

The Influence of Host Sex on the L3 to L4 Molt of *Brugia malayi*

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ABSTRACT: Immunocompetent male mice are more susceptible to experimental infection with *Brugia* spp. than are females. Because permissive male SCID mice (severe combined immunodeficient mice), which lack T and B cells, also possess higher worm burdens, the mechanism is not solely immune mediated. Recovery of fewer adult worms from the female SCID mouse suggests that females do not provide sufficient nutrients for larval growth. This study assessed the potential of the female SCID mouse to support the L3 to L4 molt of *Brugia malayi*. Unexpectedly, worms grown in females molted at earlier time points of recovery than those harvested from males. This suggests that the early stage of development of *B. malayi* is delayed in the male murine host. To determine whether the effect of host sex on molting may be similar in humans, worms were cultured in media supplemented with serum from male or female donors. Worms grown in serum obtained from female donors exhibited a significantly higher percentage of complete molts over those cultured with serum from males. Host-derived molecules required for the L3 to L4 molt may be more abundant in the female, perhaps allowing the worms to survive a vulnerable developmental stage in a less permissive environment.

It has been shown in many studies that the mammalian male has a higher prevalence and intensity of nematode infections (Klein, 2004). Composite data suggest that this trend results from either innate male susceptibility or a behavior-related increase in exposure to parasites (Zuk and McKean, 1996). Interestingly, animals under the controlled conditions of the laboratory also exhibit a male/female dichotomy in worm burdens, indicating that the physiology of the host contributes to susceptibility (Dobson, 1966; Ash, 1971). For example, male mice support larger numbers of *Brugia* spp. in experimental infection than do females (Nakanishi, 1987; Rajan et al., 1994). Because permissive male scid/scid mice (herein SCID: severe combined immunodeficient mice), which lack T and B cells, also possess larger numbers of worms, the mechanism is not solely immune mediated (Rajan et al., 1994). Additionally, we recently reported that endogenous sex hormone levels in individual mice do not correlate with worm burdens (Ganley and Rajan, 2001). Thus, it appears that some aspect of male physiology results in the higher worm burdens, which may or may not depend on androgens.

A decrease in surviving adult worms recovered from the female SCID mouse suggests that females do not provide sufficient nutrients for larval development. One of the major developmental events that takes place soon after infection is the L3 to L4 molt (Smyth, 1990). Molting is a synchronized, multifaceted process requiring both host- and parasite-derived molecules (Smyth, 1990; Smith et al., 2000; Rajan et al., 2003; Rajan, 2004). The first stage, apolysis, involves the separation of the hypodermis from the L3 cuticle and requires collagenases and other proteases (Guiliano et al., 2004). The L4 cuticle, which is composed of mainly collagens, is then synthesized by the hypodermal cells under the L3 cuticle (Kingston, 1991). Ecdysis, which is the separation of L4 cuticle from the L3 cuticle, follows and also requires several proteases (Davis et al., 2004). Proteolysis, combined with spasmodic contractions of the pharynx, breaks the L3 cuticle. The worm then sheds the L3 cuticle from head to tail as it moves, and the L4 emerges (Smyth, 1990).

The present study assessed the potential of the female SCID mouse to support the L3 to L4 molt of *Brugia malayi*. The L3 to L4 molt occurs approximately 7–10 days postinfection (PI) in vivo. Thus, the first objective was to define the length of time required for the L3 to remain in the host to acquire the crucial nutrients to achieve maximum molting potential ex vivo. The molting efficiency of worms recovered from male C.B-17 or BALB/c SCID mice on days 1–10 PI was assayed.

Mice were injected intraperitoneally with L3 larvae. All mice were maintained in the American Association of Laboratory Animal Care

accredited animal facility of the University of Connecticut Health Center. Five mice underwent necropsies at each time point. Worms were recovered by soaking the open peritoneal cavities, scrotum, and minced testes in phosphate-buffered saline. Between 50 and 100% of inoculated larvae were recovered. Larvae were then placed in culture to evaluate molting potential ex vivo. Serum-free culture medium consisted of Roswell Park Memorial Institute (RPMI) 1640 supplemented with 20 ng/ml of arachidonic acid as previously described (Smith et al., 2000). Medium supplemented with arachidonic acid was shown to sustain healthy L3 survival but does not by itself induce molting (Smith et al., 2000). Worms recovered from individual mice were cultured separately in 48-well plates and incubated at 37 C in a 5% CO₂ atmosphere. The percentage of molted worms was evaluated when postinfective L3 were 10 days old. For example, if worms were recovered on day 3 PI, molting was evaluated on the seventh day in culture. Molting efficiency was determined by the number of cast cuticles/number of worms. A complete molt was defined as the presence of an intact cast cuticle that was clear of debris (Smyth, 1990). Incomplete molts were defined as cast cuticles containing debris, worms that appeared to have undergone ecdysis (separation of L3 and L4 cuticle) but were unable to exit the cuticle, or worms that had exited from the cuticle but retained the cuticle at the posterior end (Smith et al., 2000). The percentage of recovered worms that molted was calculated for each mouse. The results of these experiments are summarized in Figure 1 and are similar to previous studies on other filarial species (Chen and Howells, 1979; Pogonka et al., 1999). Thus, worms primed in the host for less than 5 days demonstrated either no molting or a small percentage of incomplete molts. Worms recovered at day 5 demonstrated a very small percentage of complete molts (<10%), although most were incomplete molts. Molting potential increased when worms were recovered on day 6 and peaked when worms were recovered on days 7 and 8. The percentages of cast cuticles decreased when worms were recovered on days 9 and 10, as many of the worms had molted in vivo and were recovered as L4s.

Subsequently, the time course of the L3 to L4 molt was assayed in the female host. Male and female C.B-17 or BALB/c SCID mice were infected with 50 L3s; worms were recovered and cultured on days 7, 8, and 9 PI; worms were then evaluated for molting. A total of 5 male and 5 female mice were evaluated at each time point. Male and female mice yielded similar numbers of worms at these time points. The data for molting efficiency are presented as the mean of the percentages for each cage of mice (Fig. 2). Surprisingly, worms recovered from females at day 7 demonstrated enhanced molting potential, $P = 0.0052$ (Student's *t*-test; $t = 5.533$), and fewer incomplete molts, $P = 0.0001$ ($t = 14.555$), than worms recovered from males at the same time point. Only a small number of larvae harvested from females on days 8 and 9 molted ex vivo, as most worms had already molted in vivo and were recovered as L4s. Thus, worms recovered from male mice at days 8 and 9 demonstrated a significantly higher percentage of molting than did those from female mice ($P < 0.002$, $t > 7.296$). These results suggest that there is a delay of the L3 to L4 molt within the male host compared to that observed in the female host.

