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Project Summary

Determination of the Use of Solid Particle Samplers for *Giardia* Cysts in Natural Waters

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Parasitic flagellates in the genus *Giardia* are distributed worldwide and are now the most commonly reported intestinal parasites in the United States and Britain. Twenty-three waterborne outbreaks of giardiasis affecting over 7000 people occurred in various states in the United States between 1965 and 1977. Because of this significant increase in the incidence of waterborne outbreaks of giardiasis, efforts have been made to develop reliable and/or sensitive methods to determine the presence or absence of *G. lamblia* cysts in water supplies. The primary objective of this study was to improve the current methodology for concentrating, recovering and detecting cysts of *G. lamblia* in water supplies.

Two sampling processes for the concentration of cysts were examined. One process was diatomaceous earth filtration while the second was that of cyst concentration onto charged particles. Cysts of *G. muris* were used to determine the retention efficiency of ion-exchange resins and each type of diatomaceous earth filter examined. Cyst desorption efficiencies were evaluated for ion-exchange resins that best retained cysts, while backwashing parameters were optimized for diatomaceous earth filters. Results of cyst retention experiments indicated that two processes, anion-exchange concentration of cysts and diatomaceous earth filtration, had the potential to be developed into field methods. Comparison of these two processes at low cyst inoculum concentrations (1×10^3 cysts/liter) indicated that a greater number of cysts could be recovered from the diatomaceous earth filters. When 40 liter samples of tap water containing between 1.0×10^4 and 7.0×10^5 cysts were passed through diatomaceous earth filters, 5.2 to 31.1% of the cysts were recovered in the backwash. As a result, the diatomaceous earth filter was comparable to microporous filtration and may have application in sampling finished water supplies. However, its utility in raw sampling was limited since turbidity severely reduced the recovery efficiency of cysts.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The primary objective of this study was to improve the current methodology for concentrating, recovering, and detecting cysts of *G. lamblia* in water supplies. Two sampling processes for concentration of *G. lamblia* cysts were examined. One process was diatomaceous earth filtration while the second process examined was that of cyst concentration onto charged particles. The first process was based on the hypothesis that cysts could be efficiently trapped on the surface of diatomaceous earth filters and subsequently recovered through backwashing the filter with a small volume of water. The second process was based on the hypothesis that cysts could be attracted to charged surfaces, since they have been shown to have a charge of approximately -25 mV at pH 5 5 and to increase their electronegativity as the pH rises to 8 0 In

addition, charge-attraction techniques have been applied to concentration of viruses and bacteria from water, to the concentration of trypanosomes, and to the concentration of *Plasmodium* in clinical samples.

Parasitic flagellates in the genus *Giardia* are distributed worldwide and are now the most commonly reported human intestinal parasites in the United States and Britain. The cycle of this parasite is composed of two stages: the cyst stage and the trophozoite stage. Transmission of *Giardia* most often occurs when viable cysts are ingested directly or through water contaminated with feces. The average incubation period for human giardiasis is 8 days, with a range of 3-42 days. While most infections are asymptomatic, some people have a short-lasting acute diarrheal disease, nausea, and anorexia. A small percentage develop an intermittent or protracted course characterized by diarrhea, cramping, abdominal pain, bloating, and flatulence. Diarrhea with or without overt malabsorption may last months or even years.

As a measure of the significance of giardiasis in the United States, 23 waterborne outbreaks affecting over 7000 people have been caused by this infection between 1965 and 1977. Epidemic outbreaks have been reported in New York, New Hampshire, Pennsylvania, Colorado and Washington. The majority of these outbreaks were attributable to public consumption of minimally treated water which was fecally contaminated. In addition, sporadic epidemics have occurred among infants and children in hospital nurseries, custodial and residential institutions, and in day-care centers where personal hygiene standards were not stringent. Water supplies were most likely contaminated by untreated human waste or by aquatic mammal waste since asymptomatic carriers and aquatic mammals have been identified as the major reservoirs.

