ADVANCES IN *GIARDIA* RESEARCH, p. 159-163, ed. P.M. Wallis & B.R. Hammond, University of Calgary Press, Calgary, 1988.

Immunofluorescence Differentiation Between Various Animal and Human Source *Giardia* Cysts Using Monoclonal Antibodies

Henry H. Stibbs,* Edward T. Riley, Joseph Stockard, John L. Riggs, Peter M. Wallis and Judy Isaac-Renton

U.S. - Japan Biomedical Research Laboratory, Department of Medicine, Tulane University, Hebert Centre, Bldg 30, Belle Chasse, LA. 70037 U.S.A.

Mouse monoclonal antibodies have been developed against cysts of *Giardia muris* and of *Giardia simoni* isolated from a wild Norway rat captured on the University of Washington campus. The four anti-*G. muris* antibodies reacted positively in indirect immunofluorescence with the rat source cysts in addition to the homologous *G. muris*, but not with cysts isolated from beaver (2), dog (4), human (8), muskrat (3), or Richardson's vole (1). The one anti-rat cyst monoclonal antibody reacted only with rat and cow (CW-1; Alberta) source cysts. The anti-*G. lamblia* cyst monoclonal antibody provided by J. Riggs was found to react with all human, beaver, and dog source cysts, and with rat *Giardia*, but not with *G. muris* or cysts of muskrat or Richardson's vole origin. The results suggest that systematic differences occur in the cyst surface membrane antigens of various *Giardia* strains, and that monoclonal antibodies may prove useful in developing an antibody typing system for *Giardia* strain and animal source identification.

INTRODUCTION

Waterborne *Giardia* cysts have been implicated as the cause of over 80 outbreaks of giardiasis in the United States over the past 30 years (4,11,15,22). *Giardia* infected mammals, most notably the beaver, have been implicated as the probable source of *Giardia* cysts infecting humans in several of these outbreaks (1,6,15,28,30). It has never, however, been possible to prove whether the infecting *Giardia* strains in these outbreaks were of animal or human origin or both; or to identify with absolute certainty which species of animal(s) (if any) contributed the human infective cysts. *Giardia* infections are common in a wide variety of wild and domestic mammals and birds (1,3,5,8,14,20,27,28,30); therefore, it is reasonable to assume that in most North American watersheds some animal species will be *Giardia*-infected and excreting *Giardia* cysts at any point in time. A portion of the excreted cysts will find their way into the surface runoff. Thus, *Giardia* sp. cysts can probably be considered omnipresent and ubiquitous in natural surface waters in North America.

The infectivity for man of the many animal strains or species of *Giardia* found in North American watersheds is an almost completely unexplored subject, about which many assumptions and guesses have been made over the years. Controlled experiments involving human subjects have never been performed. Studies involving cross-infectivity between animal host species have revealed great variability in host-species-specificity, so that one may not confidently make predictions about the infectivity for man of any animal isolates (1,14,30).

* Corresponding author.

Effective methods have been developed for filtering surface water for the purpose of concentrating *Giardia* cysts and for identifying the cysts in the recovered particles (10,12). *Giardia* cysts have been identified in surface waters in many areas of North America, and in some cases quantitative data on the concentration of cysts in surface water have been obtained (19,28). However, in using light microscopy or immunofluorescence to examine cysts recovered from filtered surface water, one cannot reliably identify the animal origin, strain or species of the cysts. This is due to the fact that *Giardia* cysts of all animal and human origins are anatomically very similar (14) and also the fact that *Giardia* cysts of most, if not all, mammalian sources are equally well visualized by immunofluorescence methods performed with polyclonal anti-cyst serum (21,22,26).

