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Amoeba & Biofilms in UK Chlorinated Drinking Water Distributions Systems: Impact on Water Safety

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Introduction

Since the 1970s, the UK has registered cases of diseases caused by pathogenic amoebas such as Primary Amoebic Meningoencephalitis (PAM), caused by *Naegleria fowleri*, and *Acanthamoeba* keratitis (AK), caused mainly by members belonging to *Acanthamoeba* genus. These illnesses have been related with the presence of free-living amoeba (FLA) in domestic water, including tap water [1,2].

The presence of some pathogenic FLA in drinking water distribution systems (DWDS) is favoured by different factors such as: distance from the drinking water treatment plant, stagnation events, reduction of residual chlorine or water temperature increasing [3].

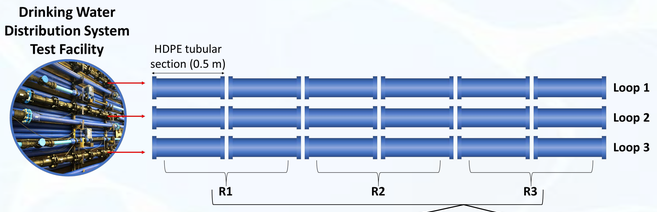
Moreover, several studies have indicated that pipe wall biofilms, which represent more than 95% of the total biomass in DWDS, are able to support the growth of amoebas by providing a food source and protecting them against disinfectants like chlorine [3].

Although pathogenic amoebas previously were found mostly in warmer areas, climate change appears to be contributing to its geographic spread [4].

The **objectives** of this research were:

1. Identify the species of amoebas in biofilms using a UK full scale DWDS facility, representative of real environments in distribution networks.
2. Study the viability of amoeba species in DWDS
3. Find possible interactions between bacterial communities with biofilm-associated drinking water amoebas.

Methodology



The experiment was carried out using a DWDS test facility at The University of Sheffield, UK. The facility is within a temperature-controlled room and it has 3 independent loops. Each loop is fed with local water which can be recirculated at different flow rates and pressure.

The 3 loops were operated under the same conditions for a growth phase of 30 days to allow for the biofilm development in the internal pipe walls. For this growth phase a low varied flow hydraulic profile which follows daily patterns observed in real DWDS in the UK was applied. To enable the *in-situ* sampling of biofilms, each loop has 6 removable HDPE pipe sections of 0.5 m long. Water samples were taken every 10 days to analyze a range of water quality parameters and amoeba viability and quantification. Biofilm samples were recollected at the end of the experiment to analyze same amoeba parameters and bacterial communities' characterization.

Physicochemistry

Free and Total Chlorine
Water Temperature
Turbidity
pH

The University of Sheffield, UK

Microbiology

Amoeba viability (NNA-*E.coli* at 42°C, 30°C and RT).
Total cell count (Flow Cytometry)
Amoeba quantification (qPCR)
Biofilm characterization (16S rRNA gene sequencing)

CSIRO Land and Water, AUS.

