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Understanding human infectious *Cryptosporidium* risk in drinking water supply catchments

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ABSTRACT

Treating drinking water appropriately depends, in part, on the robustness of source water quality risk assessments, however quantifying the proportion of infectious, human pathogenic *Cryptosporidium* oocysts remains a significant challenge. We analysed 962 source water samples across nine locations to profile the occurrence, rate and timing of infectious, human pathogenic *Cryptosporidium* in surface waters entering drinking water reservoirs during rainfall-runoff conditions. At the catchment level, average infectivity over the four-year study period reached 18%; however, most locations averaged <5%. The maximum recorded infectivity fraction within a single rainfall runoff event was 65.4%, and was dominated by *C. parvum*. Twenty-two *Cryptosporidium* species and genotypes were identified using PCR-based molecular techniques; the most common being *C. parvum*, detected in 23% of water samples. Associations between landuse and livestock stocking characteristics with *Cryptosporidium* were determined using a linear mixed-effects model. The concentration of pathogens in water were significantly influenced by flow and dominance of land-use by commercial grazing properties (as opposed to lifestyle properties) in the catchment ($p < 0.01$). Inclusion of measured infectivity and human pathogenicity data into a quantitative microbial risk assessment (QMRA) could reduce the source water treatment requirements by up to 2.67 log removal values, depending on the catchment, and demonstrated the potential benefit of collating such data for QMRAs.

1. Introduction

Cryptosporidiosis is a diarrhoeal disease caused by protozoan parasites of the genus *Cryptosporidium*, and is a major treatment issue for drinking water utilities in both industrialised and developing countries. A recent review of waterborne disease outbreaks suggested *Cryptosporidium* spp. was the responsible agent for 63% of the reported epidemics between 2011 and 2016 (Efstratiou et al., 2017). While commonly contracted via person-to-person transmission, contact with infected animals or ingestion of recreational water (Feltus et al., 2006), widespread disease outbreaks have been attributed to contamination of drinking water supplies (Baldursson and Karanis, 2011; Efstratiou et al., 2017). This, partnered by the pathogen's ability to persist in the environment (King and Monis, 2007), difficulty to remove via conventional treatment processes and resistance to chlorine disinfection (Peeters et al., 1989), results in *Cryptosporidium* risk mitigation remaining a key focus area for water utilities globally.

Understanding risk to humans requires an appreciation of the diversity within the *Cryptosporidium* genus; the 29 species and ~50 genotypes (Xiao, 2010; Ruecker et al., 2012; Zahedi et al., 2016) vary markedly in their host specificity and subsequently, virulence to human health. *Cryptosporidium hominis* and *C. parvum* are responsible for the majority of human infections (Xiao, 2010), while species such as *C. bovis* or *C. ryanae* (generally associated with adult cattle), or *C. fayeri* (generally associated with wildlife) are of a lesser concern to humans (Zahedi et al., 2016).

Generalising *Cryptosporidium* spp. host specificity enables, to some degree, the use of the pathogen to track contaminant sources within the catchment (Yang et al., 2008; Wilkes et al., 2013a). Different land uses, such as those found in mixed-use surface water catchments, will often reflect the microbial pollution present from livestock, wildlife, or human faecal material (Yang et al., 2008; Robinson et al., 2011; Ruecker et al., 2007, 2012; Wilkes et al., 2013a; Swaffer et al., 2014). Surface water runoff from areas where development is low or negligible tend to contain *Cryptosporidium* shed from wildlife (Wilkes et al., 2013a; Swaffer et al.,

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2014), whereas streams from dairy and beef farms stocked with mature cattle often have a higher frequency of species such as *C. andersoni* (Ruecker et al., 2007; Ryan and Power, 2012).

Adding to the complexity of pathogen dynamics, it is generally understood that the quantity and source of *Cryptosporidium* has a temporal, in addition to spatial, trend within a landscape (Lapen et al., 2016). This is partly due to the seasonality of rainfall, resulting in surface water runoff delivering pathogens into streams (Kistemann et al., 2002; Robinson et al., 2011; Swaffer et al., 2014), but also further influenced by the breeding cycle of hosts within the catchment. *Cryptosporidium parvum* prevalence varies markedly by the age and species of its host, especially in cattle. Young (<1 month) beef and dairy calves often exhibit high rates of infection (Coklin et al., 2007; Rieux et al., 2014) that are almost exclusively caused by *C. parvum* (Santìn et al., 2004; Rieux et al., 2014). However, as individuals age, the frequency of infection decreases (Santìn et al., 2004, 2008), and the *Cryptosporidium* species shifts to favour *C. bovis*, *C. ryanae* and/or *C. andersoni* dominance (Fayer et al., 2007, 2010a; Robinson et al., 2011; Ryan and Power, 2012). Sheep also demonstrate age-related differences in pathogen prevalence, with lambs having higher rates of infection than adults (Sweeny et al., 2011; Yang et al., 2014); however, importantly, *C. parvum* is less common, and infections tend to be attributed to *C. xiaoi* and *C. ubiquitum*, regardless of age (Sweeny et al., 2011; Ryan and Power, 2012; Yang et al., 2014). *Cryptosporidium* in sheep has therefore been suggested to present less of a concern to human health (Ryan et al., 2005; Sweeny et al., 2011) although recent reports suggest *C. ubiquitum* is an emerging pathogen of significance to humans (Li et al., 2014).