Methods capable of distinguishing antigenically, biochemically, or genetically between the *Giardia* cysts produced by different animals and by man would enable one to identify by animal source the *Giardia* cysts that have caused a human outbreak of giardiasis (by testing cysts recovered from patients), and also to identify the animal or human origin of cysts recovered from surface water. Identifying the source(s) of the cysts in water would allow one to estimate the degree to which human fecal pollution of a watershed is contributing human source cysts to the surface water, and also to estimate the relative contributions of various animal species. At present, however, almost nothing is known of the possible antigenic, biochemical, or genetic differences that may exist between cysts of various animal and human source *Giardia*. Riggs, however, has already reported the use of a mouse monoclonal antibody against human source *G. lamblia* cysts in differentiating between human and animal *Giardia* cysts by immunofluorescence (21). This antibody has been found to bind to all human source cyst isolates tested as well as to cysts from beavers and dogs;

it also has reacted positively with *Giardia* cysts from one cattle specimeh from Yosemite National Park and with cysts from a coyote and a chipmunk. We have also found (see results below) that this antibody reacts strongly with *Giardia* cysts of the *duodenalis* morphological type recovered from a wild Norway rat in Washington state. The Riggs antibody does not, however, bind to *Giardia* cysts produced by a wide variety of other animals, including deer, muskrats, and different types of voles and mice (see results). Smith et al. (25) also reported production of a mouse monoclonal antibody that would react in immunofluorescence with *G. lamblia* cysts but not cysts from mice (*G. muris*) or dogs (*G. canis*). The mice used as the source of spleen cells for the hybridoma fusion had in this case been immunized with *in vitro* trophozoites of *G. lamblia*.

The existence of antigenic diversity among cysts of human and animal *Giardia* isolates seems likely in light of previous discoveries of antigenic diversity among trophozoites of human and animal isolates (13,16,18,24) and of differences in agarose gel electrophoresis banding patterns of restriction endonuclease fragments of DNA from human and animal isolates (17). Diversity in isozyme patterns among *Giardia* trophozoites of human and animal origins has also been reported (2).

We have now produced mouse monoclonal antibodies against *Giardia muris* cysts isolated from mice and against *Giardia* cysts isolated from wild, live-trapped Norway rats in an effort to see if antibodies can be produced which bind to some or all of the animal source cysts but not to human source cysts. These antibodies will also be used to identify the animal origin of *Giardia* cysts through the creation and use of an antibody typing system for cysts of this parasite.

METHODS

Giardia isolates used in immunizing mice. *Giardia muris* was acquired from Dr. Martin Heyworth of the Division of Cell Biology, VA Medical Center, San Francisco, and maintained in BALB/c, Swiss-Webster, and nude (nu/nu) mice. This isolate had originally been procured from an infected golden hamster at Case Western Reserve University (23).

Two wild Norway rats were live-trapped along a drainage canal located on the University of Washington campus in Seattle and were found to be infected with a *Giardia* strain of the *duodenalis* morphological type (presumably *G. simoni*). The rats were maintained in the laboratory for several months on a diet of commercial rat chow, apples, carrots, and water. Cyst excretion seemed to be relatively constant over the months that the rats were maintained in captivity. Gerbils (Tumblebrook Farms Co., Massachusetts) were inoculated with cysts from the rats, and cysts isolated from successfully infected gerbils used to supplement the cysts obtained directly from the rats for the purpose of immunizing mice. We were not able to infect weanling Sprague-Dawley or Long-Evans rats with this wild rat *Giardia* isolate.

Purification of cysts. Mouse or rat source *Giardia* cysts were purified from feces by centrifugation over 1 M sucrose in water (10 min, 500 x g) followed by step gradient centrifugation over two layers of Percoll (Sigma Chemical Co., St. Louis, MO.) of specific gravities 1.05 and 1.09 (15 min, 500 x g). Occasionally, cysts were purified by flotation on zinc sulfate (specific gravity, 1.18) followed by centrifugation over 1 M sucrose.

