FULL PAPER ***Parasitology***

Removal Effect of the Water purifier for Home Use against *Cryptosporidium parvum* Oocysts

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ABSTRACT. The removal effects of the faucet mounted type water purifier for home use were examined against *Cryptosporidium parvum* oocysts. The water purifier is composed of a layer of granular activated carbon and the hollow fiber membrane filter. The cartridges were unused, 25%, 50% and 75% flow down by Arizona-dust of U. S. A. Two respective cartridges were used of the examination. The faucet and the water purifier were connected by anti-pressure tube, and 3.0×10^7 oocysts of *Cryptosporidium parvum* were injected into anti-pressure tube while water was running. Twenty liter of collected purified water was examined under the fluorescent microscope. Any oocysts in the purified water collected from all cartridges were not found. Therefore, we considered this purifier as an effective one in removing *Cryptosporidium* oocysts from drinking water.

KEY WORDS: *Cryptosporidium oocyst*, removal effect, water purifier.

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Cryptosporidiosis occurred in immunocompromised patients as one of the opportunistic diseases [5] and among veterinarians and students via infected cattle as a zoonosis [3] in 1980s. After that, a waterborne outbreak occurred by *Cryptosporidium* in man [6]. In Japan, the waterborne outbreak with *Cryptosporidium* parvum occurred and about 9,000 people were infected in the small town of Ogose-cho near by Tokyo in 1996 [7]. Recently, Fujino *et al.*, reported that the hot-water heater were useful for disinfection of contaminated cowsheds, sheepfolds and chicken houses with *Cryptosporidium* because the infectivity of cryptosporidian oocysts was lost after exposure at 70°C for 5 sec [2]. However, we were not able to find any practical way for removing oocysts from community drinking water. Therefore, the removal effect of the water purifier for home-use against *Cryptosporidium* oocysts was examined.

MATERIALS AND METHODS

Water Purifier for home use: The faucet mounted type water purifier was used. It is composed of two parts, one is the body for setting a flow to drinking and purified water, and the other part is the cartridge for filtration of potable water to purified water. The cartridge is composed of granular activated carbon layer and hollow fiber membrane filter with multi layer of pores of 0.1 μm unit. Water passed through the layer of granular activated carbon, and then through the hollow fiber membrane filter with multi layer is purified as clean water (Fig. 1). The standard for this purifier is as follows: a) the flow is 3 liter/min, b) the stagnation of water is 90 m*l* and c) the ability of chlorine and turbidity reduction is 1,500 liter. The cartridges used were unused cartridges (No. 1, 2), 1,500 liter of water through cartridges (No. 3, 4), 25% flow down cartridges by Arizona-dust of U.S.A (No. 5, 6), 50% flow down cartridges (No. 7, 8) and 75% flow down cartridges (Nos. 9, 10). Two respective cartridges were used of the examination. Arizona-dust of U.S.A. were obtained from a commercial source.

Cryptosporidian oocysts: *C. parvum SC*1 strain used in this study was first isolated from the feces of naturally infected siberian chipmunks purchased from commercial sources [4]. It was maintained by passage in the SCID mice. The oocysts were collected by the sugar flotation method (sp. gr. of sugar, 1.15) and used within 1 month.

Experimental procedure: The experimental procedure was shown in Fig. 2. Y-shaped anti-pressure tube was

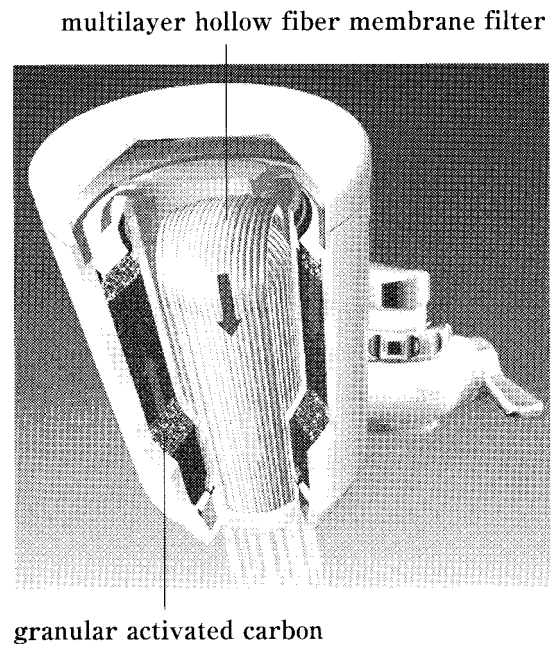

Fig. 1. The formation of the water purifier for home use.