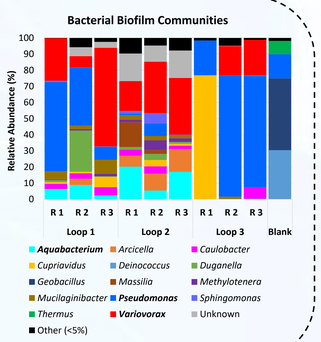
Results

Water Samples

		Room Temperature (°C)	Water pH	Water Temperature (°C)	Free chlorine (mg/L)	Total chlorine (mg/L)	Turbidity (NTU)	Amoebas in bulk water
Loop 1	Day 0	13.00 ± 2.19	7.37 ± 0.17	15.80 ± 0.46	0.50 ± 0.05	0.67 ± 0.09	0.53 ± 0.18	Non-viable amoebae were detected in the bulk water
	Day 10	16.17 ± 0.20	7.3 4 ± 0.07	16.17 ± 0.06	0.33 ± 0.09	0.41 ± 0.17	0.70 ± 0.34	
	Day 20	21.17 ± 0.21	7.33 ± 0.15	16.80 ± 0.56	0.26 ± 0.07	0.33 ± 0.01	0.40 ± 0.28	
	Day 30	24.20 ± 0.00	7.40 ± 0.03	23.40 ± 0.10	0.29 ± 0.01	0.30 ± 0.00	0.11 ± 0.05	
Loop 2	Day 0	13.00 ± 2.19	6.91 ± 0.08	16.30 ± 0.61	0.44 ± 0.05	0.59 ± 0.05	0.47 ± 0.05	
	Day 10	16.17 ± 0.20	6.70 ± 0.15	15.97 ± 0.06	0.50 ± 0.16	0.44 ± 0.05	0.53 ± 0.11	
	Day 20	21.17 ± 0.21	7.04 ± 0.05	17.00 ± 0.17	0.24 ± 0.06	0.32 ± 0.04	0.28 ± 0.06	
	Day 30	24.20 ± 0.00	7.00 ± 0.06	23.30 ± 0.15	0.30 ± 0.05	0.36 ± 0.005	0.13 ± 0.02	
Loop 3	Day 0	13.00 ± 2.19	6.92 ± 0.17	15.87 ± 0.12	0.32 ± 0.16	0.47 ± 0.08	0.36 ± 0.32	
	Day 10	16.13 ± 0.23	6.96 ± 0.08	16.00 ± 0.00	0.31 ± 0.02	0.46 ± 0.02	0.49 ± 0.22	
	Day 20	21.17 ± 0.21	6.98 ± 0.04	17.03 ± 0.15	0.28 ± 0.02	0.31 ± 0.05	0.37 ± 0.01	
	Day 30	24.20 ± 0.00	6.70 ± 0.09	23.23 ± 0.32	0.26 ± 0.03	0.32 ± 0.02	0.12 ± 0.03	

Biofilm Samples

		Amoeba identification/viability		Biofilm total cells (Cells/cm²)
Loop 1	R1	No detected	X	3,37E+05
	R2	*Naegleria dobsoni*	X	
		Naegleria australiensis	X	1,23E+06
		Vermamoeba sp.	✓	
	R3	*Naegleria australiensis*	X	
		Naegleria clarki	X	3,50E+05
		Vermamoeba sp.	✓	
		Acanthamoeba sp.	X	
Loop 2	R1	*Naegleria clarki*	✓	2,62E+05
	R2	*Naegleria dobsoni*	X	2,89E+05
	R3	*Naegleria dobsoni*	X	1,69E+04
Loop 3	R1	*Naegleria australiensis*	X	1,65E+05
	R2	*Naegleria australiensis*	X	1,84E+05
	R3	*Naegleria clarki*	X	2,70E+05
		Naegleria lovaniensis	X	



Amoebas were detected only in the biofilm samples and belonged to the genera *Naegleria, Acanthamoeba* and *Vermamoeba*. Only *Vermamoeba* sp. was viable in Loop 1 and *N. clarki* in Loop 2. *Aquabacterium*, *Pseudomonas* and *Varivorax* were the bacterial genera highly represented in all the samples. All amoeba genera detected could act as reservoirs for pathogenic bacteria such as *Legionella* and *Mycobacterium* [5]. Moreover, *Acanthamoeba* genera has been described as a potential pathogenic amoeba. Also, *Pseudomonas* could represent a risk since some members of this genus are human pathogens as well as their ability to be and amoeba-resistant microorganisms [5].

Conclusions

Biofilm samples contained multiple amoeba (viable and non-viable) which are potential pathogens and able to support the presence of amoeba resistant bacteria, such as *Legionella* and Nontuberculous Mycobacteria. No amoeba were detected in the water samples, but amoeba in the biofilm could be potentially released through physical disturbance or though migration and enter the bulk water phase. The biofilms were also had a high relative abundance of *Pseudomonas*. In conclusion, there may be a **potential risk** due the presence of amoeba in UK-DWDS biofilm samples that can be vectors of pathogenic bacteria. Further studies are needed to assess the presence, abundance and composition of amoeba and associated bacteria in UK water supply systems as well as potential changes in their presence due to climate change impacts.

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