Production of monoclonal antibodies. Female BALB/c mice were immunized against freshly isolated cysts through a series of four to five intraperitoneal injections of 1-3 x 10^6 cysts/animal/injection over 6 weeks followed by 1 or 2 intravenous tail injections, 4 days apart, of 2-3 x 10^6 cysts in sterile normal saline. Four or 5 days after the last intravenous injection, mice were sacrificed and spleen cell:myeloma cell fusions carried out. Spleen cells from the immunized mice were fused with NS-1 (P3-NS-1-Ag 4.1) mouse myeloma cells grown in RPMI 1640 medium with 15% fetal bovine serum, using 40% polyethylene glycol 1500, using conventional methods (9). Cells were plated onto 96-well culture plates and hybridoma growth selected for using RPMI 1640 medium (15% fetal bovine serum) supplemented with HAT (hypoxanthine/aminopterine/thymidine). Growth was enhanced by using mouse thymus cells as feeder cells.

Hybridomas secreting antibodies to cysts were detected by indirect immunofluorescence using the homologous cysts air-dried onto the bottoms of flat-bottom, 96-well ELISA plates (Falcon 3915) at 5,000 to 10,000 cysts per well, and also (with G. *muris* cysts) by ELISA, again with cysts dried down onto the wells of 96-well plates, using 25,000 cysts per well. Stable hybridomas were cloned by limiting dilution three times. Ascites fluid was sometimes produced in BALB/c mice pretreated with Pristane (Sigma Chemicals). Both culture supernatant and ascites fluid were used in the immunofluorescence cross-testing studies described below.

Immunofluorescence cross-testing. The *Giardia* isolates used in the immunofluorescence cross-testing experiments with the monoclonal antibodies had diverse animal origins (see Tables 1 and 2). Muskrats were live-trapped on the University of Washington campus (same location where the Norway rats were trapped) and in eastern Washington outside of Ellensburg (the NA, or Naneum, isolates), and were maintained in rabbit cages on a diet of apples, carrots, celery, lettuce, and alfalfa hay. The MR-6 muskrat isolate, the C-3 vole (*Clethrionomys gapperi*) isolate, and the CW-1 cow isolate were acquired from Peter Wallis in Calgary, Alberta, and were maintained in Swiss-Webster mice, or, in the case of the CW-1, in gerbils. *Giardia* infected *Microtus ochrogaster* were provided by Stanley Erlandsen of the University of Minnesota; the animals had originated in Missouri. The Manastash beaver isolate was from the Ellensburg area of eastern Washington and was provided by Glen Clark of Central Washington University. Dr. Clark also provided the feces of *Microtus richardsoni* which had been collected at Paradise Creek in Mr. Rainier National Park. The M beaver isolate originated in British Columbia and was provided as cultured trophozoites by Judy Isaac-Renton. The D-3 dog isolate was adapted to culture and provided by Peter Wallis. The H-2, H-3, and H-4 human isolates were provided by Charles Hibler of Colorado State University in infected gerbils, and were adapted to culture in our laboratory. The TB human isolate came from a patient in Seattle and was adapted to culture; on one occasion it was used to infect a Long- Evans rat, from which cysts were obtained. The dog *Giardia* specimens listed in Table 1 came from dogs housed in the University of Washington vivarium and used for other research purposes. In a number of cases cysts for cross-testing were obtained from gerbils that had been inoculated with either cultured trophozoites or with cysts and immunosuppressed with Dexamethazone (Intensol; Roxane Laboratories, Columbus, OH.; 1.5 mg into 100 ml drinking water); these gerbil-derived cysts are indicated as such in the Tables.

Cysts for immunofluorescence testing were usually purified by the procedures described above; sometimes, however, cysts were spotted onto the test wells as an aqueous fecal slurry. Cysts were spotted onto eight-spot Teflon-coated slides (Bellco, Vineland, N.J.), air dried, and fixed in acetone at room temperature. The slides were usually stored at -75°C with dessicant in tightly sealed boxes until testing. Hybridoma supernatant or ascites was allowed to react with the cysts for about one hour at 37°C (or overnight at 4°C) followed by two brief rinses in 0.0175 M phosphate-buffered saline, pH 7.4 (PBS), and a further one-hour incubation with FITC-labeled goat anti-mouse

immunoglobulin antibody (Cappel Laboratories, West Chester, PA.) at 1:80 dilution in PBS with 2% normal goat serum. After rinsing, the slides were mounted with 90% glycerol/PBS containing 0.5 mg/mL *p*-phenylenediamine, and viewed using epifluorescence.