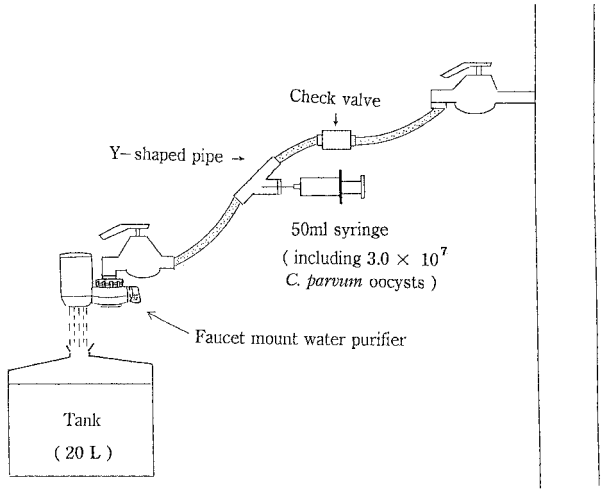

Fig. 2. The experimental procedure.

mounted to a faucet and one of the other tube ends to a faucet with the water purifier. After 1,000 m*l* of water pass through the cartridge, 50 m*l* water including 3.0×10^7 oocysts were injected from the other end of the Y-shaped tube while water was running. Continuing this process 20 liter of purified water was collected. In addition, 20 liter of purified water pass through the new cartrige were contained 5.5×10^1 oocysts as oocyst positive controls (No. 11, 12). Procedures for each cartridge are same as the above.

Each purified water was through the Millipore Filter with 1.2 μm porosity by aspiration. They were dissolved by acetone and centrifuged for 10 min by 2,500 rpm. After the top clear layer was aspirated, acetone was added and centrifuged. The top clear layer was aspirated again, and 95% ethyl alcohol and water were added, and this process of centrifugation and aspiration was repeated. Hydrophilicity Polytetrafluoroethylene membrane filter was used as a sample filter with 1.2 μm porosity, and drew a circle on it by hydrophobic ink. It was on the filter holder of aspirator with a same size support filter, and the sediment of centrifugation was placed inside the circle by aspiration. After rinsing with PBS, 1% BSA and 10% Normal Goat Serum, the filters were moved with sample on a slide glass. They were incubated with the primary antibody and the labeling reagent of fluorescent (Ensys Inc U. S. A.) for 25 min each. Then sample filter was placed on a slide glass for mounting with 1,4-diazabiccyclo-2,2,2-octane-Glycerol medium, and examined using fluorescent microscope. When Oval body of 3.5 ~6.5 μm size was found with strong green apple color periphery, phase contrast is used to identify the presence of *Cryptosporidium* oocysts. The water materials were made in Department of Infectious Diseases, Kyorin University, and the oocysts were examined in Research and Study Department, Japan Association of Parasite Control under the blind numbers.

Table 1. Removal effect of the water purifier for home use against *Cryptosporidium parvum* oocysts

Cartriges condition	No.	Detection of oocysts
Unused	1	–
	2	–
1,500 l of water through	3	–
	4	–
25% flow down by Arizona dust	5	–
	6	–
50% flow down by Arizona dust	7	–
	8	–
75% flow doun by Arizona dust	9	–
	10	–
Oocyst positive control	11	+
	12	+

–: No oocysts were detected.
+: Oocysts were detected.

RESULTS AND DISCUSSION

The results were shown in Table. 1. Any oocysts were not detected in the purified water from all cartridges, such as unused cartridges (No. 1, 2), 1,500 liter water through cartridges (No. 3, 4), using Arizona-dust of U.S.A. 25% flow down cartridges (No. 5, 6), 50% flow down cartridges (No. 7, 8) and 75% flow down cartridges (No. 9, 10). The standard on a water purifier for *Cryptosporidium* oocysts is absent in Japan. National Sanitation Foundation made the water purifier standard related to removal of *Cryptosporidium* oocysts in the U.S.A. [1]. In this standard, the water purifiers are 25%, 50% and 75% flow down using 10 Nephelomotric Turbidity Unit water with Arizona-dust of U.S.A. The standard acceptance line is over 99.95% of removal rate for the water which contain *Cryptosporidium* oocysts over 5×10^4/liter. The standard was utilized for the present experiments. If number of detected *Cryptosporidium* oocysts are under 3.0×10^4, the water purifier used in the present experiment was acceptable one, because 3.0×10^7 *Cryptosporidium* oocysts were contained in water used for the present experiments. The oocysts were not detected in the purified water from all cartridges. It's generally known that disinfections commonly used for water purification are not completely effective in removing or inactivating oocysts [6]. Therefore, the present purifier was considered effective in removing *Cryptosporidium* oocysts from drinking water and useful safeguard of outbreaks of human and cattle.

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