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The role of domestic tap water in *Acanthamoeba* contamination in contact lens storage cases in Korea

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Abstract: A survey was carried out from August to December 2004 in Pusan, Korea to document the presence of free-living amoeba (FLA), including the genus *Acanthamoeba*, in both contact lens storage cases and domestic tap water. *Acanthamoeba* was isolated from 5 (4.2%) in 120 contact lens storage cases. Four house tap water samples from residents, whose contact lens storage cases had been contaminated by *Acanthamoeba*, were also found to be contaminated with *Acanthamoeba*. Therefore, the contamination rate of FLA and *Acanthamoeba* in domestic tap water was investigated in order to examine the role of domestic tap water in *Acanthamoeba* contamination of contact lens storage cases. FLA and *Acanthamoeba* were identified in 97 (46.8%) and 16 (7.7%) of the 207 domestic tap water samples, respectively. There were no significant differences between the contamination rates of FLA in tap water according to the filtration plant of origin. No FLA was detected in the tap water directly supplied by the water purification plants. Water storage tanks appear to promote FLA colonization, including *Acanthamoeba*, in domestic tap water. This increases the risk of *Acanthamoeba* contamination in contact lens storage cases as well as increasing the risk of *Acanthamoeba* keratitis.

Key words: *Acanthamoeba*, free living amoeba, contact lens storage cases, domestic tap water

INTRODUCTION

Acanthamoeba spp. are amphizoic protozoan parasites that are found in the environment as well as in tap water, contact lens cases, soil, dust, and air (Mergeryan, 1991; Armstrong, 2000). These amoebae are the causative agents of amoebic keratitis (AK), pneumonitis, and granulomatous amoebic encephalitis in immunocompromised hosts (Sell et al., 1997; Niederkorn et al., 1999; Rivera and Padya, 2002). AK was firstly reported by Nagington et al. (1974) in Great Britain and by Jones et al. (1975) in the United States. An AK epidemic occurred in the mid-1980s, which was attributed to the increased use of contact lenses and poor lens hygiene (Seal and Hay, 1993, 1994). Recent studies have shown that the rate of *Acanthamoeba* contamination in contact lens storage cases in Korea was 10.6-15.7% (Lee et al., 1997; Yu et al., 2001; Kong et al., 2002), which is a much higher rate than that reported in the west. Poor hygiene practices, such as the rinsing and storing of lenses in non-sterile saline or tap water are the main risk factors for lens case contamination (Stehr-Green et al., 1987; Seal et al., 1992). Prior to this report, there was no study on the source of *Acanthamoeba* contamination in contact lens storage cases in Korea. This study investigated the prevalence of *Acanthamoeba* in contact lens storage cases and domestic tap water in the Pusan province in an attempt to explain the source of *Acanthamoeba* cont-

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Table 1. Prevalence of *Acanthamoeba* and FLA contamination in domestic tap water samples

Filtration plants	Survey area in Pusan	No. of contaminated samples with			
		Acanth.[a)]	FLA[b)]	Acanth. + FLA[c)]	Total
Hwamyoung/ Myoungjang	Buk-gu (6)[d)]	0	2	0	2
	Dongrae-gu (6)	0	2	0	2
	Gumjung-gu (6)	1	3	0	4
	Haeundae-gu (7)	0	2	0	2
	Yeonje-gu (7)	0	3	0	3
	Subtotal (32)	1 (3.1%)	12 (37.5)	0 (0.0%)	13 (40.6%)
Duksan	Busanjin-gu (7)	0	0	0	0
	Dong-gu (4)	0	3	0	3
	Jung-gu (5)	0	4	0	4
	Nam-gu (15)	0	5	0	5
	Saha-gu (26)	1	8	0	9
	Sasang-gu (3)	0	2	0	2
	Seo-gu (108)	9	45	4	58
	Youngdo-gu (7)	1	2	0	3
	Subtotal (175)	11 (6.3%)	69 (39.4%)	4 (2.3%)	84 (48.0%)
	Total (207)	12 (5.8%)	81 (39.1%)	4 (1.9%)	97 (46.9%)

a) *Acanthamoeba* spp.
b) free living amoeba except *Acanthamoeba*
c) *Acanthamoeba* and free living amoeba double contaminations
d) Number of samples

amination in contact lens storage cases in Korea.

MATERIALS AND METHODS

Acanthamoeba isolation from contact lens storage cases

Acanthamoeba was isolated from 120 contact lens storage cases from students residing in Pusan according to the method described by Lee et al. (1997).

Tap water sample collection

A total of 207 domestic tap water samples were collected from the resident student houses in the Pusan province from August to December 2004. Six water samples were colleted from 3 water purification plants (Duksan, Hwamyoung, Myoungjang), which supplied tap water to the survey area, at each filtration step.

Acanthamoeba and FLA isolation

One liter of each water sample was filtered through a 5.0 μm pore size cellulose filter (Milipore, Bedford, Madison, USA) under a weak vacuum. The filters were inverted on heat inactivated *E. coli* treated 1.5% non-nutrient agar plates and incubated at 25°C. After 3-4 days, the plates were monitored microscopically for *Acanthamoeba* and FLA outgrowth. The genus *Acanthamoeba* was identified from the FLA based on its distinctive features of trophozoites and cysts, particularly the double-walled cyst shape.