RESULTS

Four stable, cloned hybridoma lines secreting immunofluorescence-positive and ELISA-positive monoclonal antibodies were produced against *G. muris* cysts. All were of the IgG1 class. One stable hybridoma line secreting immunofluorescence-positive IgG1 antibody against the rat source *Giardia* cysts was also produced.

The results of cross-testing these antibodies by indirect immunofluorescence with cysts from the many animal and human origins are shown in Tables 1 and 2. The anti-*G. lamblia* cyst monoclonal antibody of Riggs, directly labeled with fluorescein, was also tested against all of the cyst isolates; the results are included in Table 1. The four *G. muris* monoclonals all showed an identical pattern of reactivity with the various cyst isolates. All four reacted strongly with cysts of the homologous isolate and also with the wild Norway rat-source *Giardia*. No binding to cysts of human, beaver, dog, muskrat, or vole origins was observed. Riggs monoclonal reacted strongly with cysts of human, beaver, dog, and rat origins. The rat *Giardia* monoclonal bound only to cysts of the homologous rat-source isolate and, oddly, to cysts of the cattle (CW-1) isolate from Alberta. We have recently found that neither the anti-rat *Giardia* monoclonal nor the Riggs monoclonal will bind to *muris* type cysts that have been recovered from other Norway rats trapped from the same location as the first two.

TABLE 1. Immunofluorescence reactivity patterns obtained with *Giardia* cysts of various animals and human sources, after incubation with four anti-*G. muris* monoclonal antibodies and with the anti-*G. lamblia* monoclonal of Riggs.

Source of *Giardia*	Antibodies 4-7B	1-6C	10-10B	2-11B	Riggs
Beaver (M;B.C.;gerb.)	-	-	-	-	++
Beaver (Manast.;Wa.;beav.)	-	-	-	-	++
Dog 1 (U.W. vivarium)	-	-	-	-	++
Dog 2 "	-	-	-	-	++
Dog 3 "	-	-	-	-	++
Dog 4 "	-	-	-	-	++
Human 1 (patient)	ND	-	-	-	++
Human 2 (TB;rat)	-	-	-	-	++
Human 3 (H2;Co.;gerb.)	-	-	-	-	++
Human 4 (H3;Co.;gerb.)	-	-	-	-	++
Human 5 (H4;Co.;gerb.)	-	-	-	-	++
Human 6 (patient)	-	-	-	-	++
Human 7 (patient)	-	-	-	-	++
Human 8 (patient)	-	-	-	-	++
Norway rat (U.W.1,2)	++	++	++	++	++
Mouse (*G. muris*; orig. hamster)	++	++	++	++	-
Muskrat (U.W.1,2)	-	-	-	-	-
Muskrat (E. Wa.;NA2)	-	-	-	-	-
Muskrat (MR-6;Alb.;mice)	-	-	-	-	-
Vole (*M. richardsoni*;Mt. Rainier)	-	-	-	-	-
Vole (*M. ochrogaster*;Mo.)	-	-	-	-	-
Vole (*C. gapperi*;Alb.;mice)	-	-	-	-	-

++ Strong reaction between antibody and cysts
- Negative reaction
ND No data available

TABLE 2. Immunofluorescence reactivity of MAb 6-E10 (anti-Norway rat *Giardia* cyst) with cysts of other animal and human sources.

Source of *Giardia*	IFA reaction (-,+,++)
Norway rat (*G. simoni;UW2;in feces*)	++
Mouse (G. muris;orig. hamster)	-
Vole (*M. richardsoni*;Mt. Rainier)	-
Vole (*C. gapperi*;C-3;Alb.;mice)	-
Vole (*M. ochrogaster*;Mo.)	-
Muskrats (UW-5,UW-2)	-
Muskrat (MR-6;Alb.;mice)	-
Beaver (M;B.C.;gerb.)	-
Beaver (Manastash;E. Wa.;gerb.)	-
Dog (D-3;Alb.;gerb.)	-
Cattle (CW-1;Alb.;gerb.)	++
Human (2 patients; Wa.)	-
Human (H-2;Colorado;gerb.)	-