Abortive molt attempts, such as those observed under certain culture conditions, result from several developmental defects, including insufficient L4 cuticle synthesis (Smith et al., 2000). To determine if worms primed in the male host demonstrated differences in development, larval morphology was assessed by electron microscopy. A total of 5 male and 5 female C.B-17 SCID mice were injected with 50 L3s. Worms were recovered on day 7 and fixed overnight in 4% glutaraldehyde, stored in 0.2 M sodium cacodylate buffer at 4 C, routinely processed for electron microscopy, and cut at 60 μm. Worms grown in male and female mice had similar L4 cuticular morphological characteristics, in-

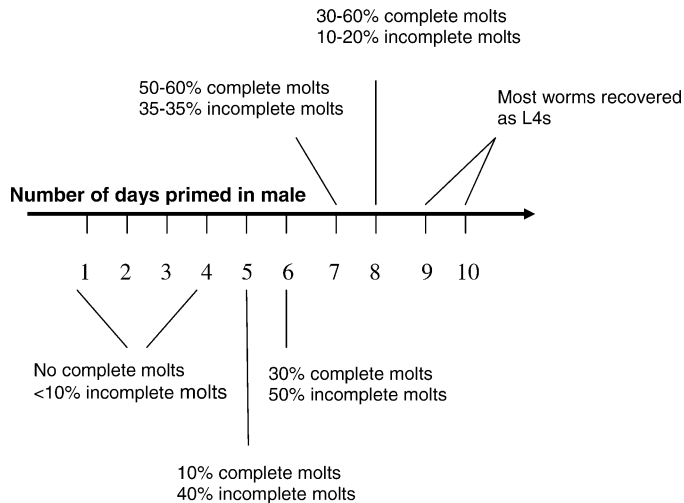


FIGURE 1. Summary of composite data characterizing the kinetics of the L3 to L4 molt in male severe combined immunodeficient (SCID) mice. Mice were injected with 50 L3 intraperitoneally and underwent necropsies on the days indicated on the figure. Recovered worms were cultured in RPMI supplemented with 20 ng/ml arachidonic acid. Molting was assessed when postinfective larvae were 10 days old. Five male BALB/c or C.B-17 SCID mice were assayed for each time point using at least 2 batches of L3.

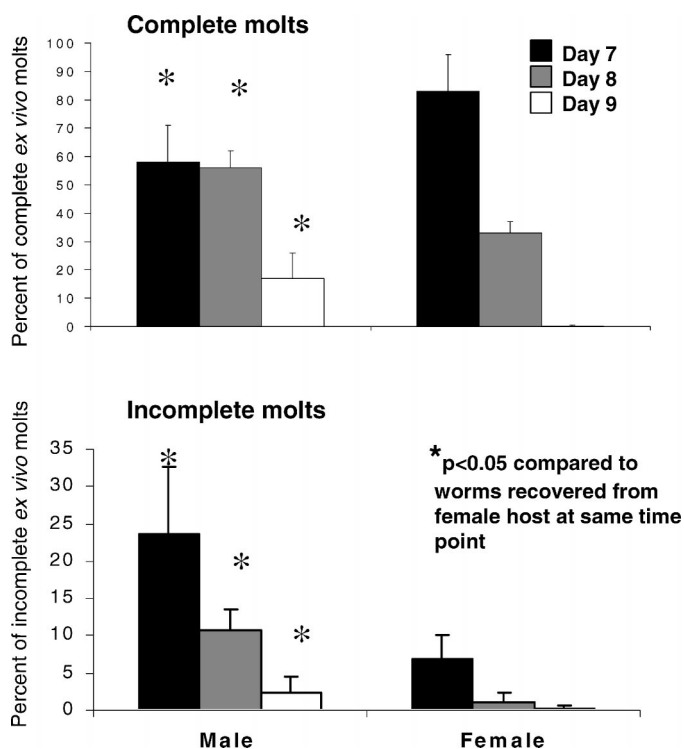
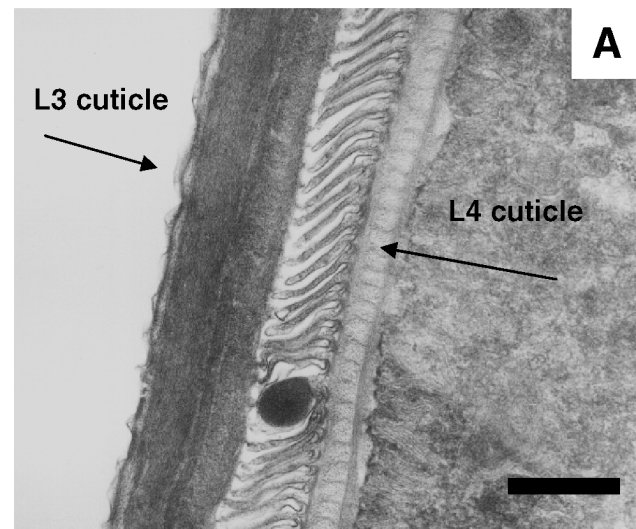


FIGURE 2. Ex vivo molting potential of worms recovered from male and female severe combined immunodeficient (SCID) mice. Mice were injected with 50 L3 intraperitoneally and underwent necropsies on the days indicated on the figure. Recovered worms were cultured in RPMI supplemented with 20 ng/ml of arachidonic acid. Molting was assessed when postinfective larvae were 10 days old. Bars represent mean percentage of molting \pm SD by worms recovered from individual mice with 5 mice per group and are characteristic of 12 experiments.

Pre *in vivo* molt



Post *in vivo* molt

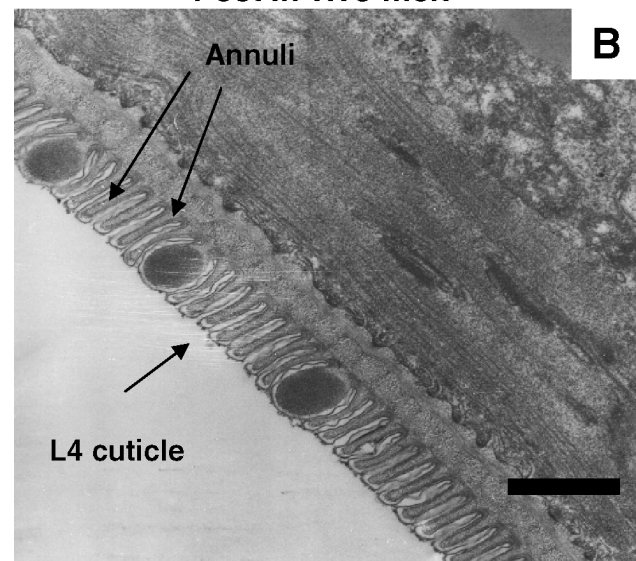


FIGURE 3. Male and female mice support L4 cuticle development. A total of 5 male and 5 female C.B-17 SCID mice were injected with 50 L3, and worms were harvested on day 7 postinfection. Worms were routinely processed for electron microscopy and cut at 60 μ m. (A) Representative L3 recovered from male host at day 7 before molting that demonstrates the L3 cuticle over an intact L4 cuticle. (B) Representative L4 recovered from a female host at day 7 following the L3 to L4 molt that demonstrates an intact L4 cuticle. There were no overt differences in morphology between worms recovered from male and female hosts, except that fewer molted L4s were recovered from the male host. Scale bar = 0.5 μ m.

cluding well-formed and uniformly distributed annuli (Fig. 3A, B). This result suggests that worms recovered from male mice at day 7 that demonstrate partial molts ex vivo have a deficiency in ecdysis rather than an underdeveloped L4. Thus, a host factor is likely required for the exit of the L4 from the L3 cuticle.

Studies on molting in *Caenorhabditis elegans*, a free-living nematode, indicate that incomplete molting results from starvation. For example, mutants of *C. elegans* that lack Gp330/megalin-related protein, or LPR-1, cannot endocytose sterols that are essential nutrients (Yochem et al., 1999). Though LPR-1 $-/-$ worms rotate in an attempt to shed

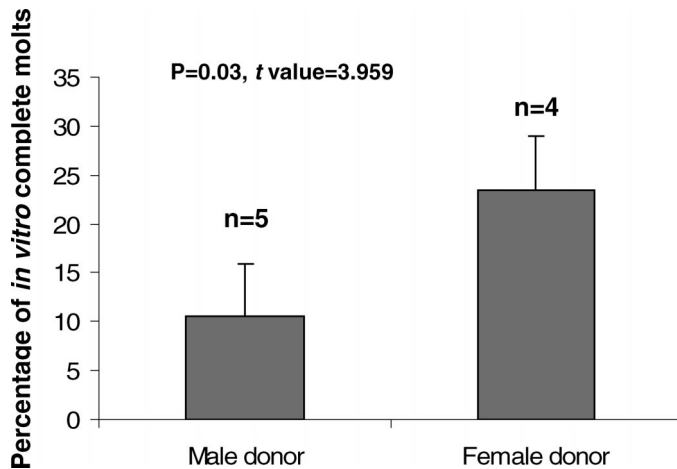


FIGURE 4. Worms grown in serum samples obtained from human female donors molt more efficiently than those cultured in serum acquired from male donors. Serum samples were obtained from 5 male and 4 female healthy, noninfected donors. Thirty worms per well were cultured in 48-well plates in RPMI supplemented with 10% heat-inactivated human serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, 40 μ g/ml gentamicin, 2 μ g/ml ceftazidime, and 20 μ g/ml ciprofloxacin. Worms were grown in individual donor serum separately and evaluated for percentage of molts after 10 days in culture. Bars represent mean percentage of complete molts for each sex \pm SD and are representative of 3 experiments.

