Original article

Presence of *Acanthamoeba* spp. in water purification plants in southern England

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Abstract

Objective: To identify the prevalence of *Acanthamoeba* in drinking water treatment plants during the course of the purification processes. **Methods**: Samples were taken from two drinking water purification plants and monitored for the presence of *Acanthamoeba* in order to estimate the removal capacity of treatment methods employed. Water samples were collected at each step in the purification, during the one year survey, and analysed for the presence of *Acanthamoeba* spp. by plating on bacterial-seeded plates. **Results**: The results showed that amoebae were present in surface raw waters in 100 % of the samples tested. *Acanthamoeba* spp. were isolated from 71 % and 57 % of the water samples collected from post flat-bottom clarifier 1 and post-sedimentation plant respectively. Considering the outflow drinking waters, the removal capacity was 100 % in both purification plants monitored. The occurrence of *Acanthamoeba* was not associated with seasonality. **Conclusion**: These findings confirm that water purification plants employing methods of flocculation, sedimentation, and filtration in combination with activated charcoal filtration, ozonisation and chlorination exhibited sufficient *Acanthamoeba* removal capacity and the presence of amoebae in the tap water may be due to older plumbing, water storage tanks, tap water hygiene, and/or environmental settings.

Keywords: *Acanthamoeba*; Drinking water; Purification plant

INTRODUCTION

Acanthamoeba is a protozoan pathogen capable of producing serious and sometimes fatal human and animal infections[1-3]. *Acanthamoeba* is one of the most abundant protozoa in the environment that can remain viable for several years, under adverse environmental conditions, while maintaining their pathogenicity[4]. In addition, *Acanthamoeba* cysts are resistant to many disinfectants at the concentrations and exposure times commonly used[1-3], suggesting that *Acanthamoeba* represents a significant threat to public and animal health.

The ubiquitous nature of *Acanthamoeba* has been well documented, however, there are several theories of who gets this and how. For example, *Acanthamoeba* keratitis is frequently associated with the use of contact lenses and tap water[5,6]. In support, several lines of evidence have shown the presence of *Acanthamoeba* in domestic water supplies in the UK (up to 30 % of domestic water samples were positive for *Acanthamoeba* [6]), Korea (7.7 % of tap water samples contained *Acanthamoeba*[7]), Jamaica (36.1 %[8]), Tenerife, Spain (59.5 %[9]), USA (2.8 %[10]) and Hong Kong, where up to 10 % of tap

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water samples were found to be contaminated with *Acanthamoeba*[11]. The large variation in the presence of *Acanthamoeba* has been attributed to older plumbing, poorly maintained water storage tanks and the drinking water supplies. Noteworthy, it is suggested that higher prevalence of *Acanthamoeba* in UK tap water, in comparison to the USA, might explain the higher incidence of *Acanthamoeba* keratitis in the UK[6,10]. Here, we surveyed the presence of *Acanthamoeba* from two drinking water purification plants, differing in relation to the treatment methods employed, in order to estimate their removal capacity at various stages during the course of purification. In addition, we investigated a possible association between different seasons and the presence of *Acanthamoeba*.

MATERIALS AND METHODS

Two water purification plants in southern England were examined for the presence of *Acanthamoeba*, up to seven times during one year period. The source of water for purification plant A is surface water, whereas for purification plant B has input of both surface and ground water. The water samples were collected aseptically in 1L sterile screw cap bottles (Aurora Scientific, Bristol, UK). Samples were taken close behind each purification step with continuous water flow and processed within 24 hrs at the laboratory. Both purification plants used methods of flocculation, sedimentation, filtration, ozonisation, granular activated charcoal filtration, and chlorination (Table 1).

The water samples were analysed aseptically using sterile filter units with 47 mm diameter gridded cellulose nitrate membrane filters with 0.45 μm pore size (Sartorius Biotech GmbH, Goettingen, Germany). The filter membrane was cut into quarters and placed on to the non-nutrient agar plates seeded with Gram negative bacterial lawn (i.e., *Escherichia coli* K-12 strain HB101). The plates were incubated at 30℃ in a humidified incubator and observed for the presence of *Acanthamoeba* spp. trophozoites and cysts daily for up to 14 days using a light microscope. For positive control, approx. 10 000 amoebae were added to sterile distilled water in a 1L bottle and processed as above. The identity of *Acanthamoeba* was further confirmed using polymerase chain reaction (PCR) as previously described[12].

RESULTS

Acanthamoeba spp. were isolated from all samples taken from the river Thames that entered purification plant at water purification plant A (Table 1). The identity of *Acanthamoeba* was confirmed with PCR methods using genus-specific primers (data not shown). In addition to samples from the river, 100 % of water samples were positive for *Acanthamoeba* spp. taken from lakes, as well as at the post pre-ozonisation step (Table 1). *Acanthamoeba* spp. were isolated from 71 % and 57 % of the water samples collected from post flat-bottom clarifier 1 and post-sedimentation plant respectively. However, none of the water samples taken from post-intermediate ozonisation and post-rapid gravity filters containing sand and anthracite as well as later stages of the treatment showed any presence of *Acanthamoeba* spp. (Table 1). For water purification plant B, *Acanthamoeba* spp. were isolated from 100 % of the water samples taken from the river (Table 1). Up to 57 % of the water samples taken from the settling reservoirs were positive for the presence of *Acanthamoeba*. In addition, *Acanthamoeba* spp. were isolated from 29 % of the water samples collected from pre- and post-rapid gravity filters (Table 1). Later purification steps completely eliminated *Acanthamoeba* spp. from water samples. Of note, we did not observe any seasonal dependence in the prevalence of *Acanthamoeba* and amoebae could be isolated from the surface waters throughout the year (data not shown).

Table 1 Distribution of *Acanthamoeba* in the course of water purification at water treatment plants in southern England.

	% of samples positive for *Acanthamoeba* spp.	
	Plant A	Plant B
River	100	100
Raw (from lakes)	100	57
Post pre-ozone	100	29
Post-flat bottom clarifier 1	71	29
Post-sedimentation plant	57	0
Post rapid gravity filter and intermediate ozonisation	0	0
Post-carbon filters (activated charcoal)	0	0
Post-chlorination	0	0
Final treated water	0	0

DISCUSSION

For the first time in the UK, two drinking water purification plants were monitored for the presence of *Acanthamoeba* in order to estimate the removal capacity of treatment methods employed. Our findings revealed that *Acanthamoeba* is prevalent in 100 % of surface water samples confirming further that *Acanthamoeba* is widely distributed in the environment. Prevalence of *Acanthamoeba* in the course of the purification-process demonstrated that none of the water samples taken from post-intermediate ozonisation and post-rapid gravity filters containing sand and anthracite as well as later stages of the treatment showed any presence of *Acanthamoeba* spp. This is in contrast to previous findings of Hoffmann and Michel[13], who examined water purification plants in Germany and showed the presence of *Acanthamoeba* spp. post-filtration and even in the purified water, which may be due to difference in parasite density, sample volume, or treatment plant. However, for both purification plants, our findings that the purified water did not contain *Acanthamoeba* spp. are consistent with previous observations in Korea[7]. In conclusion, these findings confirm that the aforementioned water purification plants employing methods of flocculation, sedimentation, and filtration in combination with activated charcoal filtration, ozonisation and chlorination exhibited sufficient *Acanthamoeba* removal capacity. The fact that amoebae have been isolated from the tap water is mostly likely due to older plumbing, water storage tanks, tap water hygiene, and/or environmental settings and these issues will be addressed in the future studies.

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