Legionella and Protozoa in Cooling Towers: Implications for Public Health and Chemical Control

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In recent years, technical and regulatory controls on the operation and maintenance of cooling towers within Australia have increased. Chemical treatments for microbial control have primarily focused on effects against bacteria and in some instances, specifically on Legionella. *There is strong evidence to suggest that the presence of protozoa contributes significantly to the survival of* Legionella *in cooling towers and might be an important consideration for health risk management strategies.*

Key words: *Amoebae; Biocides; Cooling Towers;* Legionella*; Protozoa*

The presence of *Legionella* in cooling towers has long been associated with outbreaks of disease. This was recently demonstrated by the Melbourne Aquarium outbreak in 2000 where 125 cases of Legionnaires' disease and four deaths were reported (Greig et al. 2004). Protozoa are likely to contribute to the survival of *Legionella* in cooling towers and their control has been proposed as a major method for minimising *Legionella* proliferation (Fields et al. 2002). However, the microbial ecology of cooling towers is complex, and requirements for effective protozoan control strategies are not fully understood.

Cooling Tower Operation

Cooling towers are designed to cool water and dissipate heat to the environment and are often associated with air conditioning, refrigeration systems and other large plant. In typical operation, warm water from a heat exchanger is sprayed into the top of a large chamber over packing material known as fill. The water droplets partially evaporate and lose heat to the surrounding air by conduction and convection as they fall through the tower. The water collects in the basin where it can be recirculated to the heat load. Typical water temperatures in cooling towers range from 25°C in cool areas to up to 35°C at heat exchange surfaces. The constant fall of water through the fill creates aerosol, of which, a proportion is lost from the cooling tower through 'drift'. Drift will contain any bacteria, and any organic and inorganic material present in the water. The transmission of aerosol over distances of up to 12km from cooling devices has been reported (Nguyen et al. 2006). The loss of aerosols as drift is minimised by the installation of 'drift eliminators', which are required to minimise drift loss to less than 0.002% of the circulating flow rate, as required by AS/NZS 3666.1 (AZ/NZS 2002). The continual evaporation also results in a constantly increasing salt concentration, controlled by running water off from the tower, known as the 'bleed' or 'blow down', and replacing it from the mains supply. One disadvantage of this process is the loss and dilution of chemicals for water treatment such as biocides, corrosion and scale inhibitors.

Microbial Colonisation of Cooling Towers

Micro-organisms enter cooling towers through the mains supply, the intake of air or during cooling tower construction.

The constant fall of water within cooling towers acts as an efficient 'air scrubber' and introduces large amounts of organic, inorganic particulates and micro-organisms. Within cooling towers, the elevated water temperatures, high humidity and large surface areas provide ideal conditions for the growth of micro-organisms. The extent of microbial colonisation is variable and dependent on many environmental factors. Temperature, pH, salinity and chemical additives have all been demonstrated to influence colonisation (Bentham 1993).

Micro-organisms present in cooling towers can be separated into two distinct but related populations. Firstly the microbial flora in the planktonic phase which may be transient or actively multiplying, and secondly the microbial flora in biofilm. The development of biofilm within cooling towers accounts for the persistence of micro-organisms on surfaces. Organisms present in biofilm contribute the majority of biomass within cooling towers. The extent of biofilm formation directly influences the extent of colonisation in the planktonic phase (Bentham et al. 1993). Biofilm facilitates the development of localised environmental conditions on surfaces that are extremely different from the planktonic phase (Donlan et al. 2002). More importantly, biofilm provides a mechanism that inhibits the penetration of biocides and other chemical treatments to the contained cells (Gilbert et al. 2001). This mechanism can also support the existence of micro-organisms in environments where they might not normally survive.

Biofilm can readily detach from surfaces in response to water turbulence, changes in nutrient supply, chemical treatment, physical disturbances, microbial grazing and biological stimuli (Morgenroth & Wilderer 2000; Murga et al. 2001; Rice et al. 1999; Sawyer & Haermanowicz 1998). The detachment of biofilm provides microbial inocula for the circulating water phase and acts as a continuous seed. Biofilm detachment is actively promoted by intermittent cooling tower operation (Bentham & Broadbent 1993).

The types of micro-organisms found within cooling towers are diverse and include bacteria, algae, fungi, protozoa and viruses (Bentham 2000; La Scola et al. 2003; Shelton et al. 1994; Sungur & Cotuk 2005; Thomas et al. 2006). The majority are heterotrophic and require organic carbon as a nutrient and energy source. *Legionella* are commonly isolated from cooling towers and present significant implications for public health by their potential to cause disease (Fields et al. 2002).

