Water Research 110 (2017) 15–26

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Elimination of *Naegleria fowleri* from bulk water and biofilm in an operational drinking water distribution system

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ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form
7 October 2016
Accepted 25 November 2016
Available online 27 November 2016

Keywords:
Naegleria fowleri
Chlorine
Biofilm
Drinking water distribution system
Amoeba

ABSTRACT

Global incidence of primary amoebic meningoencephalitis cases associated with domestic drinking water is increasing. The need for understanding disinfectant regimes capable of eliminating the causative microorganism, *Naegleria fowleri*, from bulk water and pipe wall biofilms is critical. This field study demonstrated the successful elimination of *N. fowleri* from the bulk water and pipe wall biofilm of a persistently colonised operational drinking water distribution system (DWDS), and the prevention of further re-colonisation. A new chlorination unit was installed along the pipe line to boost the free chlorine residual to combat the persistence of *N. fowleri*. Biofilm and bulk water were monitored prior to and after re-chlorination (RCl), pre-rechlorination (pre-RCl) and post-rechlorination (post-RCl), respectively, for one year. A constant free chlorine concentration of > 1 mg/L resulted in the elimination of *N. fowleri* from both the bulk water and biofilm at the post-RCl site. Other amoeba species were detected during the first two months of chlorination, but all amoebae were eliminated from both the bulk water and biofilm at post-RCl after 60 days of chlorination with free chlorine concentrations > 1 mg/L. In addition, a dynamic change in the biofilm community composition and a four log reduction in biofilm cell density occurred post-RCl. The pre-RCl site continued to be seasonally colonised by *N. fowleri*, but the constant free chlorine residual of > 1 mg/L prevented *N. fowleri* from recolonising the bulk and pipe wall biofilm at the post-RCl site. To our knowledge, this is the first study to demonstrate successful removal of *N. fowleri* from both the bulk and pipe wall biofilm and prevention of re-colonisation of *N. fowleri* in an operational DWDS. The findings of this study are of importance to water utilities in addressing the presence of *N. fowleri* and other amoeba in susceptible DWDSs.

1. Introduction

Current chlorine disinfection regimes control the majority of pathogens that occur in drinking water distribution systems (DWDSs). However, some organisms such as *Acanthamoeba* spp. (De Jonckheere and Van de Voorde, 1976), *Vermamoeba* sp. (Kuchta et al., 1993) and *Naegleria* spp. (including *N. fowleri*) (Cursons et al., 1980; Trolio et al., 2008) are known pathogens or are known to harbour intracellular pathogenic bacteria (Abd et al., 2003, 2005; Barker and Brown, 1994; Molmeret et al., 2005), and are not adequately controlled by current disinfection regimes. They therefore present a significant risk to human health if not controlled or eliminated from DWDSs. Pathogenic *Naegleria fowleri* has been isolated from operational DWDSs in Australia, USA and Pakistan, with all three countries reporting fatal primary amoebic meningoencephalitis (PAM) cases linked to domestic water exposure (Cope et al., 2015; Dorsch, 1982, Marciano-Cabral et al., 2003; Puzon et al., 2009; Shakoor et al., 2011; Trolio et al., 2008).

The first line of defence against DWDS-associated PAM is disinfection of drinking water throughout the entire DWDS by chlorination. Australian water utilities target a free chlorine residual of at least 0.5 mg/L, maintained at all times, throughout the entire network (National Health and Medical Research Council (2011)). However, *Naegleria* sp. can form cysts, a form known to

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http://dx.doi.org/10.1016/j.watres.2016.11.061
0043-1354/

be more resistant to disinfection (Dupuy et al., 2014) and can persist in DWDSs, tank sediments and pipe wall biofilm unless high enough disinfectant residuals are maintained (Goudot et al., 2014; Trolio et al., 2008). *N. fowleri* associated with pipe wall biofilm are capable of surviving intermittent levels of free chlorine up to 20 mg/L for short durations (Miller et al., 2015). In addition, organic matter in the bulk water and pipe wall biofilm build-up reduce free chlorine residuals and the efficacy of the disinfectant can be compromised in sections of the DWDSs (Miller et al., 2015; Momba et al., 2000; Prévost et al., 1998). Detection of *N. fowleri* in DWDSs at a density of 2 organisms per litre (or a single detection in a 500 mL sample) is the threshold for action in Australian DWDSs, given the rapid density changes that can occur in amoebae numbers. *N. fowleri* detections results in rigorous chlorine dosing at elevated concentrations of at least 1 mg/L of free chlorine, throughout the distribution system, with increased testing and surveillance, as recommended by the Australian Government National Health and Medical Research Council, 2011.

A similar response is now implemented in the USA following three DWDS-associated PAM cases in St. Bernard Parish, (Louisiana, USA). The “2013 emergency rule” stated that the new minimum disinfectant residual levels should be increased to 0.5 mg/L throughout the entire system at all times and daily records of chlorine residuals in the distribution system should be maintained to avoid “imminent peril to the public health and safety” (Louisiana Department of Health, (2013)). If *N. fowleri* is detected within DWDSs, water utilities are required to maintain a constant free chlorine residual of 1 mg/L for a 60-days. Following the implementation of the state's new public drinking water surveillance program > 27 water systems were tested for *N. fowleri*, with seven systems testing positive to date, including Terrebonne, St. Bernard and Ascension parishes (Louisiana Department of Health, 2015a). The 60-days disinfectant regime described in the 2013 emergency rule was reported to successfully control *N. fowleri* within these DWDSs until July 2014, when the St. Bernard DWDS tested positive for *N. fowleri* multiple times during the 60-days disinfectant regime (Louisiana Department of Health, 2015b). Of all the sites tested only one did not meet the required continual disinfectant residual of 1 mg/L. Given that the pipe line tested positive for *N. fowleri* during the normal disinfectant dosing (0.5 mg/L) as well as during the higher residual (1 mg/L), this suggests that the current disinfection regime including the 60-day chlorine regime of 1 mg/L was not able to fully eliminate *N. fowleri* from the DWDS.