the old cuticle, molting is incomplete. Additionally, mutants that lack CHR3, an orphan nuclear hormone receptor, are unable to molt. CHR3 belongs to a family of receptors that bind steroids, retinoic acid, and ecdysone (Kostrouchova, 1998). Mutants are unable to completely shed their old cuticles, suggesting that ecdysis is incomplete. CHR3 ligation with an exogenous steroid most likely activates a collagenase or regulates collagen gene expression required for molting. In the case of *B. malayi*, Smith and Rajan (2001) demonstrated that lipoxigenase inhibitors block the L3 to L4 molt, which suggests that products of the lipoxigenase pathway activate or release a protease. For example, mammalian proteolytic cathepsin B is released from cells by 12(S)-hydroxy-eicosatetraenoic acid (Ulbricht et al., 1996). Interestingly, Guiliano et al. (2004) have identified a gene related to cathepsin L-like proteases in *Brugia* spp. involved in ecdysis. The incubation of worms with protease inhibitors completely blocks ecdysis and results in an accordionlike phenotype where the L4 is completely encased in the L3 cuticle. Thus, it appears that a combination of both endogenous and exogenous molecules is required for ecdysis.

To determine whether the effect of host sex on the L3 to L4 molt observed in the murine host might be similar in humans, the in vitro molting potential of *B. malayi* was assessed using human sera. Serum was obtained from healthy, noninfected males (5 donors) and females (4 donors), and L3s were cultured as outlined in Smith et al. (2000) and then evaluated on day 10. Each serum sample was tested with 3 separate batches of L3. Figure 4 shows that a significantly higher percentage of worms grown in serum obtained from female donors molted compared to those grown in serum from male donors. This result suggests that the delay of the L3 to L4 molt also occurs in the natural human host. Overall, these observations imply that the L3 likely acquires an exogenous molecule(s) more readily in the female host via the existence of a larger quantity and/or an enhanced bioavailability.

These data are intriguing in the sense that not only do males support larger numbers of worms, but also that worms in male hosts are slightly larger, which suggests a more hospitable environment (Poulin, 1996). Interestingly, the molting larva has been shown to be more susceptible to host immune attack (Eisenbeiss et al., 1994). Thus, *B. malayi* may have evolved strategies to survive a vulnerable developmental stage, such as the molting process, by using morphogens more readily available in the female host. Experimentally, *Brugia* spp. can develop in a variety of mammalian hosts across several orders, including several spe-

cies of monkeys, cats, and hamsters (Partono et al., 1977; Crandall et al., 1983). Although filarial worms have a propensity for the lymphatics in the human host, it appears that these worms have the potential to develop at various anatomical sites as well. For example, *Brugia* spp. can develop to patency in the SCID mouse peritoneal cavity (Nelson et al., 1991) and have a broader tissue distribution in jirds (Carraway and Malone, 1985). Infection develops to patency in jirds as well, implying that the lymphatic system is not unique in providing growth factors. Thus, *B. malayi* requires molecules that have universal tissue distribution and that are found conserved across species of mammals other than those supplied in the culture medium. Recent reports have implicated exogenous vitamin C and nucleosides in the in vitro L3 to L4 molt of *B. malayi* (Rajan et al., 2003; Rajan, 2004). Therefore, it is possible that the female host supplies the parasite with a higher dose of nucleosides, for example, perhaps allowing the worms to better survive in a less permissive environment. Other steps in the developmental process of the larva might be delayed or inhibited in the female host.

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LITERATURE CITED

- ASH, L. 1971. Preferential susceptibility of male jirds (*Meriones unguiculatus*) to infection with *Brugia pahangi*. *Journal of Parasitology* **57**: 777–780.
- CARRAWAY, J. H., AND J. B. MALONE. 1985. *Brugia pahangi*: Comparative susceptibility of the Mongolian jird, *Meriones unguiculatus*, and the PD4 inbred hamster, *Mesocricetus auratus*. *Experimental Parasitology* **59**: 68–73.
- CHEN, S. N., AND R. E. HOWELLS. 1979. The in vitro cultivation of the infective larvae and the early mammalian stages of the filarial worm, *Brugia pahangi*. *Annals of Tropical Medicine and Parasitology* **73**: 473–486.
- CRANDALL, R. B., C. A. CRANDALL, J. T. NEILSON, J. T. FLETCHER, W. W. KOZEK, AND B. REDINGTON. 1983. Antibody responses to experimental *Brugia malayi* infections in patas and rhesus monkeys. *Acta Tropica* **40**: 53–64.
- DAVIS, M. W., A. J. BIRNIE, A. C. CHAN, A. P. PAGE, AND E. M. JORGENSEN. 2004. A conserved metalloprotease mediates ecdysis in *Caenorhabditis elegans*. *Development* **131**: 6001–6008.
- DOBSON, C. 1966. The age and sex of the host as factors affecting the host-parasite relationship of third-stage larva of *Amplificaeum robertsi* Sprent & Mines, 1960, in the laboratory mouse. *Parasitology* **56**: 399–406.
- EISENBEISS, W. F., H. APFEL, AND T. F. MEYER. 1994. Protective immunity linked with a distinct developmental stage of a filarial parasite. *Journal of Immunology* **152**: 735–742.
- GANLEY, L., AND T. V. RAJAN. 2001. Endogenous testosterone levels do not affect filarial worm burdens in mice. *Experimental Parasitology* **98**: 29–34.
- GUILIANO, D. B., X. HONG, J. H. MCKERROW, M. L. BLAXTER, Y. OKSOV, J. LIU, E. GHEDIN, AND S. LUSTIGMAN. 2004. A gene family of cathepsin L-like proteases of filarial nematodes are associated with larval molting and cuticle and eggshell remodeling. *Molecular and Biochemical Parasitology* **136**: 227–242.
- KINGSTON, I. B. 1991. Nematode collagen genes. *Parasitology Today* **7**: 11–15.
- KLEI, S. L. 2004. Hormonal and immunological mechanisms mediated sex differences in parasite infection. *Parasite Immunology* **26**: 247–264.
- KOSTROUCHOVA, M., M. KRAUSE, Z. KOSTROUCH, AND J. E. RALL. 2001. Nuclear hormone receptor CHR3 is a critical regulator of all four larval molts of the nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences USA* **98**: 7360–7365.
- NAKANISHI, H. 1987. Differences in susceptibility to *Brugia pahangi* infection between male and female BALB/c mice: Differences of effector cell responses between sexes. *Tropical Medicine* **29**: 153–163.
- NELSON, F. K., D. L. GREINER, L. D. SHULTZ, AND T. V. RAJAN. 1991.