Legionellae & Public Health

Legionella are gram negative bacteria that are ubiquitous in aquatic environments (Fliermans et al. 1981). They are motile, non-spore forming, fermentative obligate aerobes, are generally rod shaped with an optimum temperature for multiplication between 35°C and 37°C, and optimum pH for growth of 6.9 (Fields et al. 2002; Wadowsky et al. 1995). *Legionella* utilise amino acids as a carbon source and on primary isolation, have an absolute requirement for L-cysteine (Tesh & Miller 1981). The growth of *Legionella* can be stimulated by the presence of small amounts of minerals including iron, zinc, manganese, potassium, magnesium and copper (Devos et al. 2005; States et al. 1985).

There are 59 species of *Legionella* formally identified, with over 70 different serotypes recognised. Seventeen of these species have been identified or implicated as causative agents of Legionellosis, including Legionnaires' disease and Pontiac fever (Little 2003). The mode of transmission of *Legionella* from cooling towers is through the inhalation of aerosolised organisms; for example, from the inhalation of cooling tower drift (Yu 1993). The person to person transmission of *Legionella* has never been documented. *Legionellae* are able to resist destruction by macrophages in the human lung, which significantly aids their pathogenicity. The virulence of *Legionella* varies widely between

the species, serogroups and strains (Yu et al. 2002). *Legionella pneumophila* is responsible for approximately 80% of cases of Legionnaires' disease and approximately 2-15 % of pneumonia cases requiring hospitalisation (Rusin et al. 1997). *L. pneumophila* has 16 serogroups identified, the majority of which are not associated with disease. *L. pneumophila* serogroup 1 is responsible for the majority of Legionellosis worldwide and is the primary causative agent of Legionnaires' disease (Yu et al. 2002). Disease from cooling towers is almost exclusively attributable to *L. pneumophila* serogroup 1 (Fields et al. 2002).

Legionnaires' disease is a severe pneumonia that might result in multi-organ failure. It was first described after an outbreak of pneumonia affecting 182 delegates at an American Legion conference in Philadelphia during 1976 (Fraser et al. 1977). The outbreak caused the deaths of 29 people and epidemiological investigations attributed the outbreak to *L. pneumophila*. Legionnaires' disease has an incubation period between 1 and 14 days (Little 2003). Clinical presentation of the disease ranges from mild respiratory symptoms to a severe atypical pneumonia. Early symptoms include coughs, headache, malaise and fever. With disease progression, these symptoms might be followed by neurological abnormalities, gastrointestinal symptoms, chest pain, respiratory distress and organ failure. Disease is more common in males, in adults over 50 years of age, in smokers, in people with a high alcohol intake, in the immunosuppressed and in those with existing pulmonary disease (Guiguet et al.1987; Little 2003; Marston et al. 1994).

Pontiac fever is a non-pneumonic infection also caused by respiratory exposure to *Legionella*. Symptoms are similar to influenza and might include fever, tiredness, myalgia, arthralgia, headache, cough, sore throat and nausea. Pontiac fever has an incubation period of between 5 and 66 hours, demonstrates a significantly higher attack rate, however, is self-limiting and those infected fully recover. Four species of *Legionella* have been implicated as the cause of Pontiac fever: *L. pneumophila*, *L. micdadei*, *L. feeleii* and *L. anisa* (Castor et al. 2005; Fields et al. 1990; Fields et al. 2001; Herwaldt et al. 1984). It has been postulated that Pontiac fever originates from a hypersensitivity pneumonitis due to the inhalation of *Legionella* cells, rather than to the actual infection (Rowbotham 1986).

Legionellosis in Australia

In Australia, contaminated cooling towers are largely responsible for Legionellosis outbreaks. The Melbourne Aquarium was the largest outbreak of Legionnaires' disease in Australia to date (Greig et al. 2004). Over a 14-day period in April 2000, 125 cases of Legionnaires' disease occurred as a result of contamination of the Aquarium's cooling towers with *Legionella*. *Legionella* including *L. pneumophila* were reported at concentrations in the order of 10^3 - 10^4 cfu/mL. An estimated 95 people were hospitalised with the disease and four deaths resulted. During this period there were approximately 83,500 visitors to the newly opened Aquarium, equivalent to a crude attack rate of 0.13% (Greig et al. 2004). The Australian states with the highest notification rates of Legionellosis over the last three years include South Australia, Western Australia and Victoria (National Notifiable Disease Surveillance System [NNDSS] 2006). Notified cases of Legionnaires' disease in South Australia and Western Australia are predominantly associated with potting mix and compost exposure (*L. longbeachae*), rather than cooling water systems and other sources (Spencer 2005).

