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NOTES

Quantitation of *Giardia* Cysts by Membrane Filtration

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A method of fixing and staining *Giardia* cysts on a membrane filter is reported. This procedure appears to be a reliable method for the recovery and detection of cysts and also for the determination of cyst densities. Evaluation and possible applications of the technique are described.

Membrane filtration has been used extensively to quantitate bacteria and algae in water samples (2). Chang and Kabler (1) described a procedure for detecting and concentrating the cysts of *Entamoeba histolytica* by membrane filtration. A modification of this method was used by Moore et al. (4) in examining water and sewage samples for *Giardia* cysts. These studies show that cysts can be collected via membrane filtration. After the filtering process, the sediment is removed from the membrane and preserved in Formalin for analysis. Unlike the above workers, we have employed a method of fixing and staining *Giardia* cysts on the membrane filter. This technique has proven successful in quantitating cysts in feces from a variety of sources.

To prepare membranes for study, between 0.1 and 1.0 g of fresh fecal material was thoroughly emulsified in a small volume of buffered water and then diluted to a final volume of 100 ml. After thorough mixing of this suspension, cysts were trapped on membranes by filtering 1- to 10-ml samples through a 5-μm membrane filter (Gelman Metricel, MA-1, 25 mm). The amount of particulate matter in the fecal suspension determined the quantity of the suspension which could be passed through each membrane before the pores clogged. Large particles were removed from the fecal suspensions by use of two nylon mesh prefilters (pore sizes, 40 and 30 μm, respectively). These prefilters were placed directly on top of the membrane filter and were separated from it and from each other by woven mesh Dacron spacers (Millipore type AP 32). Immediately after filtration, the membrane was removed, fixed in hot Schaudinn fixative (50°C), and stained by a trichrome staining procedure. After dehydration, the membrane was cleared in xylene and placed on a glass slide, and a cover glass was permanently applied (Fig. 1). The entire membrane was examined, and cysts were counted using a light microscope with a ×400 magnification (Fig. 2). When it was necessary to confirm the identity of cysts, a ×1,000 magnification was used (Fig. 3). The characteristics used to identify *Giardia* cysts were those described by Schaefer and Rice (5). These characteristics are the correct shape and size (10 to 20 μm long by 5 to 15 μm wide) and at least two of the following anatomical features: two to four nuclei, axonemes, and median bodies. The number of cysts per membrane was counted, using these criteria.

To determine the reproducibility of the counting method, a series of slides was counted twice, and the number of cysts was compared. The duplicate counts were found to be in close agreement. Coefficients of variation between counts of the same slide ranged from a low of 0.0126 to a high of 0.0290, thus showing a high degree of reproducibility in counting *Giardia*

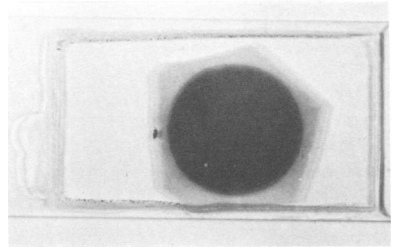

FIG. 1. Stained and covered membrane filter preparation of fecal sample.

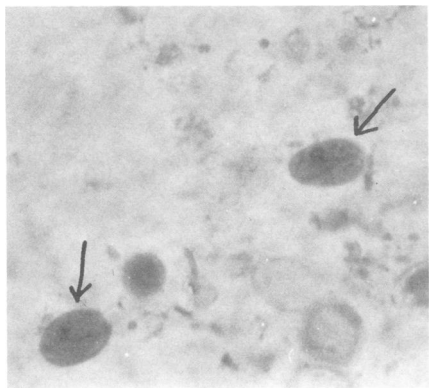

FIG. 2. Stained *Giardia* cysts trapped on membrane filter. Magnification, ×400.

cysts on membranes. To measure the precision of the method, slides from five different specimens were prepared in quadruplicate and each slide was counted. Cyst densities on these slides ranged from a low of 16 cysts per membrane to a high of 1,380. The coefficient of variation among replicate counts of each specimen was low, indicating little variation in counts from the same source (Table 1). The membrane filter procedure thus appears to be a reliable method for the recovery and detection of cysts from specimens showing considerable variation in cyst numbers.