- The immunodeficient scid mouse as a model for human lymphatic filariasis. *Journal of Experimental Medicine* **173**: 659–663.
- PARTONO, F., D. T. DENNIS, P. ATMOSOEDJONO, AND S. ATMOSOEDJONO. 1977. *Brugia timori*: Experimental infection in some laboratory animals. *Southeast Asian Journal of Tropical Medicine and Public Health* **8**: 155–157.
- POGONKA, T., U. OBERLANDER, T. MARTI, AND R. LUCIUS. 1999. *Acanthocheilonema viteae*: Characterization of a molt-associated excretory/secretory 18-kDa protein. *Experimental Parasitology* **93**: 73–81.
- POULIN, R. 1996. Helminth growth in vertebrate hosts: Does host sex matter? *International Journal for Parasitology* **26**: 1311–1315.
- RAJAN, T. V. 2004. Exogenous nucleosides are required for the morphogenesis of the human filarial parasite *Brugia malayi*. *Journal of Parasitology* **90**: 1184–1185.
- , F. K. NELSON, L. D. SHULTZ, K. L. SHULTZ, W. G. BEAMER, J. YATES, AND D. L. GREINER. 1994. Influence of gonadal steroids on susceptibility to *Brugia malayi* in scid mice. *Acta Tropica* **56**: 307–314.
- , N. PACIORKOWSKI, I. KALAJCIC, AND C. MCGUINNESS. 2003. Ascorbic acid is a requirement for the morphogenesis of the human filarial parasite *Brugia malayi*. *Journal of Parasitology* **89**: 868–870.
- SMITH, H. L., N. PACIORKOWSKI, S. BABU, AND T. V. RAJAN. 2000. Development of a serum-free system for the in vitro cultivation of *Brugia malayi* infective-stage larvae. *Experimental Parasitology* **95**: 253–264.
- , AND T. V. RAJAN. 2001. Inhibitors of the lipoxygenase pathway block development of *Brugia malayi* L3 in vitro. *Journal of Parasitology* **87**: 242–249.
- SMYTH, J. D. 1990. Cultivating parasitic helminthes in vitro: Advantages and problems. In *In vitro cultivation of parasitic helminths*, J. D. Smyth (ed.). CRC Press, Boca Raton, Florida, p. 1–5.
- ULBRICHT, B., W. HAGMANN, W. EBERT, AND E. SPIESS. 1996. Differential secretion of cathepsins B and L from normal and tumor human lung cells stimulated by 12(S)-hydroxy-eicosatetraenoic acid. *Experimental Cell Research* **226**: 255–263.
- YOCHER, J., S. TUCK, I. GREENWALD, AND M. HAN. 1999. A gp330/megalin-related protein is required in the major epidermis of *Caenorhabditis elegans* for completion of molting. *Development* **126**: 597–606.
- ZUK, M., AND K. MCKEAN. 1996. Sex differences in parasitic infections: Patterns and processes. *International Journal for Parasitology* **26**: 1009–1024.

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Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in Five Marine Mammal Species

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ABSTRACT: *Cryptosporidium* spp. and *Giardia* spp. are protozoan parasites that are often associated with severe diarrheal disease in a variety of mammals. Although these parasites have been extensively studied in terrestrial ecosystems, little is known about either parasite in the marine environment. Therefore, the objective of this study was to determine the prevalence of both *Cryptosporidium* spp. and *Giardia* spp. in 5 marine mammal species. Fecal samples were collected from 39 bowhead whales (*Balaena mysticetus*), 49 North Atlantic right whales (*Eubalaena glacialis*), 31 ringed seals (*Phoca hispida*), 22 bearded seals (*Erignathus barbatus*), and 18 beluga whales (*Delphinapterus leucas*) between 1998 and 2003. Using an immunofluorescent assay, parasites were detected in the feces of bowhead whales, right whales, and ringed seals, while neither parasite was detected in samples from bearded seals or beluga whales. Overall, prevalences were highest in ringed seals (*Cryptosporidium* spp., 22.6%; *Giardia* spp., 64.5%) and right whales (*Cryptosporidium* spp., 24.5%; *Giardia* spp., 71.4%) and lowest in bowhead whales (*Cryptosporidium* spp., 5.1%; *Giardia* spp., 33.3%). To our knowledge, this is the first report of *Cryptosporidium* spp. and *Giardia* spp. in either whale species and of *Cryptosporidium* spp. in the ringed seal.

Cryptosporidium spp. and *Giardia* spp. are well-known protozoan parasites of terrestrial mammals, reptiles, and birds. Using molecular techniques, at least 14 species of *Cryptosporidium* and several genetic types (genotypes) have been identified (Ryan et al., 2004; Xiao et al., 2004). Genetic characterization of *Giardia intestinalis* has not progressed as far as it has for *Cryptosporidium* spp.; however, 7 genetic assemblages (genotypes) have been identified, including Assemblage A, the most common type recovered from humans (Ey et al., 1997; Monis et al., 2003).

Although these parasites have been extensively studied in terrestrial ecosystems, little is known about either parasite in the marine environment. The first report of *Cryptosporidium* sp., later determined to be

Cryptosporidium hominis (= *Cryptosporidium parvum* genotype 1), was from a dugong (*Dugong dugon*) (Hill et al., 1997; Morgan et al., 2000; Morgan-Ryan et al., 2002). Subsequently, both *C. parvum* and *G. intestinalis* were reported from California sea lions (*Zalophus californianus*), but neither parasite was detected in Pacific harbor seals (*Phoca vitulina richardsi*) or northern elephant seals (*Mirounga angustirostris*) (Deng et al., 2000). *Giardia* spp. have also been detected in the feces of ringed seals (*Phoca hispida*), harp seals (*Phoca groenlandica*), harbor seals (*Phoca vitulina*), and gray seals (*Halichoerus grypus*), but neither *Cryptosporidium* spp. nor *Giardia* spp. were detected in beluga whales (*Delphinapterus leucas*) or a single bottle-nosed whale (*Hyperoodon ampullatus*) (Olson et al., 1997; Measures and Olson, 1999).

Not only do these parasites occur in marine mammals, they have also been detected in marine waters and have been shown to survive in this environment (Fayer et al., 2004). The identification of *C. hominis* and *G. intestinalis* Assemblage A in marine waters implicates human waste, possibly through the discharge of municipal wastewater or wastewater from boats, as sources of contamination. In addition to these potential sources, the identification of *C. parvum* and *Cryptosporidium baileyi* implicates agricultural runoff (Graczyk et al., 1999; Deng et al., 2000; Morgan et al., 2000; Fayer et al., 2002). Whether marine mammals are infected by these species or have their own host-specific isolates is unknown (Measures and Olson, 1999). However, the detection of *C. hominis* in a dugong indicates some anthroponotic transmission.

As a step toward understanding the impact of these parasites in the marine ecosystem, the objective of the present study was to determine the prevalence of *Cryptosporidium* spp. and *Giardia* spp. in fecal samples from 5 species of marine mammals.

Fecal samples were collected from ringed seals (*P. hispida*), bearded seals (*Erignathus barbatus*), beluga whales (*D. leucas*), and bowhead whales (*Balaena mysticetus*) from northern Alaska during subsistence hunts over a 4-yr period (Table I). Sampling was performed in the fall and spring for bowhead whales, in the spring and summer for seals, and

TABLE I. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in five marine mammal species as detected by immunofluorescent assay.

| | Collection years | n | % <i>Cryptosporidium</i> prevalence (95% CI) | % <i>Giardia</i> prevalence (95% CI) |
|---|------------------|----|---|---|
| Ringed seal (<i>Phoca hispida</i>) | 1998–2000 | 31 | 22.6% (9.6–41.1) | 64.5% (45.4–80.8) |
| Bearded seal (<i>Erignathus barbatus</i>) | 1999–2001 | 22 | 0.0% (0–15.4) | 0.0% (0–15.4) |
| Beluga whale (<i>Delphinapterus leucas</i>) | 1998–1999 | 18 | 0.0% (0–18.5) | 0.0% (0–18.5) |
| Bowhead whale (<i>Balaena mysticetus</i>) | 1998–2001 | 39 | 5.1% (0.6–17.3) | 33.3% (19.1–50.2) |
| North Atlantic right whale (<i>Eubalaena glacialis</i>) | 2002–2003 | 49 | 24.5% (13.3–38.9) | 71.4% (56.7–83.4) |

in the summer for beluga whales. Feces were collected from seals near Barrow, from bowhead whales near Barrow and Kaktovik, and from beluga whales near Barrow and Point Lay. Fecal samples were obtained 4–24 hr postmortem directly from the rectum or colon when possible, placed in individually labeled containers, and either frozen (–20 C) or placed in 10% formalin (1998 ringed seals only). When feces were not available, entire intestinal tracts were frozen (–20 C), and samples were collected from the small intestine or colon immediately before processing. When possible, gender was recorded for each individual. Seals were grouped as <5 yr (sexually immature), 5–7 yr (becoming sexually mature), and >7 yr (sexually mature) (Reeves, 1998). Age classes of bowhead whales were based on body length (George et al., 1999).