The concentrations of *Legionella* found in cooling towers associated with illness are extremely variable and range between 10^3 and 10^6 cfu/mL (Greig et al. 2004; Shelton et al. 1994). There have been no relationships detected between concentrations of heterotrophic bacteria and *Legionella*. High concentrations of *Legionella* have been isolated from cooling towers

which demonstrated no visible evidence of contamination and reported low plate counts (Bentham & Broadbent 1993). *Legionella* has also been shown to have symbiotic relationships with some algae and cyanobacteria (Bohach & Snyder 1983; Tison et al. 1980). Fields (2002) also reported that *L. pneumophila* was unable to grow and multiply in biofilm in the absence of protozoa. In contrast, Surman et al. (2002) demonstrated *L. pneumophila* could proliferate in biofilm in the absence of protozoa. These reports indicate that *Legionella* are opportunist organisms adapted to heterotrophic communities from which they derive their organic requirements. This in turn suggests that a number of mechanisms might operate in the colonisation and multiplication of *Legionella* in cooling towers. Effective control must address each of these survival strategies.

Protozoa in Cooling Towers

Cooling tower waters with their typically high microbial load provide ideal conditions for colonisation by free living protozoa including amoebae, ciliates and flagellates (Barbaree et al. 1986; Newsome et al. 1998). Free living protozoa feed predominantly on bacteria, fungi and algae and organic detritus through phagocytosis (engulfment). However, some micro-organisms have evolved that are able to evade protozoan predation (Matz & Kjelleberg 2005). These organisms are either not able to be ingested by protozoa or are able to survive, multiply and exist within the protozoa after internalisation. *Legionella* demonstrates this capacity and can survive and multiply in the cytoplasm of free living protozoa (Abu Kwaik et al. 1998). In response to environmental variables, this endocytic relationship might range from commensalism to parasitism.

Protozoa including *Naegleria*, *Hartmanella*, *Vahlkampfia*, *Acanthamoeba*, *Tetrahymena* and *Cyclidium* spp. have been extensively isolated from cooling tower waters (Newsome et al. 1998). Within cooling towers, protozoa are primarily found in association with biofilm on surfaces, sediment or microbial flocs. Surface grazing ciliates are almost exclusively found in association with biofilm (Huws et al. 2005). Amoebae readily expel vesicles from within the cell, especially prior to encystment. Vesicles expelled by amoebae do not adhere to surfaces and generally exist free in solution. Grazing by protozoa can produce rapid changes in the morphological and taxonomical properties of both biofilm and planktonic communities (Hahn & Hofle 2001). The spatial distribution of protozoa within biofilm is also complex. Some protozoa possess the ability to 'burrow', which might relate to their survival in biofilm (Huws et al. 2005). The temperatures that favor the growth of *Legionella* might also provide optimum conditions for the proliferation of protozoa (Berk et al. 2000). Protozoa have growth rates similar to bacteria and can multiply exponentially in short time periods. Adverse environmental conditions such as changes in temperature, pH, osmotic pressure and nutrient supply can stimulate some amoebae to encyst (Byrne & Swanson 1998). Cysts are walled dormant cells that might remain viable in the environment for many months, and can excyst again when conditions become favourable.

There are at least 13 species of amoebae and 2 species of ciliated protozoa that support intracellular replication of *Legionella* (Little 2003; Newsome et al. 1998). In many outbreaks of Legionnaires' disease, protozoa capable of harbouring *Legionella* have been isolated from the reservoir of infection (Barbaree et al. 1996; Fields et al. 1990). The encapsulation of *Legionella* within protozoa can provide protection from the external environment within cooling towers, including protection from biocides. Garcia et al. (2007) reported intracellular *L. pneumophila* within *A. polyphaga* was resistant to 1024 ppm sodium hypochlorite. Following intracellular replication, *Legionella* cells exhibit a dramatic increase in resistance to

conditions such as high temperatures, acidity and high osmolarity (Byrne & Swanson 1998; Abu Kwaik et al. 1997). *Legionella* released from protozoa has been reported to have significantly different morphological and chemical characteristics compared to cultured cells, demonstrating more resistance to antimicrobial agents (Cirillo & Tompkins 1994; Greub & Raoult 2003). Relationships between *Legionella* and protozoa might also be species dependent (Fields et al. 1990). The virulence of *L. pneumophila* is also maintained or even increased when grown in co-culture with amoebae (Neumeister et al. 2000). Although the regulation of virulence factors in *Legionella* is not fully understood, the potential for protozoa to increase the virulence of *Legionella* has serious public health implications. Changes in the osmolarity of cooling tower water have been suggested to increase the intracellular replication within protozoa, which might produce populations more tolerant of cooling tower environments (Neumeister et al. 2000).