All of the antibodies, including the one of Riggs, appeared to bind strongly to the cyst wall of those isolates that reacted positively, the pattern of immunofluorescence being evenly distributed around the entire surface of the cysts. No internal structures of the cysts could be seen fluorescing. We did not observe in any case partial reactions of cyst populations with any of these antibodies. However, because some of the test cyst preparations were cysts contained in fecal slurries, it was impossible to preclude by immunofluorescence the possibility that a fraction of the *Giardia* cysts in some preparations were unreactive.

On several occasions, cysts of either mouse or rat origin, stored in a dry state on test slides for six weeks or more at 4°C or thawed and refrozen a number of times from -75°C, failed to react with the antibodies. These cysts would continue to react positively, although weakly, with polyclonal rabbit antisera to *G. lamblia* cysts. Also, cysts of the H-2 isolate, harvested from gerbils, did not react with the Riggs antibody after storage for several weeks in 5% formalin in PBS at 4°C. The monoclonal antibodies also tended to lose reactivity with their homologous antigen after repeated freezing and thawing.

DISCUSSION

The cross-testing results show that antigenic differences exist between cysts of various animal origins and of human origin, and suggest that it may eventually be feasible to identify cysts in environmental samples (water or feces) according to their animal-source by testing their immunofluorescence reactivity with a battery of selected monoclonal antibodies prepared against cysts. The results also show that vole and muskrat (i.e.

microtine) *Giardia* isolates seem to segregate antigenically from the others in that they did not react with any of the monoclonals used. The fact that the rat and the mouse-source *Giardia* isolates both bind the anti-*muris* monoclonals indicates antigenic relatedness; however, the *muris* cysts did not bind the anti-rat *Giardia* monoclonal. Incidentally, we have also found that all of the cyst isolates shown in the Tables react strongly in indirect immunofluorescence with polyclonal rabbit antiserum against human *G. lamblia* cysts (data not included in Tables), an observation that corroborates previous results reported by ourselves (26) and by Sauch (22). Therefore, while it appears that all of the cyst isolates share some antigens or at least some epitopes (determinants), cross-testing with monoclonal antibodies has revealed that differences exist in the distribution of certain epitopes between isolates.

We still do not know anything of the physico-chemical nature of the antigens recognized by these monoclonal antibodies, nor do we know at this time whether the four anti-*muris* monoclonals recognize the same or different epitopes. The precise ultrastructural location of the antigens on or in the cysts also remains to be determined. The antigens may be components of the thin (0.15-0.2 μm thick) filamentous outer coat of the cyst or of the underlying outer cyst membrane, structures described by Erlandsen et al. (7). In addition, the antigens may have some biochemical or ultrastructural relationship to the chitin present in the cyst walls (29). Finally, the possibility that some of the recognized antigens, particularly if located on the outer filamentous coat of the cyst, may be host-derived cannot yet be discounted.

Two other important considerations that relate to the possible practical utility of these antibodies in differentiating between *Giardia* cyst isolates in nature are (1) the possible cyst antigenic variability that may exist in the *Giardia* populations found within one host species, especially among host populations from geographically diverse origins; and (2) the possible loss of immunofluorescence reactivity of cysts after storage in various fixatives (e.g. formalin) or in water, and at various temperatures and other conditions of storage and shipping.

In conclusion, it appears that antigenic differences exist between *Giardia* cysts of isolates from various animal and human sources. The use of monoclonal antibodies in differentiating between cyst isolates and thus in identifying the animal or human source of an unknown test cyst isolate may prove to be feasible, although much more needs to be done to study the variables mentioned above and to develop standard practical methods for testing. We are currently continuing this work by trying to develop monoclonal antibodies against the microtine types of *Giardia* cysts (in particular, cysts from muskrats in Washington) as well as against human and beaver isolates, so that a more extensive battery of antibodies can be used in developing an antibody typing scheme for *Giardia* cyst identification. While the identification scheme we are proposing would identify the *Giardia* isolates as to host species origin, it is possible (provided extensive antigenic variation does not occur within isolates) that this information on antigen or epitope distribution among cyst isolates may be helpful in establishing species designations within the genus *Giardia*.

