Efficacy of a small-quantity water treatment method for inactivating *Giardia* cysts in raw water

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Abstract

CHLORFLOC (Control Chemicals, Bramley, RSA) is a commercial product developed for clarifying and disinfecting small quantities of raw water. It consists of a mixture of sodium dichloro-S-triazinetrione dihydrate as a source of chlorine for disinfection and a proprietary flocculant to clarify raw water. We investigated the efficacy of the disinfecting process with regard to *Giardia muris* cysts. The ability of viable *G. muris* trophozoites to excyst under defined conditions was used as the criterion for viability before and after treatment. Experiments were performed using artificially polluted water at 25°, 15°, 10° and 5°C. In each of these temperature regimens the time and dosage required to effect a 99,9% reduction in the number of viable cysts was determined. At 25°C one tablet/ℓ was effective after 7 min, whereas at 15°C and 10°C the exposure time had to be extended to 15 min. At 5°C, two tablets/ℓ and a 15 min exposure time were required.

Introduction

The CHLORFLOC method is essentially a small-quantity water treatment method. It is primarily intended as a disinfection and clarification method to be used with raw untreated water. It is therefore aimed at rural areas where water users are reliant on natural water. It can also be used in military and recreational situations where water of variable quality is often encountered.

This study was concerned with the ability of the product to inactivate cysts of *Giardia* spp. The effectiveness of the flocculation and filtration system for removing parasitic ova and cysts has been investigated separately (unpublished data) while the efficacy of the product to disinfect water containing bacteria and viruses has been investigated by Kfir *et al.* (1989).

Outbreaks of water-borne giardiasis are well documented with the frequent implication of a spring, borehole or recreational water as the source of infection (Craun, 1986; Moore *et al.*, 1969 and Porter *et al.*, 1988). In a report on water-related disease outbreaks in humans, the Centre for Disease Control, Atlanta, USA reported *Giardia lamblia* as the most frequently identified pathogen for seven consecutive years (St Louis, 1988); they reported three outbreaks associated with the drinking of chlorinated but unfiltered water and two outbreaks associated with swimming pools.

Water-borne transmission of giardiasis has also been indicated in non-outbreak related cases. Birkhead and Vogt (1989) studied 1 211 isolated cases and found a higher infection rate in areas where people were receiving natural or unfiltered municipal water.

In southern Africa with its vast expanse of under-developed rural areas, the aetiological importance of giardiasis has been inadequately investigated, one of the possible reasons being the high prevalence of other more debilitating water-borne diseases (Sinclair *et al.*, 1982). The authors recently found 13% of young schoolchildren in a rural school near Durban to be infected with *G. lamblia* (unpublished data). Schutte *et al.* (1977; 1987) reported prevalences of 15,4% amongst schoolchildren aged 7 to 20 at Adams Mission near Durban and 4% in schoolchildren of the Eastern Caprivi.

The American Environmental Protection Agency (EPA) (April, 1986) provided guidelines for minimum performance standards of acceptable water treatment methods, the relevant specification here being that there should be a 99,9% reduction in viable *Giardia* cysts from an initial cyst load of 1×10^6/ℓ. *Giardia* cysts are selected as the "cyst challenge representative because of its widespread disease impact" and also its well documented resistance to water treatment disinfectants at commonly used concentrations (Leahy *et al.*, 1987; Hale *et al.*, 1985). Furthermore, *Giardia muris* (a murine parasite) has been found to be an acceptable model for inactivation studies on *G. lamblia* on account of its slightly greater resistance to disinfection agents (Wickramanayake *et al.*, 1984). In our experience *G. lamblia* cysts obtained from donors produce variable excystment rates and unless cysts with extremely high excystment rates are used for disinfection studies, unacceptably variable results are obtained.

Materials and methods

CHLORFLOC was supplied in 600 mg tablet form by Control Chemicals, Bramley, Transvaal. They were supplied in packs of 10 tablets individually sealed in a weatherproof, foil covered blister pack. The tablets are described as containing 15 mg sodium dichloro-S-triazinetrione dihydrate as chlorine-based disinfectant and a proprietary flocculant. To use the product the following procedure is prescribed by the manufacturer:

- Add 1 tablet to one litre of water
- Shake for 1 min
- Swirl for a few seconds
- Allow to settle for 4 min
- Swirl for 10 s, leave standing for 1 min and filter through a flannelette sock supplied, into a clean container
- Should the water still be murky, the process is repeated with another tablet.

G. muris cysts were obtained from Prof E A Meyer of the Dept of Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon, USA. These cysts were used to infect inbred BALB/c mice and repeat infections were carried out at 21 d intervals. Cysts were harvested from fresh faeces of infected mice using 1M sucrose as described by Roberts-Thomson *et al.* (1976).

