies from liver sections of SI-804, R-8904, and AI-1736 at 7 days after sporozoite inoculation showed large, dense nuclei (Figs. 2, 3, 4, respectively). WR-216 showed very few liver-stage parasites and they appeared poorly defined at 8 days (Fig. 5). Also at 8 days, AI-2152 showed liver stages containing large, dense nuclei (Fig. 6), and those from SI-674 were observed to be either differentiating (Fig. 7a) or disseminating merozoites into the surrounding tissue (Fig. 7b).

Sporozoite inoculation of *P. gonderi* via 3 different routes (IH, IV, and mosquito bite) has been shown to produce liver-stage parasites in *Aotus*, *Saimiri*, and rhesus monkeys. The size and route of the inoculum, as well as the species of monkey, appear to have little effect on whether EE bodies were produced, although SI-682, with an IV inoculation of 245,000 sporozoites, produced the most heavily infected liver sections. Sections from SI-682 also showed EE bodies at slightly different stages of development within the same liver, indicating that the rate of maturation of the parasites within a host's liver does not necessarily occur at a uniform rate. All species of monkeys inoculated developed EE bodies: 1 *A. nancymai* monkey, AI-1735, failed to show liver infection, but another *A. nancymai* monkey, AI-1736, did become infected. Blood stages of *P. gonderi* were seen only in the rhesus monkey, even though

EE bodies were rare at the time of biopsy on day 7. Since parasites were observed in the blood of this animal 11 days after sporozoite inoculation, it is possible that EE bodies had already matured and ruptured by the time the liver biopsy was taken. As in the case of *P. fieldi* and *P. ovale* sporozoites inoculated into *Aotus* and *Saimiri* monkeys, infection of erythrocytes by merozoites from liver stages apparently did not occur. The question remains, why do some parasites develop in the liver, but fail to develop in the erythrocytes? The results suggest that this model could be useful in delineating receptors involved in the invasion process of merozoites arising from schizonts in the liver. Despite the lack of a patent blood parasitemia, infections in these animals may prove of use for drug or immunologic studies on the liver stages of *P. gonderi*. It remains to be determined if sporozoites of other Old World nonhuman primate malarias, i.e., *P. simiovale* and *P. coatneyi*, will infect New World monkeys.

This work was supported in part by IAA 936-6001 between the U.S. Agency for International Development and the Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention. We thank the staff of the Animal Resources Branch, Scientific Resources Program, Centers for Disease Control and Prevention for their help.

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J. Parasitol., 88(2), 2002, pp. 425–427
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Negative Influence of *Gammarinema gammari* (Nematoda) on the Fecundity of *Microphallus papillorobustus* (Trematoda): Field and Experimental Evidence

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ABSTRACT: The gammarid amphipod *Gammarus insensibilis* frequently harbors adult individuals of the ectoparasitic nematode *Gammarinema gammari* as well as metacercariae of the trematode *Microphallus papillorobustus*. After the demonstration in a previous study of a negative relationship between the abundance of these 2 parasites, the nature of the relationship between these 2 parasites was explored in more detail by studying, in the field and in the laboratory, the influence of nematode abundance on trematode fecundity. In gammarids collected in the field, a negative relationship between metacercarial fecundity and the number of co-occurring nematodes was found. By manipulating the nematode abundance in the laboratory, it was confirmed that *G. gammari* has a negative effect on egg production in *M. papillorobustus*.

Demonstrating the existence of interspecific interactions among parasites is an important step toward understanding the structure of parasitic communities (Poulin, 1998). Among the different kinds of interactions between parasite species, interspecific competition is undoubtedly the interaction that has received the most attention from parasitologists. Much of the evidence for this phenomenon within parasitic communities relies on 2 types of observations, which are traditionally called numerical and functional responses. In the first situation, i.e., the numerical response, a reduction in the number of individuals of 1 parasite species, or a reduction in the fecundity of these individuals, is observed when another parasite species is present. In the second situation, i.e., the functional response, a parasite changes the way it uses host resources when a second species is present, for instance, by restricting its ecological niche inside the host (Rhode, 1994). Although it is important to demonstrate numerical effects before inferring the existence of competition (Thompson, 1980), parasitologists have in recent years devoted more effort to the study of functional responses (Poulin, 1998).