RESULTS

Acanthamoeba in contact lens cases in the Pusan area

In this study, *Acanthamoeba* was isolated from 5 (4.2%) in 120 contact lens storage cases, and four house tap water samples from residents, whose contact lens storage cases had been contaminated with *Acanthamoeba*, were also polluted with this organism.

Acanthamoeba and FLA in tap water in the Pusan area

The FLA were isolated from 97 (46.9%) of the 207

Table 2. Prevalence of *Acanthamoeba* and other free-living amoeba contamination in domestic tap water samples from 2 house types

House type	No. of contaminated samples with			
	Acanth.[a)]	FLA[b)]	Acanth. + FLA[c)]	Total
Community dwelling (137)[d)]	9 (6.6%)	56 (40.9%)	2 (1.5%)	67 (48.9%)
Independence (70)	3 (4.3%)	25 (35.7%)	2 (2.9%)	30 (42.9%)

[a)] *Acanthamoeba* spp.
[b)] free living amoeba except *Acanthamoeba*
[c)] *Acanthamoeba* and other free living amoeba double contaminations
[d)] Number of water samples

Table 3. Contamination from *Acanthamoeba* (above diagonal) and other free living amoeba (below diagonal) in water samples from each purification step of 3 water purification plants

Water purification plants	Water sample was obtained from					
	Pre-treatment	After precipitation	After sand filtration	After carbon filtration	Pure water	Direct domestic tap water[a)]
Duksan	+/+	+/+	-/-	-/-	-/-	-/-
Myoungjang	+/+	+/+	-/+	-/-	-/-	-/-
Hwamyoung	+/+	+/+	+/+	-/-	-/-	-/-

[a)] This water do not pass though in storage tank.

domestic tap water samples, of which 12 (5.8%) contained *Acanthamoeba*, in the Pusan area (Table 1). The *Acanthamoeba* and FLA contamination rates at each water collection site were similar in this study (Table 1). However, they were more frequently isolated in community dwelling house types than in independent ones (Table 2).

Acanthamoeba and FLA at filtration steps in the purification plants

The level of FLA contamination was examined at each filtration step in the purification plants using a three step system, which supplies domestic tap water to the survey area. Most FLA, including *Acanthamoeba*, were completely eliminated by the carbon filtration steps (Table 3). No FLA was detected in the water before it was supplied to houses.

DISCUSSION

AK is an infection that is associated with the wearing of contact lenses. There have been significant improvements in contact lens care systems. This study investigated the possible role of domestic tap water as a reservoir for *Acanthamoeba* contamination in contact lens storage cases.

In this study, *Acanthamoeba* was isolated from 5 (4.2%) in 120 contact lens storage cases. The contamination rates were lower than those reported in other provinces in Korea (Lee et al., 1997; Yu et al., 2001; Kong et al., 2002), but were similar to those reported in other countries (Larkin et al., 1990; Watanabe et al., 1994).

The contamination rate of FLA, including *Acanthamoeba*, in domestic tap water was investigated in order to determine the role of domestic tap water in *Acanthamoeba* contamination in contact lens storage cases. The FLA were isolated from 46.9% of the domestic tap water samples, of which 5.8% contained *Acanthamoeba*, in the Pusan area. These results demonstrate that domestic tap water is a significant source of these organisms. These results show a much lower *Acanthamoeba* contamination rate than in other countries. Kilvington et al. (2004) reported that FLA were cultured from one or more taps from 24 (89%) in 27 households of AK patients in UK. Of these, 8 (26.9%) contained *Acanthamoeba*. In Spain, Lorenzo-Morales et

al. (2005) reported that *Acanthamoeba* contamination in 88 out of 148 (59.5%) tap water samples. The difference in the prevalence of *Acanthamoeba* contamination in tap water in different countries might be due to the difference in the tap water hygiene in each country. The *Acanthamoeba* and FLA were more frequently isolated in community dwelling house types than in independent ones. Community dwelling type houses and most independent ones in this survey area have water storage tanks, which are not often tightly covered. Therefore, environmental organisms can easily contaminate them.

Most FLA, including *Acanthamoeba*, was completely eliminated by the water purification steps in water purification plants. This shows that FLA contamination, including that from *Acanthamoeba*, in domestic tap water does not originate from the purification plant, but from the water storage tanks.

Previous studies have shown that many *Acanthamoeba* isolated from tap water and seawater sources might have some pathogenic ability (Kilvington et al., 2004; Lorenzo-Morales et al., 2005). In addition, some *Acanthamoeba* isolated in this study have similar molecular characteristics to those of the clinical isolates (data not shown).

In conclusion, domestic tap water, especially when supplied from roof storage tanks, is a source of *Acanthamoeba* contamination. Contact lens wearers should be aware of the risks associated with *Acanthamoeba* in tap water supplied from water storage tanks. More education about the hygienic maintenance of water storage tanks is recommended.

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