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Pooled and washed cysts were stored in aged tap water at 4°C. Prior to use, cysts numbers were assessed and adjusted by means of a haemocytometer. Only cysts with excystment rates >90% were used.

Standard test water was prepared according to the EPA (April, 1986) minimum standard for stress testing of halogen disinfectants as follows:

Humic acid, 12 mg/ℓ of water (Fluka, Switzerland)
Total dissolved solids: 1 560 mg/ℓ
Turbidity: 38 NTU made up with Bentonite powder (aluminium silicate)
pH adjusted to 9,0 with 1M NaOH

All treatment experiments were performed using this water in narrow-necked, capped bottles that had been presoaked in a dilute chlorine solution followed by rinsing in warm water.

Excystment solutions

Induction medium

A modified induction medium based on that described by Schaefer *et al.* (1984) was used. Twenty-five mℓ of Hanks solution was made up to 100 mℓ with distilled water, autoclaved for 10 min at 103,43 kPa, rapidly cooled and 0,40 g L-cysteine hydrochloride and 0,42 g sodium bicarbonate added. The pH was adjusted to 2,0 with hydrochloric acid.

Our modifications of the method of Schaefer *et al.* (1984) entailed autoclaving the dilute Hanks solution to dispel excess oxygen and increasing the concentration of L-cysteine hydrochloride and sodium bicarbonate to ensure that the medium was optimally reduced.

Excystment medium

A modified medium based on HSP-1 medium, first described by Meyer (1976), was used. Forty mℓ of Hanks solution was made up to 100 mℓ with distilled water, 1 g trypticase (BBL) and 0,5 g glucose added, and autoclaved for 10 min at 103,43 kPa, rapidly cooled and the following added:

0,4 g L-cysteine hydrochloride
0,4 mℓ of a 0,025% resazurin solution
0,25 g trypsin
15 mℓ decomplemented horse serum

The pH was adjusted to 7,5 with 1 M NaOH and the medium distributed in 3 mℓ aliquots in glass tubes, overlayed with liquid paraffin and incubated at 37°C until the resazurin had been reduced to its colourless state. Excess medium was used for washing the cysts after the induction step.

This medium differs from the HSP-1 culture medium described by Meyer (1976) in that no antibiotics were added and an increased amount of L-cysteine hydrochloride was added. In addition resazurin was used as an indicator of reduction in the medium.

Excystment method

A modified procedure based on that described by Bingham and Meyer (1979) and Schaefer *et al.* (1984) was used. Excystment was induced by adding freshly prepared induction medium to a pellet of cysts, the tube was filled to the top, capped and incubated at 37°C for 20 min. Thereafter the tube was centrifuged at 800 g for 3 min, the supernatant aspirated and discarded; the tube was filled with excystment medium, centrifuged and the supernatant removed. The resultant cyst pellet was then transferred by means of a Pasteur pipette to the pre-incubated aliquots of excystment medium and allowed to incubate for 30 min at 37°C. Thereafter the tubes were centrifuged at 800 g for 3 min, followed by aspiration of the liquid paraffin and supernatant to waste. The pellet was then resuspended in a small volume of excystment medium and the volume adjusted so as to obtain suitable numbers for counting. A drop of the suspension was then transferred to a slide and covered with a coverslip. A compound microscope with 400x magnification was used for counting cysts and trophozoites.

Since each cyst produces a trophozoite which almost immediately divides into two, a distinction had to be made between intact cysts, excysted trophozoites and excysted, separated trophozoites, when counting. Numbers were adjusted and expressed as the total number of 'undivided' trophozoites and intact cysts. In contrast with observations by other workers (Schaefer *et al.,* 1984), who allowed encystment to take place in a depression slide sealed with a coverslip and vaseline, we found that empty cyst shells often disintegrated or dissolved and were then not readily recognisable. This was possibly due to the action of the product being tested, but more likely to the excystment method used by us. We preferred to let excystment take place in controlled conditions in a test-tube prior to assessing excystment levels. This allowed us to adjust for optimal trophozoite numbers per microscopic field.

Water treatment method

A 500 mℓ water sample was allowed to stabilise at the required temperature using a cooling/heating waterbath (± 1°C); this was seeded with *G. muris* cysts and adjusted to a final concentration of 3 000 cysts/mℓ. The water was then treated according to the instructions described above; the filtration step was omitted, since filtration efficiency was assessed separately. The action of the chlorine was stopped at 7 or 15 min by the rapid addition of 0,1M sodium thiosulphate.