Fecal samples from North Atlantic right whales (*Eubalaena glacialis*) were collected during 2002 and 2003 (Table I) from floating fecal matter shortly after defecation using a 300- μ m mesh nylon dip net. Collections were made in the Bay of Fundy, Canada, between August and September of both years, with a single sample collected in the Great South Channel east of Cape Cod in 2003. All samples were placed in individually labeled containers and held on ice until frozen at –20 C (2002) or stored at approximately 4 C (2003) until processed. By means of photoidentification analysis (Kraus et al., 1986) and corresponding life history records in the North Atlantic Right Whale Catalog (Hamilton and Martin, 1999), animal identification, age (or estimated age)/age-class, and gender of individual right whales were determined when an individual whale was observed defecating.

Fresh and frozen fecal samples were concentrated by mixing 3 g of fecal material with 4.5 ml of phosphate-buffered saline (0.01 M, pH 7.2) containing 0.01 M EDTA (PBS-EDTA), expressing the mixture through 2 layers of sterile cheesecloth, and centrifuging the filtrate at

1,200 g for 10 min at room temperature. The supernatant was then decanted, and the pellet was resuspended in 1 ml of PBS-EDTA. Formalin-fixed fecal samples were processed in the same manner, except that an estimated 3-g sample was centrifuged first to remove the formalin before washing with PBS-EDTA. Samples were examined for the presence of the parasites using an immunofluorescent assay procedure (Meridian Bioscience, Cincinnati, Ohio) in accordance with the manufacturer's instructions, except that 15 μ l of the concentrated sample was divided among 2 or 3 wells on the slides provided. Because some samples adhered poorly to slides, the procedure was altered when necessary to allow reactions to occur in microcentrifuge tubes rather than on the slides. In these cases, 50 μ l of the resuspended sample and 8 μ l of each of the positive and negative control samples (supplied with the kit) were transferred to separate microcentrifuge tubes, and 1 drop of detection reagent was added to each. The solution was gently mixed, and the tubes were incubated at room temperature in the dark for 30 min. Following a quick spin, 30 μ l of test sample supernatant and 15 μ l of control sample supernatant were discarded and replaced with equal volume of wash buffer, and the samples were resuspended. After a quick-spin, the process was repeated. The same volume was again discarded after the final quick spin, and 15 μ l from each tube was then divided equally between 3 wells on the slide. The slides were air dried, mounting media was added, and a cover slip was placed on the slide. The procedure was optimized using *Cryptosporidium* sp.-spiked bowhead whale and right whale samples prepared in duplicate. In all cases, a sample was considered positive if one or more cysts/oocysts exhibiting an apple green color and morphological characteristics consistent with *Giardia* spp. or *Cryptosporidium* spp. were present when viewed by fluorescent microscopy.

Prevalence and 95% confidence intervals around the prevalence were calculated as previously described (Rickard et al., 1999). Sample size limitations prevented further statistical comparisons.

Both *Cryptosporidium* spp. and *Giardia* spp. were detected in right whales, bowhead whales, and ringed seals (Table I), while neither parasite was detected in beluga whales or bearded seals. Overall, *Cryptosporidium* spp. and *Giardia* spp. were more prevalent in ringed seals and right whales than in bowhead whales. Given the results of photoidentification analysis for 2002, multiple sampling of 1 right whale did occur. Samples were collected 6 days apart, and *Giardia* spp. cysts were detected in both samples. Because only 50% of the 2002 samples and none of the 2003 samples were linked to individual right whales at the time of this publication, it is possible that other multiple sampling is represented in this data set.

Estimated ages for bearded seals ranged from 2 to 20 yr. Most animals were in the <5-yr age class (n = 10), with fewer animals in the 5- to 7-yr (n = 5) and >7 yr (n = 6) age classes. Ringed seals ranged in age from <1 to 23 yr, with fewer animals in the 5- to 7-yr age class (Table II). The prevalence of *Cryptosporidium* spp. in ringed seals increased with age class, unlike the prevalence of *Giardia* spp., which was highest in the 5- to 7-yr age class. The majority of bowhead whales were in the <25-yr age class, which was also the class in which the highest prevalences of *Cryptosporidium* spp. and *Giardia* spp. occurred (Table II).

All of the 18 beluga whales sampled were male, whereas more female bearded seals were sampled than male bearded seals (12 and 9, respectively). The prevalence of *Giardia* spp. in both ringed seals and bowhead whales was higher in males than in females (Table II). The prevalence of *Cryptosporidium* spp. was also higher in male bowhead

TABLE II. Prevalence of *Cryptosporidium* spp. and *Giardia* spp. by age class and gender in ringed seals and bowhead whales.

| | n | <i>Cryptosporidium</i> | <i>Giardia</i> |
|---|----|------------------------|----------------|
| Ringed seal (<i>Phoca hispida</i>) | | | |
| Age class | | | |
| <5 yr | 13 | 1 (7.7%)* | 6 (46.2%) |
| 5–7 yr | 7 | 2 (28.6%) | 6 (85.7%) |
| >7 yr | 11 | 4 (36.3%) | 7 (63.6%) |
| Gender | | | |
| Male | 22 | 5 (22.7%) | 16 (72.7%) |
| Female | 8 | 2 (25.0%) | 3 (37.5%) |
| Bowhead whale (<i>Balaena mysticetus</i>) | | | |
| Age class | | | |
| <25 yr | 34 | 2 (5.9%) | 12 (35.3%) |
| >25 yr | 5 | 0 | 1 (20.0%) |
| Gender | | | |
| Male | 22 | 2 (9.1%) | 9 (40.9%) |
| Female | 17 | 0 | 4 (23.5%) |

* No. of animals in which the parasite was detected (prevalence).

whales but was approximately equal between genders for ringed seals (Table II).

Few studies have assessed *Cryptosporidium* and *Giardia* spp. in marine mammals. The only report from the Southern Hemisphere is that of *C. hominis* from a dugong in Hervey Bay, Queensland (Hill et al., 1997; Morgan et al., 2000; Morgan-Ryan et al., 2002). Within the Northern Hemisphere, *Giardia* spp. cysts have been detected in ringed seals from the western arctic region of Canada, and harp seals, harbor seals, and gray seals in the Gulf of St. Lawrence, St. Lawrence estuary, and coastal Newfoundland in eastern Canada (Olson et al., 1997; Measures and Olson, 1999). Deng et al. (2000) later detected *C. parvum* oocysts and *G. intestinalis* cysts in California sea lions from areas of northern coastal California. The present study extends the known host range of *Cryptosporidium* spp. and *Giardia* spp. to include bowhead and right whales and the geographic range to include the Chukchi and Beaufort seas and the Bay of Fundy.

