A Research Note

Concentration of *Giardia* Cysts from Water by a Centrifugal Cream Separator

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**ABSTRACT**

A method utilizing centrifugal separation followed by membrane filtration recovered 31 to 61% of *Giardia* cysts from 38-L samples of water.

*Giardia lamblia* was the most commonly identified cause of water-related disease in the United States from 1972 to 1977 (2). *G. lamblia* has now been identified as a causative agent of waterborne, foodborne and sexually transmitted infectious diarrhea (8).

The causative agent of giardiasis is a flagellate protozoan (4). This is shed in the feces of man and animals, most often in the cyst stage, although with cases of severe watery diarrhea the fragile trophozoite reproductive stage may be shed.

As few as ten cysts ingested in capsules have been found to be infective for humans and it is possible that fewer viable cysts ingested in drinking water may be sufficient to initiate infection. An infected individual may shed more than 10^6 cysts per gram of stool. Cysts may survive for as long as 2 months in drinking water at 8°C. Therefore, fecal contamination of water supplies by infected persons could lead to waterborne transmission of giardiasis (4).

According to the tentative filtration method described in *Standard Methods for Examination of Water and Wastewater* (4), the volume of water to be sampled depends on the intent of the investigation; a minimum sample size of 380 L is suggested.

Holman et al. (5) filtered large samples of water and then found processing of the filter extract by the algal centrifuge was superior to membrane filtration in recovering *Giardia* cysts.

**MATERIALS AND METHODS**

Human fecal sample (0.2 ml) preserved with formaldehyde and containing 550,000 *Giardia* cysts/ml (or 110,000 cysts/0.2 ml), the average of six hemacytometer counts, was added to 38 L of “city” water and mixed. This mixture was processed through a gravity fed cream separator (4,530 rev/min; DeLaval Model 514) with a capacity of ca. 4 L/min. At the completion of separation, the liquid remaining in the separator bowl (ca. 300 ml) was poured rapidly into a glass jar and filtered through a 47-mm diameter membrane filter apparatus. The apparatus was assembled with a 45-μm pore screen (Tetko, Lancaster, NY; HD-3-45) at the top, a 30-μm pore screen (Tetko HC 3-30) in the middle, and a 5-μm pore membrane (Millipore, Bedford, MA; SMWP 0 4700) at the bottom. After filtration, the 45-μm screen was discarded. The lower surface of the 30-μm screen and the upper surface of the 5-μm membrane were exposed for rinsing on the interior wall of a 100-ml beaker. Rinsing was accomplished with 1 ml of distilled water and a Pasteur pipette equipped with a rubber bulb. Both the membrane and the screen were rinsed 25 times each. Six hemacytometer counts of the unstained *Giardia* cysts in this liquid were averaged. This average represented the number of *Giardia* cysts recovered from the liquid retained in the separator bowl when concentrated to 1 ml. The hemacytometer was used according to the method described in Linne and Ringsrud (6), except that all 0.04-mm squares were counted.
The separator bowl and its 30 plates were disassembled and rinsed piece by piece with ca. 500 ml of distilled water dispensed from a plastic squirt bottle. This rinse liquid was processed through a second membrane filter apparatus assembled as described previously. Rinsing of the 5-μm membrane and 30-μm screen, counting and obtaining the average of cysts recovered were done as described above.

The sum of the average recoveries from the two rinses gave the total number of *Giardia* cysts recovered for one trial when a 38-L water sample was concentrated to 2 ml. These trials determined that approximately half of the cysts were recovered from each of the two rinses.

**RESULTS AND DISCUSSION**

The data obtained from seven trials are shown in Table 1. The recovery of cysts varied from 30 to 61%, or approximately 0.82 to 1.8 cysts recovered of 2.9 cysts/ml inoculated into the 38-L water sample. The data suggest that centrifugal cream separators are useful in concentrating *Giardia* cysts in water samples. Centrifugal clarifiers, designed to remove sediment from milk, also merit consideration.

One factor contributing to the variation in cyst recovery was the apparent imprecision of hemacytometer counts at this level of organisms. A second and significant factor contributing to inconsistence in cyst recovery and to total cyst recovery was the presence of varying amounts of ferric iron in the "city" water on the same day and on different days. Ferric iron accumulated on the 5-μm membrane and hindered filtration of the sample. Recovery of cysts appeared to be inversely related to the amount of ferric iron in the water.

Further studies are needed to identify improved techniques to avoid the interference of ferric iron in recovering cysts from the separated samples. This might also permit the concentration of larger samples of water. One possibility is to substitute the extract concentration method for *Giardia* described in *Standard Methods for Examination of Water and Wastewater* (4) for the membrane filtration technique employed in this study.

**TABLE 1. Recovery of 110,000 *Giardia* cysts from 38-L water samples.**

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Number</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43,000</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>40,000</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>68,000</td>
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<tr>
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<td>51,000</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>34,000</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>53,000</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
<td>53,000</td>
<td>48</td>
</tr>
</tbody>
</table>

*Numbers of cysts added and recovered are averages of six hemacytometer counts.*

**ACKNOWLEDGMENTS**

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**REFERENCES**


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