Chemical Control of Protozoa

Chemical treatments applied to cooling towers include biocides to inhibit microbial growth, dispersants, corrosion and scale inhibitors. Chemical biocides include metals, oxidising, non-oxidising antimicrobials and other agents such as surfactants and dispersants. Active ingredients of oxidising biocides include chlorine, bromines, chlorine dioxide, monochloramine, ozone and hydrogen peroxide. Non-oxidising biocides commonly include quaternary ammonium compounds, isothiazolinones, halogenated amides, guanidines, thiocyanates, thiocarbamates, halogenated glycols, aldehydes and other organic formulations. Dispersants, corrosion and scale inhibitors might include metals such as molybdenum, zinc and chromium, organics and phosphate polymers. Some of these chemicals might be stimulatory to *Legionella* growth and have the potential to influence biocide performance (States et al. 1985).

Biocides that have demonstrated activity towards protozoa include ozone, peroxides, chlorine, halogenated bisphenols and guanidines (Barker et al. 1992; Cursons et al. 1980; Kuchta et al. 1993; Sutherland & Berk 1996). Bromines have demonstrated some activity towards vesicles from protozoa, but recent reports suggest application of these formulations might be ineffective in spa pools (Surman-Lee & Bentham 2006). However, previous research has suggested cooling tower biocides might not always have desirable effects on protozoa. Srikanth and Berk (1994) reported the exposure of *A. hatchetti* and *Cochliopodium bilimbosum* to thiocarbamate, quaternary ammonium and isothiazolinone biocides increased their resistance upon exposure to other biocides. There are also significant differences between the efficiency of cooling tower biocides to amoebae in non-cysted and encysted forms. Trophozoites have far greater susceptibility to biocides than cysts (Sutherland & Berk 1996). Cysts of *Acanthamoeba* spp. have been reported to survive disinfection by free chlorine up to 40 mg/L, far greater than concentrations employed *in situ* (De Jonckheere & van De Woorde 1976). Encystment can also provide significant protection if *Legionella* cells are contained during this process. *Legionella* cells encysted within *Acanthamoeba* spp. have been demonstrated to survive disinfection by chlorine at 50mg/L (Kilvington & Price 1990).

Research has demonstrated that amoebae expel vesicles containing *Legionella* and other bacteria prior to encystment (Berk et al. 1998). Ciliates also expel vesicles during active grazing of biofilm (Berk et al. 1998). Vesicle production might be stimulated by the presence of cooling tower biocides, which encourage amoebae to encyst (Sutherland & Berk 1992). The expelled vesicles containing *Legionella* might also demonstrate some resistance to biocides. Berk et al. (1998) investigated the resistance of *L. pneumophila* contained in vesicles produced by two *Acanthamoeba* species against non-oxidising

biocides. After exposure to recommended dosing concentrations for contact times of 4 and 24 hours, the viability of *L. pneumophila* cells was still maintained. This has important public health implications as vesicles have been reported to contain up to 10^4 bacteria and the exposure dose of *Legionella* from inhalation of cooling tower drift is potentially increased (Rowbotham 1986). Rowbotham (1986) proposed that the mechanism of infection for Legionnaires' disease was through the inhalation of protozoa or vesicles containing concentrated *Legionella* cells as opposed to individual cells.

The effects of biocides against protozoa have also been reported to be species variable, which might have ramifications considering the microbial diversity of cooling towers (Cursons et al. 1980). Many of the published biocide assessments have also been performed under axenic and controlled laboratory conditions using laboratory strains, and are unlikely to be comparable to conditions in cooling towers. There is little known about biocide performance in the presence of chemical treatments such as scale and corrosion inhibitors, which might have antagonistic or synergistic effects. *In situ*, the chemical and physical characteristics of the water and the engineered environment, including water temperature and pH, will additionally influence the effectiveness of biocides. The chemical control of protozoa in the presence of biofilm has been the subject of limited investigation (Brown & Barker 1999). Successful control will necessarily address both planktonic and sessile populations of microorganisms in the system.

Conclusion

The presence of *Legionella* in cooling towers presents significant implications for public health. Protozoa are likely to contribute to the survival of *Legionella* and their control has been proposed as a major method for minimising *Legionella* proliferation. Little effort has been directed towards the synergistic or antagonistic effects of chemical treatments in cooling tower microbial control. Cooling water systems are complex microbial ecosystems in which predator-prey relationships play a key role in dissemination of *Legionella*, leading to public health risk. Understanding the relative physical, chemical and biological contributions to these ecosystems is the pre-requisite for providing informed management strategies to protect public health.

Acknowledgements

The authors gratefully acknowledge the support of the Victorian Department of Human Services in the review of the manuscript and funding of a Public Health Research Project into chemical treatments for the control of protozoa in cooling towers.

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