Presence of *Giardia* spp. and Absence of *Salmonella* spp. in New Jersey Muskrats (*Ondatra zibethicus*)

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Of 220 muskrat fecal specimens collected from 12 sites in southwestern New Jersey, 154 (70%) were found to contain cysts of the protozoan parasite *Giardia* spp. Cysts from selected muskrat fecal specimens infected Mongolian gerbils, but attempts to cultivate trophozoites removed from these gerbils were unsuccessful. *Salmonella* spp. were not detected in any of the muskrat fecal specimens.

Evidence has been accumulating during the past decade to support the hypothesis that drinking water is a common vehicle for the transmission of giardiasis among humans (10). However, the primary source of the *Giardia* cysts that contaminate surface water is uncertain. Whether wild and domestic animals are susceptible to the same *Giardia* strains infecting human beings is controversial (3, 5, 21–23). The beaver (*Castor canadensis*) is the wild animal most frequently incriminated as a potential reservoir of *Giardia* spp. capable of infecting man via drinking water (10). Another more common and widespread aquatic mammal that has been shown to harbor *Giardia* spp. is the muskrat (*Ondatra zibethicus*) (13, 19, 20; R. L. Sautter and E. M. Knights, Letter, Lancet i:1103, 1983). Few surveys have been conducted to determine the prevalence of *Giardia* spp. in muskrats.

Another potential zoonosis that has been increasing in prevalence recently in the United States is nontyphoid salmonellosis (8). Although there is good evidence to incriminate domestic food animals as important sources of *Salmonella* spp., little information is available on the reservoir potential of wild animals. It is possible that *Salmonella* spp. from wild animals may reach human beings directly through fecal contamination of surface water or indirectly via livestock infected with bacteria from wild animals.

The purpose of the study described here was to determine the prevalences of *Giardia* and *Salmonella* infections in muskrats, which were common animals in the study area.

MATERIALS AND METHODS

Between April and August 1986, fresh specimens of muskrat feces were collected from 12 different sites, representing 5 different habitat types, in Gloucester, Salem, and Cumberland counties, located in southwestern New Jersey (Fig. 1). Specimens were placed in sterile plastic bags that were sealed and refrigerated for transport to the laboratory.

Fecal specimens were analyzed for *Giardia* cysts by the zinc sulfate centrifugal flotation technique. In addition, cysts from some specimens were concentrated (by zinc sulfate centrifugal flotation or over a 0.85 M sucrose gradient), washed, counted in a hemacytometer, and inoculated orally (via gavage) into *Giardia*-free, inbred Mongolian gerbils (*Meriones unguiculatus*) of both sexes (16). Gerbils were

bred in our laboratory from stock purchased from Tumblebrook Farms, Inc., West Brookfield, Mass. Details on measures taken to ensure that gerbils were free of *Giardia* infection were presented previously (16). The gerbils each received from 1×10^4 to 6×10^4 cysts. Gerbils have been shown previously to be susceptible to *Giardia* spp. isolated from various host species (4, 11, 16). Several attempts were made to initiate axenic cultures in modified TYI-S-33 medium (15) plus antimicrobial agents (200 U of penicillin per ml, 200 μg of streptomycin per ml, 2.5 μg of amphoteracin B per ml) with *Giardia* trophozoites isolated from the upper small intestines of infected gerbils. Furthermore, impression smears of intestinal mucosa from infected gerbils were dried, fixed with absolute methanol, stained with Giemsa stain, and examined microscopically.