Regardless of geographic location, neither *Cryptosporidium* spp. nor *Giardia* spp. has been detected in a total of 45 beluga whales (Olson et al., 1997; Measures and Olson, 1999; this study). Given the widely divergent localities from which the whales were sampled (Shingle Point, Yukon, Canada; Hendrickson Island, Northwest Territories, Canada; St. Lawrence Estuary, Canada; and Point Lay and Barrow, Alaska), it appears that beluga whales may be refractory to infection with these parasites. Conversely, these parasites have been detected in ringed seals from diverse localities in the western arctic (Holman, Northwest Territories, Canada; near Shingle Point, Yukon, Canada; and Barrow, Alaska). Although *Giardia* spp. cysts have been detected in ringed seals from all locations examined, the highest prevalence was detected in those from Alaska (this study; Olson et al., 1997). *Cryptosporidium* spp. oocysts have only been detected in the Alaskan populations (Olson et al., 1997; this study). The reasons for the different geographical patterns among ringed seals remain to be determined.

The prevalences of *Cryptosporidium* spp. in ringed seals and right whales in the present study are relatively high compared to those reported for terrestrial mammals, whereas the prevalence in bowhead whales was similar to that in previous reports (Atwill et al., 1997; Rickard et al., 1999; Siefker et al., 2002). The prevalences for *Giardia* spp. in the present study were higher than that reported for many terrestrial mammals (Isaac-Renton et al., 1987; Díaz et al., 1996; Rulofson et al., 2001; Dunlap and Thies, 2002). These relatively high prevalences may be explained by the numerous potential routes of exposure. Both parasites are easily transmitted through the ingestion of contaminated water (Fayer et al., 1997). Alternatively, certain prey, such as shrimp and zooplankton, may be capable of concentrating the infective stages in much the same manner as clams, oysters, and mussels (Graczyk et al., 1999; Gomez-Bautista et al., 2000; Fayer et al., 2004), thereby making large numbers of organisms readily available to the mammalian host. Finally, behavior that brings groups of animals together, such as hauling out at shared ice holes, would increase the likelihood of direct transmission between animals.

It is clear that *Cryptosporidium* spp. and *Giardia* spp. are present in the marine environment. However, the mere detection of parasites is not sufficient to demonstrate their impact on animal or human health; consequently, future studies on the health of marine mammals should include an evaluation of the parasites present. Genetic characterization of isolates from marine mammal species is also necessary to determine if these parasites are specific to marine mammals or originate from non-marine sources, such as human waste, agricultural runoff, or terrestrial wildlife. For example, human waste may be a factor in the relatively high prevalences of these parasites in the North Atlantic right whale because their migratory range includes densely populated coastal habitats extending from the Bay of Fundy to the eastern coast of Florida (International Whaling Commission, 2001). Molecular techniques must be used in future studies to clarify both the potential sources of environmental contamination and the routes of exposure.

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LITERATURE CITED

- ATWILL, E. R., R. A. SWEITZER, M. DAS GRACAS, C. PEREIRA, I. A. GARDNER, D. VAN VUREN, AND W. M. BOYCE. 1997. Prevalence of and associated risk factors for shedding *Cryptosporidium parvum* oocysts and *Giardia* cysts within feral pig populations in California. *Applied and Environmental Microbiology* **63**: 3946–3949.
- DENG, M., R. P. PETERSON, AND D. O. CLIVER. 2000. First findings of *Cryptosporidium* and *Giardia* in California sea lions (*Zalophus californianus*). *Journal of Parasitology* **86**: 490–494.
- DÍAZ, V., M. CAMPOS, J. LOZANO, I. MAÑAS, AND J. GONZÁLEZ. 1996. Aspects of animal giardiasis in Granada province (southern Spain). *Veterinary Parasitology* **64**: 171–176.
- DUNLAP, B. G., AND M. L. THIES. 2002. *Giardia* in beaver (*Castor canadensis*) and nutria (*Myocastor coypus*) from east Texas. *Journal of Parasitology* **88**: 1254–1258.
- EY, P. L., M. MANSOURI, J. KULDA, E. NOHÝNKOVÁ, P. T. MONIS, R. H. ANDREWS, AND G. MAYRHOFER. 1997. Genetic analysis of *Giardia* from hoofed farm animals reveals artiodactyl-specific and potentially zoonotic genotypes. *Journal of Eukaryotic Microbiology* **44**: 626–635.
- FAYER, R., J. P. DUBEY, AND D. S. LINDSAY. 2004. Zoonotic protozoa: From land to sea. *Trends in Parasitology* **20**: 531–536.
- , C. A. SPEER, AND J. P. DUBEY. 1997. The general biology of *Cryptosporidium*. In *Cryptosporidium* and cryptosporidiosis, R. Fayer (ed.). CRC Press, Boca Raton, Florida, p. 1–41.
- , J. M. TROUT, E. J. LEWIS, L. XIAO, A. LAL, M. C. JENKINS, AND T. K. GRACZYK. 2002. Temporal variability of *Cryptosporidium* in the Chesapeake Bay. *Parasitology Research* **88**: 998–1003.
- GEORGE, J. C., J. BADA, J. ZEH, L. SCOTT, S. E. BROWN, T. O'HARA, AND R. SUYDAM. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) using aspartic acid racemization. *Canadian Journal of Zoology* **77**: 571–580.
- GOMEZ-BAUTISTA, M., L. M. ORTEGA-MORA, E. TABARES, V. LOPEZ-RODAS, AND E. COSTAS. 2000. Detection of infectious *Cryptosporidium parvum* oocysts in mussels (*Mytilus galloprovincialis*) and cockles (*Cerastoderma edule*). *Applied and Environmental Microbiology* **66**: 1866–1870.
- GRACZYK, T. C., R. C. A. THOMPSON, R. FAYER, P. ADAMS, U. M. MORGAN, AND E. J. LEWIS. 1999. *Giardia duodenalis* cysts of genotype A recovered from clams in the Chesapeake Bay subestuary, Rhode River. *American Journal of Tropical Medicine and Hygiene* **61**: 526–529.
- HAMILTON, P. K., AND S. M. MARTIN. 1999. A catalogue of identified right whales from the North Atlantic: 1935–1997. New England Aquarium, Boston, Massachusetts, 27 p.
- HILL, B. D., I. R. FRASER, AND H. C. PRIOR. 1997. *Cryptosporidium* infection in a dugong (*Dugong dugon*). *Australian Veterinary Journal* **75**: 640–641.
- INTERNATIONAL WHALING COMMISSION. 2001. Report on the workshop on status and trends of western North Atlantic right whales. *Journal of Cetacean Research and Management, Special Issue 2*: 61–87.
- ISAAC-RENTON, J. L., M. M. MORICZ, AND E. M. PROCTOR. 1987. A *Giardia* survey of fur-bearing water mammals in British Columbia, Canada. *Journal of Environmental Health* **50**: 80–83.