The head of the crustacean amphipod *Gammarus insensibilis* (Gammaridae) frequently harbors 2 different parasite species, the metacercariae of the trematode *Microphallus papillorobustus* and adult individuals of the ectoparasitic nematode *Gammarinema gammari* (Monohysteridae) (Fauchier and Thomas, 2001). The trematode *M. papillorobustus* uses gammarids as second intermediate hosts and finishes its life cycle in an aquatic bird (Helluy, 1981). Conversely, the nematode *G. gammari* uses gammarids as a habitat and trophic resource base (Fauchier and Thomas, 2001). In a previous study aimed at exploring the biology of *G. gammari* (Fauchier and Thomas, 2001), a negative relationship was found between the abundance of *G. gammari* and that of *M. papillorobustus* among gammarids. This negative relationship could result merely from the nematode abundance increasing with host age (Fauchier and Thomas, 2001), whereas the trematode abundance usually shows a negative relationship with host age because of parasite-induced host mortality (Thomas et al., 1995). However, the negative

relationship between the abundance of the 2 parasites remained significant when holding constant host body size-effects, suggesting that other phenomena occur.

The aim of the present paper was to examine both in the field and in the laboratory the relationship (numerical response) between the fecundity of the trematode and the number of co-occurring nematodes within gammarid hosts. An estimate of *M. papillorobustus* fecundity is indeed possible because metacercariae experimentally removed from the amphipod's brain and placed at 37 C produce eggs that can be counted (Rebecq, 1964). To perform this study, a sample of *G. insensibilis* was first collected during February 2001 (Thau's Lagoon, southern France 43°25'N, 3°35'E). To obtain amphipods harboring metacercariae of *M. papillorobustus*, only gammarids displaying an aberrant behavior at the water surface were collected (see Thomas et al., 1996). Because the prevalence of the nematode infection is very low in female gammarids compared with males (Fauchier and Thomas, 2001), only male gammarids were considered for the present purpose. All these males were returned to the laboratory and kept alive in salt water until dissection (completed within 8 hr of capture). Gammarids were measured (from head to tip of telson) and then dissected to count the metacercariae of *M. papillorobustus* and adult nematodes of *G. gammari*. Metacercariae of this trematode form distinctive ovoid cysts, i.e., on average 270 × 350 μm (Rebecq, 1964), that cannot be confused with other trematodes infecting *G. insensibilis* in this geographic area (see Rebecq, 1964; Helluy, 1981). Gammarids that harbored other trematode species were excluded from the study. Because the infrapopulation size of *M. papillorobustus* may negatively influence the mean number of eggs produced by metacercariae, only gammarids harboring 1 metacercaria were considered (n = 41 gammarids). Metacercariae length and width were measured using a graticule on a microscope with ×200 magnification. Metacercariae volume was estimated using the formula of an ellipsoid ($[\text{length}][\text{width}]^2\pi/6$) (see Trouvé et al., 1999). To estimate the fecundity of the trematode, the metacercaria removed from the amphipod brain was immediately placed in a petri dish containing 5 ml of saline solution (NaCl 9‰) and kept at 37 C. After 6 hr, the metacercariae were gently crushed between a slide and a coverslip and the number of eggs produced during this time was counted using a microscope. Egg length and width were also measured using a graticule on a microscope with ×400 magnification. When the number of eggs was less than or equal to 5, all eggs were measured; otherwise, 5 eggs were selected at random for measurement. The volume of these eggs was estimated as before using the formula of an ellipsoid. For the nematode *G. gammari*, only adults (which are located around the mouth parts) were considered because smaller larvae could be accidentally omitted (F. Thomas, unpubl. obs.).