Water utilities are continually seeking to improve their understanding of pathogen-related human health risk, driven by tighter regulatory controls on microbial thresholds for potable water, and a desire to untangle the complex interactions between landuse, human, wildlife, seasonal and/or climatic influences that result in source water contamination. *Cryptosporidium*, in particular, are notorious for presenting a planning challenge to water suppliers due to their irregular fate and transport mechanisms within the catchments as well as uncertainties in relation to their human-infectious characteristics, especially when drinking water supply catchments are open to human activities. Quantitative microbial risk assessments (QMRA) are being increasingly used to assess the effect of human exposure to *Cryptosporidium*, however, it can overestimate human health risk if based on concentration data alone (either IFA + ve or DAPI-confirmed, with preference given to DAPI-confirmed when available) without considering the human-infective proportion (Lapen et al., 2016; Petterson et al., 2015). An increasing body of evidence demonstrates the value of refining QMRA approaches by quantifying the human-pathogenic proportion and/or the infectious fraction of oocysts. Both Wilkes et al. (2013b) and Lapen et al. (2016) found that average risk values were approximately an order of magnitude lower when considering the proportion of *C. hominis* and *C. parvum* detections using molecular approaches, compared to assuming all oocysts were human-pathogenic.

Polymerase chain reaction (PCR) approaches have been commonly used to refine QMRAs by identifying the prevalence of *C. hominis* and *C. parvum* in raw water samples (Boyer and Kuczynska, 2003; Yang et al., 2008; Chalmers et al., 2010; Wilkes et al., 2013b; Swaffer et al., 2014); however, data regarding oocyst infectivity (capacity to infect if ingested by a suitable host) is not widely available. The ability to measure oocyst infectivity has been impeded by a number of logistical and methodological issues (Hijjawi, 2010). Recent improvements in cell-culture immunofluorescence assays (King et al., 2015) have started to generate data on oocyst infectivity in both surface water (Swaffer et al., 2014) and wastewater sources (King et al., 2015, 2017). Incorporating both species/genotyping and infectivity data into QMRA approaches offers a vastly improved assessment of human health risk in source water used for drinking water supplies.

1.1. Objectives

The objectives of this study were to quantify the occurrence and infectious fraction (including identification of the infective oocyst) of *Cryptosporidium* in source water used for drinking water supplies, using water samples collected across nine locations from five catchments for up to four years. In addition, we profiled the range of *Cryptosporidium* in the water using molecular approaches, to identify species/genotypes present independently from their infective nature. Both infectivity and species/genotyping data were compared to the land use variables within the catchment, to evaluate the linkage between catchment characteristics and the magnitude and timing of infection risk in source water. Finally, the source water pathogen challenge in the catchment was calculated using a QMRA, refined by the infectivity and genotyping data, to highlight the benefit of including such data when quantifying source water treatment requirements.

2. Materials and methods

2.1. Location description

The study sites were located within the northern zone of the Mount Lofty Ranges Watershed (−34.7834, 138.8515), a multi-use region comprised of 'open' catchments and 90% private ownership (EPA, 2017). The Mount Lofty Ranges Watershed provides, on average, 60% of the source water (EPA, 2017) used to supply drinking water to approximately 1,700,000 people (SA Water, 2017). Land use is dominated by livestock grazing (either commercial or lifestyle-orientated), remnant native vegetation, low intensity residential areas and horticulture (Fig. 1). Land usage codes were used to partition livestock grazing into commercial or lifestyle-orientated properties (Government of South Australia, 2015). Climate is classified as temperate with a dry and warm summer, has an average annual rainfall of ~830 mm and annual average potential evapotranspiration of ~1300 mm (Bureau of Meteorology, 2017). The annual rainfall and potential evapotranspiration rates received during the study period, compared to the long term average, is shown in Fig. 2.

The water quality characteristics of five surface water catchments supplying two reservoirs were quantified during this investigation; 1) Little Para Reservoir via Little Para River (LPR) and Gould Creek (GC) catchments, and 2) Millbrook Reservoir via Millbrook Creek (MC), Kersbrook Creek (KC) and the Torrens River (TR). An additional four sub-catchments were also monitored upstream of Kersbrook Creek in a nested design (sub-catchment 2 (SC2), sub-catchment 3 (SC3), sub-catchment 4 (SC4) and sub-catchment 5 (SC5)). The catchment area (Ha) and dominant land-use occurring upstream of the monitored locations is described in Table 1.

Nine telemetered, automatic sampling and water quality monitoring stations were modified to collect a maximum of 24 × 10 L flow-proportional samples (Model 3700, ISCO Inc., Nebraska USA) per runoff event, while simultaneously measuring flow (Model CS541, Campbell Scientific Inc., USA) and turbidity (Sonde: MS 600 Series, YSI Australia) every five minutes. Five sites were established in 2013, and a further four sites in 2014. The duration that each station was operational is described in Table 2.