For the isolation of *Salmonella* spp., feces were incubated in selenite F enrichment broth for 24 h at 37°C, at which time a portion was streaked onto MacConkey and xylose-lysine-desoxycholate (XLD) agars. Bacteria were identified as described in detail elsewhere (6). Ninety-eight samples were enriched for *Salmonella* spp. in both modified Rappaport (14) and selenite F broths. Hektoen enteric agar was used in addition to MacConkey and XLD agars for about half of the samples. Neither Rappaport broth nor Hektoen agar improved the selection and detection of *Salmonella* spp. in this study or with known positive specimens. Thus, both media were deleted from the routine isolation protocol. The media used were all commercially prepared and certified by the manufacturer (Otisville Biotech, Inc., Otisville, N.Y.) to support differential isolation and growth of *Salmonella* spp. Furthermore, all lots of the media used supported positive control cultures of *Salmonella* and yielded isolates of *Salmonella* from feces of several domestic animals. A total of 40, 200-ml grab samples of surface water from various localities within the study area also were analyzed for *Salmonella* spp. by membrane filtration of 100-ml specimens or by selenite F enrichment. The latter technique was performed by dissolving the powdered media directly in 200 ml of sample. This culture was then incubated at 37°C for 24 h, after which portions of the culture were streaked onto the selective agars noted above.

RESULTS

Of the 220 muskrat fecal specimens examined, 154 (70%) were found to contain *Giardia* cysts. Prevalences at the 12 sites ranged from 30.8 to 100% (standard deviation, ±24.9%) (Fig. 1). From a pooled sample, 25 *Giardia* cysts were

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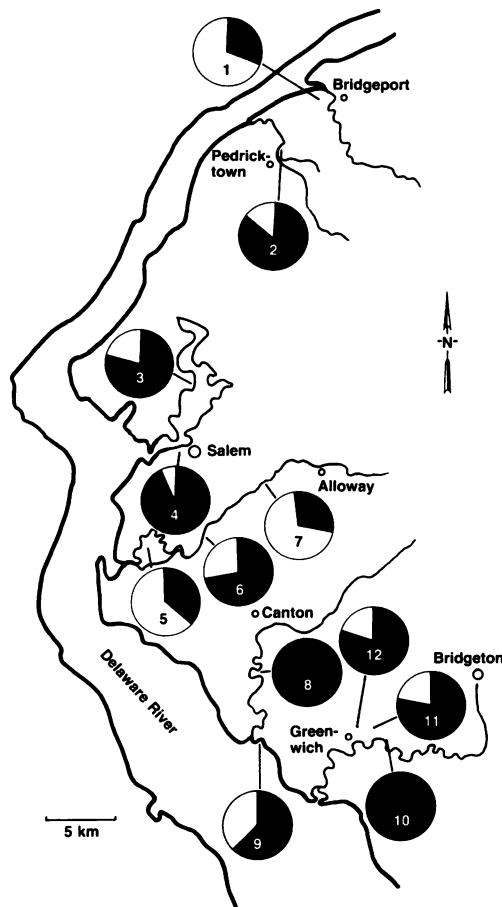† Present address: Department of Veterinary Pathobiology, University of Illinois, Urbana, IL 61801.

FIG. 1. Study area in southwestern New Jersey indicating the sites where muskrat feces were collected. The proportion of darkening of each circle indicates the proportion of specimens positive for *Giardia* cysts; site numbers are indicated on each circle. Habitat types included pond (sites 1 and 10); tidal marsh (sites 2, 3, 8, and 9); tidal creek (sites 4, 6, and 7); impounded, freshwater marsh (site 5); and freshwater (nontidal) creek (sites 11 and 12).

measured; the mean ± standard deviation length was 12.4 ± 0.8 μm, and the mean ± standard deviation width was 9.2 ± 0.6 μm.