The mean body size ($\bar{X} \pm \text{SD}$) of the 41 male gammarids harboring 1 metacercaria of *M. papillorobustus* was 15.0 mm ± 1.8. Prevalence

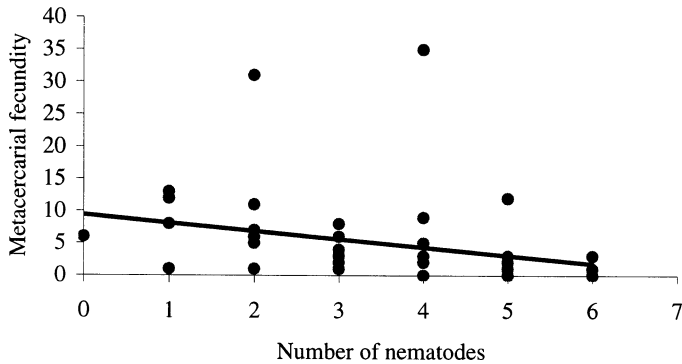


FIGURE 1. Relationship between the metacercarial fecundity and the number of nematodes.

of infection by *G. gammari* was 40/41, i.e., 1 uninfected gammarid, and the mean ($\bar{X} \pm \text{SD}$) parasitic intensity was 3.5 ± 1.5 ($n = 40$, range 1–6). Among the 41 metacercariae found, 33 produced eggs (\bar{X} number of eggs $\pm \text{SD}$, 6.3 ± 7.8 , $n = 33$, range 1–35). The mean volumes ($\bar{X} \pm \text{SD}$) of metacercariae and of eggs were $0.018 \text{ mm}^3 \pm 0.002$ ($n = 41$) and $1,176.5 \mu\text{m}^3 \pm 504$ ($n = 33$), respectively. Metacercariae volume and fecundity showed a positive relationship (Spearman rank order correlation coefficient, $r_s = 0.40$, $P = 0.0096$), indicating that larger metacercariae produced more eggs than smaller ones. There was also a positive relationship between the mean volume of eggs and their number (Spearman rank order correlation coefficient, $r_s = 0.41$, $P = 0.02$). The number of nematodes and the fecundity of metacercariae showed a negative relationship (Spearman rank order correlation coefficient, $r_s = -0.46$, $n = 41$, $P = 0.0025$; Fig. 1). The relationships between the number of nematodes and both the volumes of metacercariae and of eggs were not significant (Spearman rank order correlation coefficient, $P > 0.23$).

To test for a negative influence of nematode intensity on trematode fecundity, the following experiment was conducted in the laboratory. A new sample of male *G. insensibilis* with an aberrant behavior was collected at Thau's lagoon during April 2001. The new gammarid sample was divided into 2 equal groups of 50 individuals and were for 2 wk in 2 identical opaque plastic tanks measuring 25×15 cm, filled with constantly aerated seawater (20 C, 38‰) and fed with fish food (Tetra Ani Min). On the first day of the first and the second week, all the gammarids in the first group were placed in a tank containing freshwater for 2 5-min periods (separated by an interval of 10 min in seawater) and then returned to their initial seawater tank. This procedure serves as a very efficient method of killing nematodes without killing the gammarids or the trematode (F. Thomas, unpubl. obs.). At the end of the second week, all gammarids harboring only 1 metacercaria were dissected, i.e., $n = 24$ for the first group and $n = 16$ for the second group, and the metacercariae fecundity was estimated using the same procedure as before. The body size of each gammarid and the number of nematodes harbored were also recorded.

The efficiency of freshwater exposure in killing *G. gammari* was total because none of the gammarids from the first group had nematodes ($n = 24$), whereas prevalence was 100% in the second group (\bar{X} parasitic intensity $\pm \text{SD}$ 7.4 ± 2.6 , range 6–14, $n = 16$). The mean body size of gammarids was not significantly different between the 2 groups ($\bar{X} \pm \text{SD}$, first group, $16.3 \text{ mm} \pm 2.6$, $n = 24$; second group, $17.3 \text{ mm} \pm 1.5$, $n = 16$, unpaired *t*-test, $t = 1.42$, $\text{df} = 38$, $P = 0.16$). There was no significant difference either in the mean metacercariae volume between the 2 groups ($\bar{X} \pm \text{SD}$; first group, $0.017 \text{ mm}^3 \pm 0.002$, $n = 24$; second group, $0.017 \text{ mm}^3 \pm 0.005$, $n = 16$. Unpaired *t*-test, $t = -0.60$, $\text{df} = 38$, $P = 0.55$). However, there was a significant difference in the metacercariae fecundity between the 2 groups because metacercariae from the first group, i.e., with nematodes eliminated, produced more eggs than those from the second group, i.e., with nematodes (Mann-Whitney *U*-test, $z = -2.27$, $P = 0.023$; Fig. 2). Because exposure to freshwater per se is unlikely to have enhanced the metacercaria fecundity, this result suggests that nematode elimination results in a significant increase in trematode fecundity within a period as short as 2 wk.

