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Investigating source water *Cryptosporidium* concentration, species and infectivity rates during rainfall-runoff in a multi-use catchment

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ABSTRACT

Protozoan pathogens present a significant human health concern, and prevention of contamination into potable networks remains a key focus for drinking water providers. Here, we monitored the change in *Cryptosporidium* concentration in source water during high flow events in a multi-use catchment. Furthermore, we investigated the diversity of *Cryptosporidium* species/genotypes present in the source water, and delivered an oocyst infectivity fraction. There was a positive and significant correlation between *Cryptosporidium* concentration and flow ($\rho = 0.756$) and turbidity ($\rho = 0.631$) for all rainfall-runoff events, despite variable source water pathogen concentrations. Cell culture assays measured oocyst infectivity and suggested an overall source water infectious fraction of 3.1%. No infectious *Cryptosporidium parvum* or *Cryptosporidium hominis* were detected, although molecular testing detected *C. parvum* in 7% of the samples analysed using PCR-based molecular techniques. Twelve *Cryptosporidium* species/genotypes were identified using molecular techniques, and were reflective of the host animals typically found in remnant vegetation and agricultural areas. The inclusion of molecular approaches to identify *Cryptosporidium* species and genotypes highlighted the diversity of pathogens in water, which originated from various sources across the catchment. We suggest this mixing of runoff water from a range of landuses containing diverse *Cryptosporidium* hosts is a key explanation for the often-cited difficulty forming strong pathogen–indicator relationships.

1. Introduction

Cryptosporidium present a particularly difficult challenge for water utilities – oocysts can persist in water for long periods (Ryan et al., 2005; King and Monis, 2007), are small and can penetrate conventional filtration systems, are resistant to chlorination (Peeters et al., 1989) and consumption of as few as 9 oocysts can cause infection (Teunis et al., 2002). In response to these challenges, significant effort has focussed on improving our understanding of the origin and prevalence

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of oocysts present in source water catchments (Ryan et al., 2005; Ng et al., 2010, 2011; Robinson et al., 2011; Ryan and Power, 2012; Nolan et al., 2013) and their transport from the site of deposition into surface waters used for producing drinking water (Atwill et al., 2006; Curriero et al., 2011; Khaldi et al., 2011).

As a result, it is well understood that elevated stream flow resulting from rainfall run-off (here referred to as event-based flow) is a major driver behind the transport of oocysts to surface water (Kistemann et al., 2002; Ferguson et al., 2003; Keely and Faulkner, 2008; Curriero et al., 2011). Despite recognising the link between event-based flow and *Cryptosporidium* transport, the ability to predict the magnitude of source water contamination during high flow events remains elusive (Wilkes et al., 2011) despite continued efforts by water utilities and others seeking to avoid exposure to adverse source water quality.

Substituting *Cryptosporidium* with a reliable indicator (either microbes [Brookes et al., 2005; Wilkes et al., 2009], water quality parameters [LeChevallier et al., 1991; Kistemann et al., 2002; Brookes et al., 2005; Wilkes et al., 2011], land use factors [Wilkes et al., 2011] or hydrological indices [Kistemann et al., 2002; Wilkes et al., 2009, 2011]) has long been a potential goal (i.e. LeChevallier et al., 1991) that has so far met limited success. The difficulty in developing general indicator–pathogen relationships has been attributed to the heterogeneity found across surface water catchments (Wilkes et al., 2009) where a diverse range of pathogen host species and catchment hydrological characteristics combine to confound the development of generalist tools capable of predicting source water contamination.

In addition, the routine practice of assessing *Cryptosporidium* contamination using total oocyst count may overestimate human health risk, considering the majority of the ~26 species and ~40 genotypes (Fayer, 2010; Ruecker et al., 2012) either do not infect or rarely cause illness in humans. While eight *Cryptosporidium* species have been reported to infect humans (Chalmers and Davies, 2010; Ng et al., 2010), only two cause the majority of reported gastroenteritis cases – *Cryptosporidium hominis* and *Cryptosporidium parvum* (Ng et al., 2010; Robinson et al., 2011; Ryan and Power, 2012; Nolan et al., 2013). The introduction of identification techniques such as those based on the polymerase chain reaction (PCR) has been an important advance for water management frameworks and quantification of pathogen risk to risk to drinking water supplies (Nolan et al., 2013).

Risk assessment can be further refined by incorporating environmental inactivation (Monis et al., 2014), which has the potential to greatly reduce the number of oocysts capable of causing human illness. Therefore, measuring oocyst infectivity presents further opportunities for adjusting the risk profile of oocysts in source waters. The ability to routinely measure oocyst infectivity has been hampered by a number of issues including the distribution and low numbers of oocysts, costs and method variability (Di Giovanni and LeChevallier, 2005; Hijjawi et al., 2010; Karanis and Aldeyarbi, 2011; Johnson et al., 2012; Kothavade et al., 2012; Lalancette et al., 2010). However, recent improvements in a cell culture immunofluorescence assay (CC-IFA) have led to the development of a single format assay that provides information on method performance (recovery rate), oocyst number, oocyst infectivity and identity of infectious oocysts (King et al., 2011, 2012; Webber et al., 2013; King et al., in preparation), overcoming these obstacles.

This work is aimed at improving our understanding of the distribution of *Cryptosporidium* during elevated flow conditions caused by rainfall run-off, including characterising the species present and infectivity rates, to provide essential information to facilitate more effective drinking water risk assessments. We hypothesised that elevated flow conditions would significantly increase *Cryptosporidium* counts, but that total oocyst numbers would not be reflective of the proportion of oocysts that presented a health concern to humans.

The objectives of the study were to 1) define relationships between *Cryptosporidium* concentration and hydrological and water quality variables in surface water systems impacted by mixed land use activities and 2) investigate the proportion of total oocyst concentration that may present a health concern by investigating species and infectivity rates.

2. Material and methods

2.1. Site description

The South Australia Water Corporation is a major drinking water service provider, with around 1.5 million customers. A significant proportion of source water originates from catchments within the Mount Lofty Ranges, which are ~90% privately owned (EPA, 2007).

The study site was located at a weir along the Onkaparinga River in the southern Mount Lofty Ranges (S 35°7′0″, E 138°38′0″). The weir receives water from three contributing catchments covering an area of approximately 56 km^2 (Fig. 1). The catchment is dominated by remnant native vegetation (41%) and grazing of livestock (34%), with sparse semi-rural settlements serviced by on-site waste disposal systems.

The study site climate is temperate, with warm dry summers (Peel, 2007) and winter dominated rainfall averaging 790 mm $year^{-1}$ (1900–2011, BOM, 2012).

2.2. Water quality monitoring

A Starflow in-stream monitoring gauge (Ultrasonic Doppler Model 6529, Unidata Pty Ltd, Australia) was installed on April 30 2012 along the Onkaparinga River prior to the weir, where the channel remained narrow to ensure sensitivity to changes in flow rate. The gauge was programmed to trigger flow proportional 10 L samples at the weir using a radio signal. A sonde (MS 600 Series, YSI Australia) was installed adjacent to the flow gauge and measured turbidity (NTU), electrical conductivity (mS cm^{-1}), temperature (°C) and depth (m) every 10 min. A second sonde measuring the same parameters was installed adjacent to the automated pathogen sampling equipment at the weir wall (Fig. 1).

2.3. Cryptosporidium sampling

Cryptosporidium samples were collected at the weir using an auto-sampler (Model 3700, ISCO Inc., Nebraska USA) modified to collect 24 × 10 L samples. The sampler was controlled