The sample was then centrifuged at 800 g for 5 min and the supernatant discarded. The sludge deposit was broken up with the aid of a vortex mixer and subjected to zinc sulphate flotation (Faust *et al.,* 1974). The floating cysts were recovered by aspiration and washed three times in aged tap water by centrifugation and aspiration. The final cyst pellet was subsequently subjected to the excystment procedure described above.

A treatment control was included each time by first adding the CHLORFLOC tablet, then neutralising the chlorine with sodium thiosulphate before adding the *G. muris* cysts. A further control to determine the baseline excystment value was included each time. This latter control consisted of a water sample seeded with cysts, but not treated with CHLORFLOC. All treatment experiments were performed using the stress test water described above. Each experiment was done three times.

Results

Titrations, using an iodometric method with sodium thiosulphate (Standards Methods, 1981), revealed a mean result of 4,7 mg/ℓ for available chlorine in the case of samples treated with 1 tablet/ℓ of stress test water. The mean pH of the treated water samples was 4,5. The dosage and time-related effects of CHLORFLOC on cysts of *G. muris* seeded into an artificial raw water at different temperatures, are summarised in Table 1.

Discussion

The flocculation and filtration action has been disregarded, since

the aim of this study was to establish the disinfection efficiency when dealing with water containing protozoan cysts. Outbreaks of giardiasis have been shown to have occurred in the presence of chlorine levels sufficient to prevent the spread of bacterial disease, the likely cause being inadequate filtration or disrupted supply (Braidech and Karlin, 1985; Kent *et al.,* 1988; Craun, 1986). In assessing a multi-user product that may be subjected to variable usage conditions, greater reliance has to be placed on the disinfecting capability rather than the filtration, to render the treated water safe.

Direct comparison with other *Giardia* disinfection studies is impossible, since the stress test water used has a relatively high chlorine demand and the disinfectant value of the combined chlorine cannot be discounted. It is nevertheless useful to control the results obtained by comparing them with those of disinfection studies done elsewhere.

Leahy *et al.* (1987) observed that, at a temperature of 25°C, a pH of 5 and a residual chlorine level of 4,31 mg/ℓ, an exposure time of 16,3 min was required for a 99% reduction in viable cysts. This compares favourably with the results obtained with CHLORFLOC at a similar pH and temperature: 100% cysts were killed in 7 min with an available chlorine level of 4,7 mg/ℓ. Kong *et al.* (1988), using an organic N-halamine compound, achieved total inactivation of cysts of *G. lamblia* and *G. canis* within 2 min at 22°C, a pH range of 4,5 to 9,5 and 5 mg total chlorine per litre.

Leahy *et al.* (1987) also found that at a temperature of 5°C, a pH of 7 and a chlorine level of 23,8 mg/ℓ, a 99% reduction of viable *G. muris* cysts was obtained in 42,6 min. Kong *et al.* (1988), using an organic N-halamine compound, observed that at 4°C, a pH range of 4,5 to 9,5 and a total chlorine concentration of 5 mg/ℓ, only 10 min were required (*G. lamblia* and *G. canis*). At this temperature, we found that CHLORFLOC treatment resulted in a 100% reduction of viable cysts when using a double treatment dose (2 tablets/ℓ) and an exposure time of 15 min.

Results of this study indicate that the CHLORFLOC treatment process is highly effective in disinfecting *Giardia* cysts in a high demand water over a wide range of temperatures. It would therefore seem to be an invaluable water treatment aid to rural communities as well as to the military forces and the weekend hiker.

TABLE 1
PERCENTAGE VIABLE AND NON-VIABLE *G. MURIS* CYSTS AFTER TREATMENT WITH CHLORFLOC AT VARYING TEMPERATURES, DOSAGE LEVELS AND EXPOSURE TIMES

	% Excysted trophozoites	% Non-viable intact cysts
25°C		
Baseline excystment control	92,2	7,8
Treatment control	84,7	15,3
CHLORFLOC 1 tab/ℓ 7 min	0	100,0
CHLORFLOC 1 tab/ℓ 15 min	0	100,0
15°C		
Baseline excystment control	95,4	4,6
Treatment control	81,4	18,6
CHLORFLOC 1 tab/ℓ 7 min	9,43	90,57
CHLORFLOC 1 tab/ℓ 15 min	0	100,0
10°C		
Baseline excystment control	97,3	2,7
Treatment control	79,6	20,4
CHLORFLOC 1 tab/ℓ 7 min	37,25	62,75
CHLORFLOC 1 tab/ℓ 15 min	0	100,0
5°C		
Baseline excystment control	93,05	6,95
Treatment control	84,79	15,21
CHLORFLOC 1 tab/ℓ 15 min	23,12	76,88
CHLORFLOC 2 tab/ℓ 15 min	0	100,0

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