Several phenomena could a priori explain the negative influence of

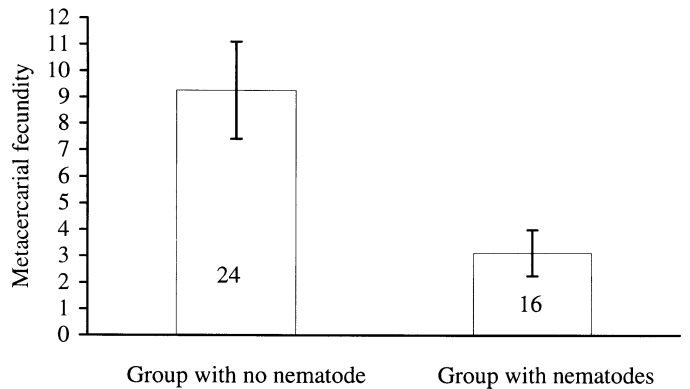


FIGURE 2. Fecundity ($\pm \text{SE}$) of metacercariae according to the presence or absence of nematodes.

the nematode abundance on trematode fecundity. It is firstly possible that competition for 1 or several resources exists between these 2 parasite species. However, at present, the resource requirements of these 2 parasites when inside gammarids are not known; hence, further investigations are thus necessary to determine the precise nature of the limiting resource(s) for which these co-occurring parasites compete. The fact that the trematode *M. papillorobustus* incurs fecundity reductions when nematodes are present does not necessarily mean that competition is asymmetrical because the reciprocal effect was not investigated, i.e., the influence of the trematode abundance on nematode fecundity. The present study, however, suggests that the negative relationship between the abundance of the 2 parasites (see Fauchier and Thomas, 2001) could result from an interspecific antagonism in which at the least the trematode suffers fitness losses when co-occurring with the nematode. Further experiments would be necessary to determine whether the negative relationship between the abundance of the 2 parasites results from a mutual or a unilateral avoidance or from an exclusion phenomenon.

Alternatively, a conflict of interest (sensu Lafferty, 1999) between *M. papillorobustus* and *G. gammari* may exist in this system because the trematode uses gammarids as vehicles to be transmitted to aquatic birds, whereas the nematode uses gammarids as a habitat and resource trophic base. Knowing that *M. papillorobustus* is a manipulative parasite that renders gammarids more vulnerable to predation by aquatic birds (Helluy, 1981), the negative relationship between the number of *G. gammari* and *M. papillorobustus* fecundity could result from the trematode investing more energy in manipulation (and hence less in fecundity) when nematodes are present. Indeed, although this hypothesis remains speculative without additional data, *G. gammari* may exert a negative influence on the trematode manipulation in order to reduce the predation risk of gammarids by aquatic birds, i.e., a particular case of the 'hijacking' hypothesis (Lafferty, 1999; Lafferty et al., 2000) that we could name the 'sabotage' hypothesis.

The fact that large metacercariae produced more eggs than small ones, as well as the positive relationship between egg volume and their number, remains an enigmatic issue worthy of further research. Note, however, that these results are in accordance with previous findings (Brown et al., unpubl. obs.). In the present study, only the egg production arising from self-fertilization was recorded, and we assume that this remains a reliable estimate of total reproductive potential. Although there is a priori no reason to believe that metacercariae adapt their relative investment in self- and cross-fertilization in response to the infestation size of *G. gammari*, it would be necessary in further studies to consider the total reproductive investment of the trematode.

Finally, we would like to underline a possible limitation in our study because what is measured in this work is the egg production by immature metacercariae over 6 hr in a nutrient-free saline solution and not their fitness. Surely, over the many hours or days of their adult life inside the nutrient-rich gut of an avian definitive host, the worms would produce 1000s of eggs, and differences between individuals of just a few eggs over the first few hours may become insignificant over time. In the absence of estimates of lifetime fecundity, we must be cautious in the interpretations of the results presented here.

We thank Sam Brown for having corrected our English.

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