Because of the significant increase in the incidence of waterborne outbreaks of giardiasis reported, efforts have been made to develop reliable and/or sensitive methods to determine the presence or absence of *G. lamblia* cysts in water supplies. The major problem associated with developing sampling technology is that cysts are assumed to be present in low numbers, therefore necessitating the need for large-volume water sampling. During the past few years, several approaches have been taken to concentrate and detect cysts in water supplies. Developed methods can be divided into three major categories: (a) membrane filtration, (b) particulate filtration, and (c) microporous filtration.

Moore et al. (1969) utilized membrane filtration to examine water and sewage samples for *Giardia* cysts. One- and two-liter samples were passed through cheesecloth and then filtered through 0.45 μm porosity membrane filters. Sediment on the filter surface was brushed into water, centrifuged and preserved in 10% formalin for microscopic examination. No cysts were observed in 10 water samples. Barbour and his coworkers (1976) used the method of Chang and Kabler to filter 22 liters of stream water following an outbreak. However, no *Giardia* cysts were found. Luchtel and colleagues (1980) utilized 293 mm diameter 5.0 μm pore size Nuclepore* filters to concentrate formalin-fixed *G. lamblia* cysts from 20 liter tap water samples. Recovery rates of approximately 75% were found under such conditions.

The Center for Disease Control developed a large-volume sampling technique. The method used a swimming pool filter in which sand was the sampling medium. During an outbreak in Rome, New York, a total volume of 1.1×10^6 liters (28,000 gal.) of water was collected at an average flow rate of 76 liters/minute (20 gal./min) through the filter daily for 10 days. The filter backwash was collected each day in two 210-liter drums and coagulated with alum. The resulting sediment was collected and aliquots were fed to beagle puppies and examined microscopically. Two of ten samples fed to dogs produced infection and a single cyst was observed in one sample. In 1976, the U.S. Environmental Protection Agency (EPA) developed a cyst concentration technique involving the filtration of a large volume of water (100 gal. or more) through a microporous orlon fiber filter. This method has been tentatively adopted as the "method of choice" for concentrating cysts from water supplies. However, the reliability and validity of the technique has yet to be fully evaluated. Limited laboratory evaluations have indicated that cyst recovery was only in the range of 3-15%, with a mean of 6.3%. The interpretation of positive or negative field data is uncertain. In the sampling methodology described above, efficient cyst concentration from water was possible under certain conditions. However, processing of the concentrate led to significant losses of cysts. Therefore, a major problem in detecting *Giardia* cysts in contaminated water supplies using any of the above techniques has been the quantitative recovery of cysts from the filter medium.

Summary

The feasibility of using diatomaceous earth filters or ion-exchange resins to concentrate cysts of *G. lamblia* from water samples and subsequently recover those cysts in a quantitative manner was evaluated with water samples experimentally contaminated with *G. muris* cysts. A series of ion-exchange resins were initially selected for evaluation. Anionic resins selected included DEAE-cellulose and two polystyrene, divinylbenzene-crosslinked resins, Dowex 1-X4* and Dowex 1-X8. Cationic resins evaluated included Dowex 50W-X4 and Dowex 50W-X8. The relative ability of each resin to capture cysts from water samples was determined by passing cysts in buffered, distilled water through ionically-charged columns and determining the fraction of cysts retained at varying inoculum concentrations. DEAE cellulose retained all the cysts passed through the column regardless of the inoculum concentration; whereas, Dowex 1-X4, Dowex 1-X8, Dowex 50W-X4, and Dowex 50W-X8 retained 101, 71, 75, and 97% of the inoculated cysts, respectively. Statistical analysis of the data indicated that there was no significant difference between the ability of DEAE cellulose, Dowex 1-X4 and Dowex 50W-X8 to capture cysts from water samples. Therefore, these resins were selected for desorption studies. Cysts concentrated on the surfaces of ion-exchange resins were eluted with 40 ml of buffer optimized to a particular pH and ionic strength (pI) and the percent recovered from each resin was compared. Approximately 49% of the cysts concentrated on Dowex 1-X4 resins could be recovered throughout the inoculum range tested; however, an average of only 38% of the inoculated cysts were recovered from the Dowex 50W-X8 columns. No cysts were recovered from DEAE cellulose resins regardless of the elution pH or pI.