- KRAUS, S. D., K. E. MOORE, C. E. PRICE, M. J. CRONE, W. A. WATKINS, H. E. WINN, AND J. H. PRESCOTT. 1986. The use of photographs to identify individual North Atlantic right whales (*Eubalaena glacialis*). Reports of the International Whaling Commission, Special Issue **10**: 145–151.
- MEASURES, L. M., AND M. OLSON. 1999. Giardiasis in pinnipeds from Eastern Canada. *Journal of Wildlife Diseases* **35**: 779–782.
- MONIS, P. T., R. H. ANDREWS, G. MAYRHOFER, AND P. L. EY. 2003. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. *Infection, Genetics, and Evolution* **3**: 29–38.
- MORGAN, U. M. L., XIAO, B. D. HILL, P. O'DONOGHUE, J. LIMOR, A. LAL, AND R. C. THOMPSON. 2000. Detection of the *Cryptosporidium parvum* "human" genotype in a dugong (*Dugong dugon*). *Journal of Parasitology* **86**: 1352–1354.
- MORGAN-RYAN, U. M., A. FALL, L. A. WARD, N. HIJAWI, I. SULAIMAN, R. FAYER, R. C. THOMPSON, M. OLSON, A. LAL, AND L. XIAO. 2002. *Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from *Homo sapiens*. *Journal of Eukaryotic Microbiology* **49**: 433–40.
- OLSON, M. E., P. D. ROACH, M. STABLER, AND W. CHAN. 1997. Giardiasis in ringed seals from the western arctic. *Journal of Wildlife Diseases* **33**: 646–648.
- REEVES, R. R. 1998. Distribution, abundance and biology of ringed seals (*Phoca hispida*): An overview. North Atlantic Marine Mammal Commission Scientific Publication **1**: 46–62.
- RICKARD, L. G., C. SIEFKER, C. R. BOYLE, AND E. J. GENTZ. 1999. The prevalence of *Cryptosporidium* and *Giardia* spp. in fecal samples from free-ranging white-tailed deer (*Odocoileus virginianus*) in the southeastern United States. *Journal of Veterinary Diagnostic Investigation* **11**: 65–72.
- RULOFSO, F. C., E. R. ATWILL, AND C. A. HOLMBERG. 2001. Fecal shedding of *Giardia duodenalis*, *Cryptosporidium parvum*, *Salmonella* organisms, and *Escherichia coli* O157:H7 from llamas in California. *American Journal of Veterinary Research* **62**: 637–642.
- RYAN, U. M., P. MONIS, H. L. ENEMARK, I. SULAIMAN, B. SAMARASINGHE, C. READ, R. BUDDLE, I. ROBERTSON, L. ZHOU, R. C. A. THOMPSON, AND L. XIAO. 2004. *Cryptosporidium suis* n. sp. (Apicomplexa: Cryptosporidiidae) in pigs (*Sus scrofa*). *Journal of Parasitology* **90**: 769–773.
- SIEFKER, C., L. G. RICKARD, G. T. PHARR, J. S. SIMMONS, AND T. M. O'HARA. 2002. Molecular characterization of *Cryptosporidium* sp. isolated from northern Alaskan caribou (*Rangifer tarandus*). *Journal of Parasitology* **88**: 213–216.
- XIAO, L., R. FAYER, U. RYAN, AND S. J. UPTON. 2004. *Cryptosporidium* taxonomy: Recent advances and implications for public health. *Clinical Microbiology Reviews* **17**: 72–97.

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Prevalence of Agglutinating Antibodies to *Toxoplasma gondii* and *Sarcocystis neurona* in Beavers (*Castor canadensis*) From Massachusetts

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ABSTRACT: The present study examined the seroprevalence of *Toxoplasma gondii* and *Sarcocystis neurona* in a population of beavers (*Castor canadensis*) from Massachusetts. Sixty-two blood samples were collected during the field seasons over 3 consecutive years from different animals. Blood was collected onto filter paper and shipped to the Department of Biomedical Sciences, Virginia Tech, Blacksburg, Virginia, for parasite testing. The samples were tested at dilutions of 1:25, 1:50, and 1:100 against each parasite antigen by modified agglutination tests to determine whether antibodies to either parasite were present in the blood. Six of 62 samples (10%) were positive for *T. gondii*, with 2 samples having titers of 1:25 and 4 having titers of 1:50. Four of 62 samples (6%) were positive for *S. neurona*, with 2 samples having titers of 1:25 and 2 having titers of 1:50.

Toxoplasma gondii is an important protozoal pathogen of humans and other warm-blooded vertebrates. Humans become infected by ingesting meat containing tissue cysts or by ingesting oocysts in the environment. Domestic cats and other felines are the only known, definitive hosts for *T. gondii*. Cats can excrete millions of environmentally resistant oocysts in their feces. Sporulated *T. gondii* oocysts have been reported to be able to survive in the environment for 1.5 yr by Frenkel et al. (1975) and for 4.5 yr at 4 C by Dubey (1998). The cat population in the United States was ~57 million in 1991 (Patronek, 1998). The prevalence of antibodies to *T. gondii* is ~58% in free-roaming cats and 37% in pet cats (Dubey, 1994). Outbreaks of *T. gondii* resulting from contaminated water have been described (Dubey, 2004). The prevalence of *T. gondii* antibodies in beavers may reflect the environmental exposure to *T. gondii* in aquatic ecosystems.

Sarcocystis neurona is an obligate, heteroxenous parasite that has the opossum as its definitive host. Intermediate hosts are infected by ingestion of sporocysts passed in opossum feces. *Sarcocystis neurona* is important because it is the causative agent of equine protozoal mye-

loencephalitis, a severe neurological disease that can occur when a horse accidentally ingests *S. neurona* sporocysts. Horses may become infected by drinking sporocyst-contaminated water.

Little is known about the prevalence of apicomplexan parasites in beavers. The present study was conducted to determine the seroprevalence of *T. gondii* and *S. neurona* in beavers from Massachusetts.

Beavers were live-trapped as part of a larger project on their demographics. Four study areas have been established across Massachusetts to represent a combination of different levels of development and different attitudes of residents regarding management of beavers (Deblinger et al., 1999). The northeastern area of Massachusetts represents heavy suburban development, and a majority of people there are opposed to recreational fur-trapping (based on how residents voted on a statewide trapping referendum). The central area represents light suburban development, and a slight majority of people there are in favor of recreational trapping. The southwestern area is rural, with a slight majority of people opposed to trapping, and the northwestern area is rural, with the majority of residents in favor of recreational trapping. Colonies were surveyed and beavers captured and marked at sites in the eastern, central, and western areas of the state. At least some of these localities are sites included in the state's long-term, beaver colony-monitoring program, which has been ongoing for the last 7–8 yr.

Bobcats (*Felis rufus*) as well as feral and pet domestic cats were present in all study areas (K. Koenen and S. DeStefano, pers. obs.). One of 9 documented mortalities of beavers included predation by a bobcat of a kit during a year of low water levels in the western study area. Bobcats are capable of dealing with human influences, but they tend to avoid agricultural areas with extensive cleared lands that eliminate other vegetative cover types. Bobcats can be classified as being common in central and western Massachusetts, present in the northeast, and rare to absent in southeastern areas of the state (Massachusetts Division of Fisheries and Wildlife, unpubl.).

Beavers were captured at multiple colonies within each of the 3 study areas. Box traps (Koenen et al., in press) and Bailey traps were used to capture beavers, and each animal was immobilized with an intramuscular injection of ketamine hydrochloride (10–13 mg/kg) and acepromazine maleate (2.5 mg) (Lancia et al., 1978). Age and sex were determined for each animal, and individuals were marked with metal and plastic ear tags as well as a tail-mounted radio transmitter. Given the difficulty of venipuncture in beavers, an alternative method for collecting samples was employed. Drops of blood were blotted onto filter paper following a cutaneous tail stick using a hypodermic needle. This is a reliable method of collecting blood for use in toxoplasmosis testing, as described in congenitally infected infants (Hsu et al., 1992; Patel and Holliman, 1994; Paul et al., 2001). It also has been used in sheep (Uggla and Nilsson, 1987) and cats (Nogami et al., 1992). After marking, beavers were placed back into box traps in a shady, protected area near the water, and individuals were not released until they had recovered fully from the drugs. Recovery was judged on the basis of the beaver's alertness, mobility, and ability to hold up its head and to move within the box trap (Koenen et al., in press).

The filter-paper blood blots were kept on ice packs and mailed to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, for testing. Sections (1 cm²) containing the dried blood were cut from the filter-paper blots and submerged in 200 μ l of phosphate-buffered saline (PBS). The paper was soaked overnight at 4 C to allow blood proteins to dissolve into the PBS. Then, the eluate was removed and placed into clean tubes for use in agglutination testing.