To determine cyst densities, between 0.1 and 1.0 g of feces was weighed and emulsified in buffered water of known volume. Measured samples of the fecal suspension were filtered and stained as described above. The cysts on each membrane were counted, and the number per gram was calculated. Feces collected from water vole (*Microtus richardsoni*), muskrat (*Ondatra zibethica*), and humans and pellets from unidentified rodents were examined by this technique, and the number of cysts was determined. Cyst densities in these specimens ranged from a low of 1.2×10^3/g in a rodent sample to a high of 1.9×10^5/g in a water vole (Table 2). For the human specimens examined, cyst densities ranged from 5×10^3 to nearly 9×10^4/g.

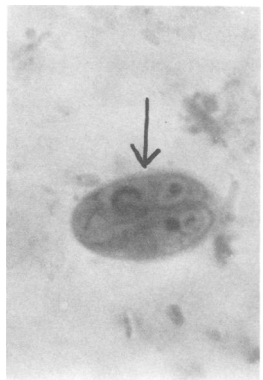

FIG. 3. Stained *Giardia* cyst trapped on membrane filter. Magnification, ×1,000.

TABLE 1. Evaluation of membrane filter method for detection of *Giardia* cysts

Count	No. of cysts per membrane				
	Microtus richardsoni			*Ondatra zibethica*	
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2
1	720	249	1,249	19	50
2	763	240	1,342	16	59
3	820	222	1,380	17	66
4	846	226	1,280	21	62
Mean	787.25	234.50	1,312.80	18.25	59.25
SD	56.67	12.20	59.20	2.22	6.80
CV[a]	0.072	0.052	0.045	0.122	0.115

[a] CV, Coefficient of variation.

Quantitating *Giardia* cysts by fixing and staining membrane filters has proven to be useful on a variety of samples and was especially useful in detecting cysts in specimens from humans and animals with mild infections. With this method, the cysts appear to be intact with a stain that demonstrates distinguishable internal characteristics. Furthermore, the recovery rate with this method should be excellent. This, in addition to the precision of the method with its good reproducibility, indicates that it may be a valuable

TABLE 2. Cyst densities obtained from various sources using the membrane filtration method

Host	No. of *Giardia* cysts per g of fecal material
Muskrat (*O. zibethica*)	1.8×10^3
Muskrat (*O. zibethica*)	1.2×10^5
Water vole (*M. richardsoni*)	1.9×10^5
Water vole (*M. richardsoni*)	9.6×10^4
Rodent[a]	1.3×10^4
Rodent	1.8×10^3
Rodent	6.8×10^3
Rodent	1.2×10^3
Rodent	4.3×10^3
Rodent	1.7×10^3
Rodent	5.8×10^3
Rodent	6.1×10^3
Rodent	3.4×10^3
Human	5.3×10^3
Human	3.3×10^4
Human	6.9×10^4

[a] Unidentified rodent pellets collected from mountain meadows.

tool for further studies with *Giardia* spp. and other similar organisms. The method also may have application in the analysis of Orlon filters used in the detection of *Giardia* cysts in water samples (3, 5).

LITERATURE CITED

1. **Chang, S. L., and P. W. Kabler.** 1956. Detection of cysts of *Endamoeba histolytica* in tap water by the use of membrane filtration. Am. J. Hyg. **64**:170–180.
2. **Greenberg, A., et al. (ed).** 1981. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
3. **Jakubowski, W., and T. H. Erickson.** 1979. Methods for detection of *Giardia* cysts in water supplies, p. 193–210. *In* W. Jakubowski and J. C. Hoff (ed.), Waterborne transmission of giardiasis. EPA-600/9-79-001. Environmental Protection Agency, Cincinnati.
4. **Moore, G. T., W. M. Cross, D. Mcquire, C. S. Mollohan, N. N. Gleason, G. R. Healy, and L. H. Newton.** 1969. Epidemic giardiasis at a ski resort. N. Engl. J. Med. **281**:402–407.
5. **Schaefer, F. W., and E. W. Rice.** 1982. *Giardia* methodology for water supply analysis, p. 143–147. *In* Proceedings of the American Water Works Association Water Technology Conference. American Water Works Association, Seattle.