Previous work using laboratory biofilm monitors and DWDSs has shown that *N. fowleri* was capable of surviving disinfection while associated with pipe wall biofilm and was able to re-emerge from biofilm into the bulk water (Biyela et al., 2012; Miller et al., 2015). As only a few of the sites tested positive for *N. fowleri* in the St. Bernard DWDS during the chlorine burn it is likely that the pathogen persisted within the pipe wall biofilm during both the regular disinfection regime and the high levels of disinfectant during the 60-day burn and later re-emerged from the biofilm after the free chlorine had been scaled back or switched to chloramine.

In addition to the USA DWDS-associated PAM cases, Karachi, Pakistan, reported 56 PAM cases since 2008. However, this number is likely to be under reported as most reports come from local media and the likelihood of misdiagnoses is high (Kazi and Riaz, 2013; Shakoor et al., 2011; Shariq et al., 2014). The continued persistence of *N. fowleri* in the city's DWDSs has been blamed on inadequate chlorination (Kazi and Riaz, 2013). DWDS-associated PAM cases in Australia reached 19 between 1961 and 1981, before the introduction of awareness campaigns (Dorsch, 1982). More recently 7 ground water/storage tank/DWDSs-associated PAM cases have been reported in Queensland since 1971, with 4 in the last 15 year and the most recent case in April 2015 (Nicholls et al., 2016) (personal communication). In Western Australia (WA), *N. fowleri* has been detected within DWDSs in rural areas (Morgan et al., 2016; Puzon et al., 2009), however, no PAM cases have been reported since the 1980s (Dorsch, 1982). The detection of any *Naegleria* spp. (density of 2 organisms/L) or water temperatures greater than 25 °C, continuously for more than 4 months a year, is considered high risk for the presence of *N. fowleri* in WA DWDSs (Trolio et al., 2008). The minimum chlorine residual required throughout DWDSs in WA is 0.6 mg/L, with the aim of maintain a minimum of 0.6 mg/L at the terminal end of the distribution system (Trolio et al., 2008). However, reaching this minimum residual is difficult due to the distance of the terminal end from preceding chlorination sites, water age, temperature, organic matter load and the presence of pipe wall biofilm reducing the free chlorine concentration within a DWDS. To address these issues, particularly in rural regions of WA, disinfectant levels are boosted within the network by staggered chlorination stations. Despite the use of chlorination stations, free chlorine residuals are still difficult to maintain in some remote regions and *N. fowleri* is occasionally detected (Morgan et al., 2016; Puzon et al., 2009; Trolio et al., 2008).

Due to an increased the number of DWDS-associated PAM cases, the need to understand the minimum level of chlorine required to eliminate *N. fowleri* from an operational DWDS and prevent its re-emergence from pipe wall biofilm is of significant importance. In this study, the efficacy of free chlorine on removing *N. fowleri* from an operational DWDS is reported. The study was designed to monitor the presence of *N. fowleri* and other amoebae in both the bulk water and pipe wall associated biofilm and determine the chlorine residual required to prevent its persistence and re-emergence. To our knowledge, this is the first field study using an operational DWDS to assess the removal of *N. fowleri* in both the bulk water and the biofilm and will further aid water utilities in their management practices with respect to *N. fowleri* and other amoebae.

2. Materials and methods

2.1. Operational DWDS site monitoring and access

This study took place in a regional water supply scheme sourced from surface water in rural Western Australia over a 19 month time span to observe the effects of a newly installed chlorinator over all seasons. The study site was selected based on persistent historical detections of *N. fowleri* and continuous low chlorine residual. The water in the pipe had previously been disinfected at several points including at its source and re-chlorination points along the DWDS. Two sampling sites were selected by their position along the pipeline relative to the location of the chlorinator. Pre-rechlorination site (pre-RCl) was located 500 m upstream from the chlorinator and hence did not receive any chlorine residual. Post-rechlorination site (post-RCl) was located 15 km downstream of the chlorinator. Pre-RCl acted as the “control”, in regards to any natural factors affecting amoebae distribution and survival (i.e. water temperature) to relate observed changes in the amoebae and bacterial communities within the DWDS with the newly established chlorine residual. Post-RCl site also had historical detections of *N. fowleri* and a continuous low free chlorine residual before the initiation of the study. Both sites had previously been monitored for 3 years before the chlorinator's installation on a seasonal basis. Bulk water and biofilm samples were analysed for presence of viable and non-viable amoeba, cell counts, free and total chlorine residuals, turbidity, adenosine triphosphate (ATP) activity and water temperature.

Bulk water samples were collected in sterile 250 mL collection bottles directly from the pipe line using a pre-sterilized spout,