The capacity of three types of diatomaceous earth filter, Celite 505, Hyflo SuperCel, and Celite 560, to concentrate cysts was evaluated by passing distilled water samples containing between 6.0 cysts/liter and 1.62×10^4 cysts/liter through each column. No significant differences between the diatomaceous earth filters were observed, with retention ranging between 66 and 100%. However

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significant differences between columns were noted when each was backwashed to recover the cysts captured on the surface. Best cyst recoveries were observed when the filters were backwashed with 2000 ml of distilled water at a flow rate of 2 liters/minute. An average of 13% of the cysts concentrated on Celite 560 columns could be recovered. However, because of the concentration of small diatomaceous earth particles or "fines" in the backwash of both Celite 505 and Hyflo-SuperCel columns, no cysts could be detected under the microscope. Therefore, Celite 560 was selected for comparative studies with Dowex 1-X4.

The utility of either Dowex 1-X4 or diatomaceous earth (Celite 560) columns as *Giardia* cyst sampling devices was compared by sampling 40 liter distilled water samples contaminated with cysts and comparing the number of cysts recovered from each sampler. The mean number of cysts recovered was not significantly different over the range of inoculum concentrations used; however, analysis of the data indicated that there was a higher probability of recovering cysts from water samples containing low concentrations of cysts ($1.0\text{-}1.5 \times 10^3$ cysts/liter) when the diatomaceous earth filter was used. Cyst recoveries from water samples passed through diatomaceous earth averaged 13%, similar to that observed when microporous filtration was evaluated. However, the efficiency of the diatomaceous earth filter was markedly decreased when turbid water was sampled. When the recovery of cysts inoculated into turbid water samples (24 FTU) was compared to that of turbid-free samples, an 85-86% reduction was observed.

Conclusions

Based on the objectives and results of this study the following conclusions can be drawn: 1) Results of resin capacity and cyst retention experiments indicated that two processes, anion-exchange concentration of cysts and diatomaceous earth filtration, had the potential to be developed into field methods. 2) Analysis of the data with respect to inoculum concentration indicated that a greater number of cysts were recovered from the diatomaceous earth filters when dilute samples (1×10^3 cysts/liter) were filtered. Based on these data diatomaceous earth filters were considered the best choice. 3) Comparison of the efficiency of the diatomaceous earth filter to the reported efficiency of the EPA method of microporous filtration (13.0 and 6.7%, respectively) indicated that the two were similar. However, it appeared that the diatomacous earth filter was more severely affected by the composition of the water sample than the microporous filter would be. 4) The diatomaceous earth filter may have application to finished water supplies; however, its use on raw water samples is limited at the present time.

Recommendations

1. The results of this study indicate that *Giardia* cysts could be efficienctly concentrated on either the surface of diatomaceous earth or on the surfaces of charged particles. However, subsequent research should be directed toward the efficient recovery of concentrated cysts. Further research should be carried out with weak ion-exchange resins that operate efficiently in a narrower pH range or with charge-modified filters similar to those used to concentrate viruses.

2. One of the major problems encountered in this study was the lack of a sensitive detection technique. Quantification of *Giardia* cysts is presently based upon microscopic identification and counting. Experimental studies should be carried out to develop accurate detection of small numbers of cysts mixed with other microorganisms and debris from aquatic habitats. Immunofluorescent techniques similar to those used for the detection of bacteria should be investigated.

3. The behavior of *Giardia* cysts on certain resins observed in this study raised some question concerning the biochemical and physiological nature of cysts. Little information is available on these subjects. Basic research on the nature of the cysts themselves would lead to rational decisions on applicable sampling methods.

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The complete report, entitled "Determination of the Use of Solid Particle Samplers for Giardia *Cysts in Natural Waters," (Order No. PB 83-246 090; Cost: $10.00, subject to change) will be available only from:*
National Technical Information Service
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