Efficiency of sand filtration for removing cryptosporidium oocysts from water

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Summary. Purified oocysts of Cryptosporidium were applied to the top of a sand filter which had been constructed in the laboratory. The filter was eluted with distilled water; fractions were collected and examined for Cryptosporidium by modified Ziehl-Neelsen technique and immunofluorescence microscopy and by an enzyme immunoassay. The results indicate that oocysts of Cryptosporidium do not easily pass through the sand filter, and that some disintegration of oocysts may occur during filtration.

Introduction

Transmission of Cryptosporidium, an important cause of diarrhoeal disease, from animals to man by contamination of drinking water supplies has been a recent cause for concern to public health authorities. Spread of Cryptosporidium by bathing in a contaminated municipal swimming pool has also been reported (Dr P. Fenton, personal communication). Cryptosporidium oocysts have been detected in water which has been adequately treated to remove bacterial and viral pathogens, and some workers have doubted the ability of sand filtration to remove oocysts effectively. The aim of this study was to assess the efficiency of sand filtration for the removal of cryptosporidium oocysts from water.

Materials and methods

Sand filter

Sand from a swimming pool filter was washed for 24 h in each of three changes of detergent solution 7X (Flow Laboratories) 1% v/v, and then in running tap water until the wash water was clear. A slurry of the washed sand and water was used to pack a 1000 x 26-mm glass chromatography column (Pharmacia/LKB) to a depth of 800 mm. A sample of sand was reserved for later examination for oocysts of Cryptosporidium. The flow rate through this filter was maintained at 1.33 ml/min throughout the experiment with a peristaltic pump (Pharmacia LKB P1) controlled by a fraction collector (Pharmacia LKB Frac 100). The void volume (Vo) of the column was determined with dextran blue (Pharmacia/LKB) as a marker. The sand column was equilibrated with 5 Vo of distilled water, and four 25-ml fractions (fractions 1–4) were collected and examined later for Cryptosporidium.

Cryptosporidium oocysts

A concentrated suspension of cryptosporidium oocysts was harvested from a human faecal sample by sucrose flotation and purified by Percoll (Pharmacia LKB) discontinuous density gradient centrifugation. Purified oocysts were then washed three times in phosphate-buffered saline, pH 7.2 (PBS) and resuspended to a concentration of 10⁶ oocysts/ml, a portion of this suspension being retained for later examination by microscopy and by enzyme immunoassay (EIA) with monoclonal antibody against oocyst antigens.

Filtration

A 1-ml volume of the oocyst suspension was applied to the top of the sand filter, during continuous elution with distilled water, by means of a 3-way chromatography valve (Pharmacia LKB). One Vo (c. 160 ml) was run to waste, and 160 fractions (fractions 5–164) of 25 ml each were then collected.

Post-filtration examination of sand

After completion of the filtration process, the sand was aspirated from the column in eight portions of 100 mm, numbered consecutively from the top of the column.

Examination of filtrate fractions. The 25-ml fractions were centrifuged at 3000 g at 4°C for 20 min, and the deposit was resuspended in 0.5 ml of PBS and examined by microscopy and EIA.

Examination of sand samples. The pre-filtration sample of sand and the eight samples removed after filtration...
were examined as follows: the sample was added to 100 ml of PBS with Tween 20 1% v/v in a plastic jar, and shaken vigorously for 5 min; the sand was allowed to settle for 5 min, and the supernate was centrifuged at 3000 g at 4°C for 20 min; the centrifuged deposit was resuspended in 1 ml of PBS and examined by microscopy and EIA.\textsuperscript{8}

### Results

Oocysts were readily demonstrated by microscopy and EIA in the purified oocyst suspension and in the first three samples of sand from the top of the filter after filtration (table). None was detected in the sand from which the filter was prepared, nor in the 164 fractions of filtrate taken before (1–4) or after (5–164) the oocyst suspension was added. The EIA gave positive results, although microscopy was negative, with sand samples 4–7 from the lower part of the column after filtration; neither test gave positive results with the lowest sand sample (no. 8).

### Discussion

Outbreaks of cryptosporidiosis associated with contaminated drinking water supplies have been a recent cause for concern to public health authorities.\textsuperscript{1–4} Cryptosporidium oocysts are resistant to many disinfectants,\textsuperscript{9} including chlorine at the level adopted because it is effective against bacterial and viral pathogens that may be present in drinking water. Therefore prevention of waterborne spread of Cryptosporidium relies on satisfactory filtration; but some workers\textsuperscript{2,10} have doubted the efficacy of sand filtration.

A recent community outbreak of cryptosporidiosis was associated with a municipal swimming pool. Investigations at the time detected Cryptosporidium oocysts in the swimming pool water\textsuperscript{8} and the efficacy of sand filtration for the removal of oocysts was questioned.

In modern swimming pools, filtration rates of 5–20 m\textsuperscript{3} of water/m\textsuperscript{2} of filter surface area/h are used.\textsuperscript{11} Therefore, we chose a filtration rate of 15 m\textsuperscript{3}/m\textsuperscript{2}/h for our experiment, and a high challenge dose of oocysts. Our results show that this type of filtration should be effective: despite elution of the sand filter with >20 Vo, oocysts could not be detected in the eluate. Post-filtration examination of the sand showed that the oocysts had been contained within the top 300 mm. The next 400 mm gave positive results in an EIA for Cryptosporidium, but did not contain whole oocysts demonstrable by microscopy, probably because of disintegration of oocysts as they passed through the sand. The EIA has been shown to react with disintegrated oocysts, and some water samples and sand samples have given positive results with the EIA although negative by microscopy.\textsuperscript{8}

However, assuming 100% efficiency of filtration and perfect circulation of water within a swimming pool, as many as 10 pool-turnover periods (the time taken to circulate one pool volume through the water treatment system) may be necessary to remove almost all particulate matter.\textsuperscript{11}

Giardia lamblia, another protozoan parasite, has also been implicated in a swimming-pool-associated outbreak of diarrhoeal disease;\textsuperscript{12} like Cryptosporidium, G. lamblia is very resistant to chlorine.\textsuperscript{13} Ozonation of water is effective in destroying both G. lamblia\textsuperscript{14} and Cryptosporidium.\textsuperscript{15} However, ozonation plants are costly to install and maintain;\textsuperscript{11} therefore, their use is not widespread. While chlorination remains the generally preferred method of chemical disinfection, the risk of swimming-pool-associated cryptosporidiosis or giardiasis can be reduced only by adequate filtration and short turnover periods for the pool water.

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### REFERENCES