ACKNOWLEDGEMENTS

This research was funded wholly by the United States Environmental Protection Agency, Office of Exploratory Research, under cooperative agreement #R811970-01 to H.H.S.; this report does not however, necessarily reflect the views of that agency and no official endorsement should be inferred. The authors gratefully acknowledge the assistance of the Department of Game of the state of Washington in providing a collecting permit for capturing some of the animals used in this work.

LITERATURE CITED

1. Bemrick, W.J. 1984. Perspectives on the transmission of giardiasis, In: *Giardia and giardiasis: biology, pathogenesis, and epidemiology*. S.L. Erlandsen and E.A. Meyer (eds). Plenum Press. New York. pp. 379-400.
2. Bertram, M.A., Meyer, E.A., Lile, J.D., and S.A. Morse. 1983. A comparison of isozymes of five axenic *Giardia* isolates. J. Parasitol. 69:793-801.
3. Clark, G.W., and R.E. Pacha. 1984. Animals associated with mountain streams and meadows as reservoirs of *Giardia*. Abstract #30, 59th Annual meeting, American Society of Parasitologists, Snowbird, Utah.
4. Craun, G.F. 1984. Waterborne outbreaks of giardiasis: current status, In: *Giardia and giardiasis: biology, pathogenesis, and epidemiology*. S.L. Erlandsen and E.A. Meyer (eds.). Plenum Press. New York. pp. 243-261.
5. Davies, R.B., and C.P. Hibler. 1979. Animal reservoirs and cross-species transmission of *Giardia*, In: *Waterborne transmission of giardiasis, proceedings of a symposium*. U.S. Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. pp. 104-126.
6. Dykes, A.C., D.D. Juranek, R.A. Lorenz, S. Sinclair, W. Jakubowski, and R. Davies. 1980. Municipal waterborne giardiasis: an epidemiologic investigation. Ann. Int. Med. 92:165-170.
7. Erlandsen, S.L., D.G. Schupp, M.M. Januschka, and W.J. Bemrick. 1986. Ultrastructure of *Giardia* spp. cysts: development and formation of membranes in the cyst wall and their loss during excystation. Abstract #95, 61st annual meeting, American Society of Parasitologists. Denver, CO.
8. Frost, F., B. Plan, and B. Leichty. 1980. *Giardia* prevalence in commercially trapped mammals. J. Environ. Hlth. 42:245-249.
9. Galfre, G., and C. Milstein. 1981. Preparation of monoclonal antibodies: strategies and procedures. Meth. Enzymol. 73:3-46.
10. Hausler, W.J., Jr., W.E. Davis, and N.P. Moyer. 1984. Development and testing of a filter system for isolation of *Giardia lamblia* cysts from water. Appl. Environ. Microbiol. 47:1346-1347.
11. Hopkins, R.S., P. Shillam, B. Gaspard, L. Eisnach, and R.J. Karlin. 1985. Waterborne disease in Colorado: three years' surveillance and 18 outbreaks. Am. J. Pub. Hlth. 75:254-257.
12. Jakubowski, W.. 1984. Detection of *Giardia* cysts in drinking water: state of the art. In: *Giardia and giardiasis: biology, pathogenesis, and epidemiology*. S.L. Erlandsen and E.A. Meyer (eds). Plenum Press. New York. pp. 286-363.