A total of 27 gerbils received *Giardia* cysts isolated from muskrat fecal specimens. Each of five groups of gerbils (n = 3 to 6 per group) were given cysts isolated from different, individual fecal specimens, and one group (n = 3) was given cysts from a pool of fecal specimens. Because of the small number of gerbils per group and the similarity of their response to *Giardia* infection, the results of these trials are combined. *Giardia* trophozoites were found in the small intestines of 10 of 10, 4 of 4, 2 of 2, and 3 of 11 gerbils killed on days 8, 9, 11, and 16 postinoculation, respectively. Cysts from all of the muskrat fecal specimens tested produced infections in gerbils. Several uninoculated, control gerbils housed in separate cages in the same room as infected gerbils were not found to contain *Giardia*. Attempts to cultivate trophozoites from 14 gerbils (killed on various days postinoculation, as noted above) were unsuccessful. The trophozoites lived for a day or two, but they did not appear to replicate. Each batch of culture medium used in these attempts was found to support the growth of passaged *Giardia* trophozoites originally isolated from some other

mammalian host species (cats [including strain ATCC 50163], a sheep, and a llama) as well as from humans (strains WB [ATCC 30957] and KS [ATCC 50114]). In all of the stained intestinal mucosal impression smears made from 14 infected gerbils, trophozoites of the *Giardia duodenalis* type, as judged by the "claw-hammer" shape of the median bodies (12), were found.

None of the fecal specimens nor any of the water samples yielded positive *Salmonella* cultures. All of the positive and negative control cultures responded appropriately.

DISCUSSION

These results indicate that muskrats may not be important in the epizootiology of salmonellosis in the study area. There is a previous report of an epizootic of salmonellosis among captive muskrats (2); however, we are not aware that infection of free-living muskrats with *Salmonella* spp. has been documented. It is possible, however, that *Salmonella* spp. may have been detected in fecal and water specimens had other or additional enrichment and selective media been employed or if greater volumes of sample had been tested.

The public health significance of finding a majority of muskrats passing *Giardia* cysts depends on the relatedness of *Giardia* spp. from muskrats and humans. The *Giardia* sp. infecting the muskrat was named *Giardia ondatrae* in 1939, in accordance with the view prevalent at that time that *Giardia* spp. are highly host specific (20). Later, it was suggested that there were no more than three distinct forms of *Giardia*, based primarily on the shape of the median bodies (12). Following this scheme, the *Giarida* spp. from muskrat and human sources both would be considered *G. duodenalis*. In recent studies in which biochemical and molecular genetic techniques have been used to determine the relatedness of cultured *Giardia* trophozoites isolated from human and some nonhuman sources, it was concluded that *Giardia* spp. were not necessarily host specific and that a particular host may harbor more than one strain (or even species) of *Giardia* (7, 18). Some rodents (i.e., gerbils and rats) can be infected experimentally with *Giardia* spp. isolated from humans (4, 9, 16). Other investigators were unsuccessful, however, in establishing an infection in adult rodents (i.e., rats, hamsters, and mice) with *Giardia* spp. from human sources (23). Some human-adapted *Giardia* strains may be less fastidious than other strains and are capable of infecting rodents, such as muskrats. If this suggestion is valid, muskrats could serve as reservoirs of *Giardia* strains capable of infecting human beings via the drinking water supply.

Surface water in the study area described here does not represent an important source of drinking water. However, the Delaware River and its tributaries within the study area do carry sewage effluents from several municipalities. In other studies (17) it has been shown that *Giardia* cysts can be detected in treated sewage effluents. Muskrats in our study area, then, could serve as sentinels of the presence of human fecal contamination as well as amplifiers of the concentration of *Giardia* cysts in water, assuming that muskrats are susceptible to human-source *Giardia* strains. Confirmation of this conclusion would require the cultivation of muskrat-source *Giardia* strains and characterization by biochemical and molecular genetic typing. Carefully controlled, cross-transmission experiments would provide additional insight into the host range limitations of these strains of *Giardia*.

Habitat-related differences in the prevalences of several helminth parasites of muskrats have been reported by others

(1). Although in this study the prevalence of *Giardia* spp. in feces varied markedly among the 12 collection sites (Fig. 1), no obvious relationship of prevalence to habitat type emerged. The collection of more samples from each habitat type and from additional sites would be necessary to reveal such a relationship for *Giardia* spp. if, indeed, one exists.

ACKNOWLEDGMENTS

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