The modified direct agglutination test (MAT) was used to examine beaver eluates for agglutinating immunoglobulin G antibodies to *T. gondii* (Dubey and Desmonts, 1987) and *S. neurona* (Lindsay and Dubey, 2001). A dilution of 1:25 was used to screen sera. Thirty-one serum samples were tested from beavers in 2002, 17 in 2003, and 14 in 2004. Positive beaver sera were examined further at dilutions of 1:50 and 1:100.

Positive *T. gondii* MAT results were found in 6 of 62 beavers (10%) from 2002. Four samples were positive at 1:50, and 2 samples were positive only at 1:25. Four of 62 beavers (6%) from 2002 were positive for *S. neurona* by MAT. Two were positive at 1:50, and 2 were positive only at 1:25. No samples from 2003 or 2004 were positive for *T. gondii* or *S. neurona*.

Toxoplasma gondii has been isolated from a beaver in Kansas (Smith and Frenkel, 1995). The prevalence of *T. gondii* in beavers reported here is low compared to that in other surveys of aquatic wildlife in the United States. One study in North Carolina found that 45% of river otters sampled were seropositive for *T. gondii* using a latex agglutination test (Tocidowski et al., 1997).

The infected beavers in the present study may be ingesting *T. gondii* oocysts excreted by wild or domestic cats from contaminated water or foliage. Bobcats are the only wild cats that live in Massachusetts, where they are most common in the central and western regions. Although to our knowledge no prevalence data exist for *T. gondii* in bobcats from Massachusetts, we are aware of studies conducted in other parts of the United States. Kikuchi et al. (2004) tested serum from 52 bobcats from various states for antibodies to *T. gondii*, and 50% of the animals were seropositive.

We are unaware of any previous reports of *S. neurona* infection in beavers. However, a recent study examined other wildlife species from Connecticut for agglutinating antibodies to *S. neurona*. Eleven of 24 skunks (46%) and 12 of 12 raccoons (100%) had positive titers to *S. neurona* (Mitchell et al., 2002). Another study found that 24 of 25 raccoons (96%) from Massachusetts were seropositive for *S. neurona* (Lindsay et al., 2001). Compared to these and other studies, the prevalence of *S. neurona* infection in beavers observed in the present study is low.

Opossums are the definitive host for *S. neurona*, and they are abundant throughout Massachusetts. Prevalence data are not available for *S. neurona* infections in opossums from Massachusetts, but studies have been conducted in Maryland and Michigan. These studies found that 6 of 11 opossums (55%) from Maryland (Dubey, 2000), and 31 of 206 opossums (15%) from Michigan (Elsheikha et al., 2004), were infected with *S. neurona*.

The prevalence of *T. gondii* and *S. neurona* in beavers from Massachusetts is lower than what has been observed in other wildlife species. However, even a small number of infected animals indicates the presence of parasites in the area.

LITERATURE CITED

- DEBLINGER, R. D., W. A. WOYTEK, AND R. R. ZWICK. 1999. Demographics of voting on the 1996 Massachusetts ballot referendum. *Human Dimensions of Wildlife* **4**: 40–55.
- DUBEY, J. P. 1994. Toxoplasmosis. *Journal of the American Veterinary Medical Association* **205**: 1593–1598.
- . 1998. *Toxoplasma gondii* oocyst survival under defined temperatures. *Journal of Parasitology* **84**: 862–865.
- . 2000. Prevalence of *Sarcocystis* species sporocysts in wild-caught opossums (*Didelphis virginiana*). *Journal of Parasitology* **86**: 705–710.
- . 2004. Toxoplasmosis—A waterborne zoonosis. *Veterinary Parasitology* **126**: 57–72.
- , AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal* **19**: 337–339.
- ELSHEIKHA, H. M., A. J. MURPHY, AND L. S. MANSFIELD. 2004. Prevalence of *Sarcocystis* species sporocysts in Northern Virginia opossums (*Didelphis virginiana*). *Parasitology Research* **93**: 427–431.
- FRENKEL, J. K., A. RUIZ, AND M. CHINCHILLA. 1975. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *American Journal of Tropical Medicine and Hygiene* **24**: 439–443.
- HSU, H., G. F. GRADY, J. H. MAGUIRE, B. J. WEIBLEN, AND R. HOFF. 1992. Newborn screening for congenital *Toxoplasma* infection: Five years experience in Massachusetts, USA. *Scandinavian Journal of Infectious Disease* **84**: 59S–64S.
- KIKUCHI, Y., B. B. CHOMEL, R. W. KASTEN, J. S. MARTENSON, P. K. SWIFT, AND S. J. O'BRIEN. 2004. Seroprevalence of *Toxoplasma gondii* in free-ranging or captive pumas (*Felis concolor*) and bobcats (*Lynx rufus*). *Veterinary Parasitology* **120**: 1–9.
- KOENEN, K., S. DESTEFANO, C. HENNER, AND T. BEROLDI. From the field: capturing beavers in box traps. *Wildlife Society Bulletin* (in press).
- LANCIA, R. A., R. P. BROOKS, AND M. W. FLEMING. 1978. Ketamine hydrochloride as an immobilant and anesthetic for beavers. *Journal of Wildlife Management* **42**: 946–948.
- LINDSAY, D. S., AND J. P. DUBEY. 2001. Direct agglutination test for the detection of antibodies to *Sarcocystis neurona* in experimentally infected animals. *Veterinary Parasitology* **95**: 179–186.
- , A. C. ROSYPAL, J. A. SPENCER, M. A. CHEADLE, A. M., ZAJAC, C. RUPPRECHT, J. P., DUBEY, AND B. L. BLAGBURN. 2001. Prevalence of agglutinating antibodies to *Sarcocystis neurona* in raccoons, *Procyon lotor*, from the United States. *Veterinary Parasitology* **100**: 131–134.
- MITCHELL, S. M., D. J. RICHARDSON, M. A. CHEADLE, A. M. ZAJAC, AND D. S. LINDSAY. 2002. Prevalence of agglutinating antibodies to *Sarcocystis neurona* in skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), and opossums (*Didelphis virginiana*) from Connecticut. *Journal of Parasitology* **88**: 1027–1029.
- NOGAMI, S., H. KAMATA, S. MARUYAMA, H. FURUYA, AND I. INOUE. 1992. Preservation of feline anti-*Toxoplasma gondii* antibody activity using blood absorbed on filter paper stored under different conditions. *Research in Veterinary Science* **52**: 387–388.
- PATEL, B., AND R. E. HOLLIMAN. 1994. Antibodies to *Toxoplasma gondii* in eluates from filter paper blood specimens. *British Journal of Biomedical Science* **51**: 104–108.
- PATRONEK, G. J. 1998. Free-roaming and feral cats—Their impact on wildlife and human beings. *Journal of the American Veterinary Medical Association* **212**: 218–226.
- PAUL, M., E. PETERSEN, AND J. SZCZAPA. 2001. Prevalence of congenital *Toxoplasma gondii* infection among newborns from the Poznan region of Poland: Validation of a new combined enzyme immunoassay for *Toxoplasma gondii*-specific immunoglobulin A and immunoglobulin M antibodies. *Journal of Clinical Microbiology* **39**: 1912–1916.
- SMITH, D. D., AND J. K. FRENKEL. 1995. Prevalence of antibodies to *Toxoplasma gondii* in wild mammals of Missouri and east central Kansas: Biologic and ecologic considerations of transmission. *Journal of Wildlife Diseases* **31**: 15–21.
- TOCIDLOWSKI, M. E., M. R. LAPPIN, P. W. SUMMER, AND M. K. STOSKOPF. 1997. Serologic survey for toxoplasmosis in river otters. *Journal of Wildlife Diseases* **33**: 649–652.
- UGGLA, A., AND L. A. NILSSON. 1987. Evaluation of a solid-phase immunoassay (DIG-ELISA) for the serodiagnosis of ovine toxoplasmosis. *Veterinary Immunology and Immunopathology* **14**: 309–318.