13. Korman, S.H., S.M. Le Blancq, D.T. Spira, J. El On, R.M. Reifen, and R.J. Deckelbaum. 1986. *Giardia lamblia*: identification of different strains from man. Z. Parasitenkd. 72:173-180.
14. Kulda, J., and E. Nohynkova. 1978. Flagellates of the human intestine and of intestines of other species. In: *Parasitic protozoa, Vol.* 2. J.P. Kreiser (ed.). Academic Press. New York. pp. 1-138.
15. Lopez, C.E., A.C. Dykes, D.D. Juranek, S.P. Sinclair, J.M. Conn, R.W. Christie, E.C. Lippy, M.G. Schultz, and M.H. Mires. 1980. Waterborne giardiasis: a communitywide outbreak of disease, and a high rate of asymptomatic infection. Am. J. Epidemiol. 112:495-507.
16. Nash, T.E., and D.B. Keister. 1985. Differences in excretory secretory products and surface antigens among 19 isolates of *Giardia*. J. Inf. Dis. 152:1166-1171.
17. Nash, T.E., T. McCutchan, D. Keister, J.B. Dame, J.D. Conrad, and F.D. Gillin. 1985. Restriction-endonuclease analysis of DNA from 15 *Giardia* isolates obtained from humans and animals. J. Inf. Dis. 152:64-73.
18. Nash, T.E., and A. Aggarwal. 1986. Cytotoxicity of monoclonal antibodies to a subset of *Giardia* isolates. J. Immunol. 136:2628- 2632.
19. National Park Service. 1985. Field survey of *Giardia* in streams and wildlife of the Glacier Gorge and Loch Vale basins, Rocky Mountain National Park. Natural Resources Report Series #85-3. Water Resources Division, Applied Research Branch, National Park Service, U.S., Fort Collins, CO.
20. Pacha, R.E., G.W. Clark, and E.A. Williams. 1985. Occurrence of *Campylobacter jejuni* and *Giardia* species in muskrat (*Ondatra zibethica*). Appl. Environ. Microbiol. 50:177-178.
21. Riggs, J.L. 1984. *Giardia* Methods Workshop Water Quality Technology Conference. American Water Works Assoc.. Denver, CO. pp. 496.
22. Sauch, J.F. 1985. Use of immunofluorescence and phase-contrast microscopy for detection and identification of *Giardia* cysts in water samples. Appl. Environ. Microbiol. 50:1434-1438.
23. Schaefer, F.W., III, E.W. Rice, and J.C. Hoff. 1984. Factors promoting *in vitro* excystation of *Giardia muris* cysts. Trans. Roy. Soc. Trop. Med. Hyg. 78:795-800.
24. Smith, P.D., F.G. Gillin, N.A. Kaushal, and T.E. Nash. 1982. Antigenic analysis of *Giardia lamblia* from Afghanistan, Puerto Rico, Ecuador, and Oregon. Inf. Immun. 36:714-719.
25. Smith, R.D., N.S. Stimler, J.A. Sauch, and F.W. Schaefer, III. 1983. Monoclonal antibodies to *Giardia lamblia*: potential for identification and speciation. Abstract #173, 58th annual meeting, American Society of Parasitologists, San Antonio, TX.
26. Stibbs, H.H. 1985. Morphological and antigenic comparisons of *Giardia* isolated from muskrat, beaver, mice, and man. Abstract, Tenth International Symposium on Intestinal Microecology. Society for Intestinal Microbial Ecology and Disease. University of Minnesota, Minneapolis, MN.
27. Wallis, P.M., J.M. Buchanan-Mappin, G.M. Faubert, and M. Belosevic. 1984. Reservoirs of *Giardia* spp. in southwestern Alberta. J. Wildl. Dis. 20:279-283.
28. Wallis, P.M., R.M. Zammuto, and J.M. Buchanan-Mappin. 1986. Cysts of *Giardia* spp. in mammals and surface waters in southwestern Alberta. J. Wildl. Dis. 22:115-118.
29. Ward, H.D., J. Alroy, B.I. Lev, G.T. Keusch, and M.E.A. Pereira. 1985. Identification of chitin as a structural component of *Giardia* cysts. Inf. Immun. 49:629-634.
30. Woo, P.K. 1984. Evidence for animal reservoirs and transmission of *Giardia* infection between animal species. In: *Giardia and giardiasis: biology, pathogenesis, and epidemiology*. S.L. Erlandsen and E.A. Meyer (eds). Plenum Press, New York. pp. 341-364.