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Application of a salivary immunoassay in a prospective community study of waterborne infections

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ABSTRACT

Quantifying sporadic waterborne infections in community settings can be challenging. Salivary antibody immunoassays are a promising non-invasive tool that can be used in prospective studies of common infections, especially those involving children.

This study was conducted in a Massachusetts city, which uses a microbiologically contaminated river as its water source, during summer-early winter periods before and after construction of a new drinking water treatment plant. Monthly saliva samples (7480 samples from 1170 children and 816 adults) were analyzed for immunoglobulin G (IgG) responses to recombinant proteins of *Cryptosporidium*, one genogroup I (GI) and two GII noroviruses. Immunoconversion was defined as at least four-fold increase in specific antibody responses between two monthly samples with a post-conversion response above a flexible age-dependent cut-off.

Episodes of gastroenteritis (diarrhea or vomiting or cramps) were associated with 3.2 (95% confidence limits 1.1; 9.5) adjusted odds ratio (aOR) of immunoconversion to *Cryptosporidium*; episodes of combined diarrhea and vomiting symptoms were associated with 3.5 (0.8; 15.0) and 4.6 (1.7; 12.6) aORs of an immunoconversion to GI and GII noroviruses, respectively. Swimming in natural water bodies or chlorinated pools was associated with 2.3 (0.4; 15.4) and 4.9 (1.6; 15.5) aORs of immunoconversion to *Cryptosporidium*, respectively. In a subset of study participants who did not use home water filters, consumption of at least some amount of non-boiled tap water reported in a monthly recall survey was associated with 11.1 (1.2; 100.0) and 0.6 (0.1; 2.5) aORs of immunoconversion to *Cryptosporidium* before and after the new water treatment plant construction, respectively. Among individuals who used home water filters, associations between non-boiled tap water consumption and *Cryptosporidium* immunoconversion were not significant before and after new plant construction with aORs of 0.8 (0.2; 3.3) and 0.3 (0.1; 1.6), respectively. The interaction effect of study phase and non-boiled tap water consumption on *Cryptosporidium* immunoconversions was statistically significant in the entire study population with aOR of 5.4 (1.1; 25.6).

This was the first study that has used a salivary antibody immunoassay to demonstrate significant associations between gastrointestinal symptoms and *Cryptosporidium* and norovirus infections, and between water-related exposures and *Cryptosporidium* infections.

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1. Introduction

Sporadic waterborne infections in tap water consumers and in recreational water users remain a considerable public health challenge in the US (Arnold et al., 2016; Ashbolt, 2015). Most previously conducted prospective epidemiological studies of sporadic waterborne infections used self-reported gastroenteritis as an

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outcome (Colford et al., 2006). However, obtaining sufficient statistical power to demonstrate waterborne transmission in non-outbreak settings can be problematic for studies which rely on non-specific symptoms as the outcome measure because a variety of pathogens transmitted through different routes can cause similar symptoms while many waterborne infections can be asymptomatic (Exum et al., 2016). Saliva sampling poses minimal risks and it is well tolerated by adults and children (Gammie et al., 2002; McKie et al., 2002). The use of saliva samples for quantitation of specific antibody responses to pathogens is a low cost, non-invasive alternative to the invasive blood sampling approach (Exum et al., 2016).

Cryptosporidium is a gastrointestinal protozoan parasite that is extremely chorine resistant in its environmental form, the oocyst (Collinet-Adler and Ward, 2010). This parasite causes approximately half of all illness outbreaks associated with recreational water in the US, and most outbreaks associated with chlorinated swimming pools (Hlavsa et al., 2015). It has also accounted for a majority of cases of illness in drinking water-related outbreaks in the US since 1971 (Craun et al., 2010). Two species cause most infections in humans: *C. parvum* can infect a wide range of animals, including humans, while *C. hominis* (previously known as *C. parvum* genotype 1) is a specialist parasite of humans (Leoni et al., 2006; McLauchlin et al., 2000).

The incidence of reported cryptosporidiosis in the US varies from approximately 1 to 4 cases per 100,000 persons per year (Painter et al. 2015, 2016). The incidence is highest in children under 10 years of age; the seasonal peak of infections typically occurs in August–October (Naumova et al., 2000). A substantial proportion of *Cryptosporidium* infections can be mildly symptomatic or completely asymptomatic while most cases of clinical cryptosporidiosis are not diagnosed or not reported to passive surveillance systems; therefore, the incidence of cryptosporidiosis is drastically underreported (Painter et al., 2016). Previous studies in Canada demonstrated that *Cryptosporidium* infections were very common in contrast with the low incidence of reported cases (Ong et al., 2005).

IgG responses to *Cryptosporidium* steeply increase within approximately two weeks after infection and then gradually decline to the pre-infection level within several months (Priest et al., 2001). Research in human volunteers demonstrated that most individuals develop antibody responses to certain immunodominant antigens following experimental infection, with IgG responses being a more accurate indicator of infection than IgA responses (Moss et al., 1998). Serum IgG responses to immunodominant *Cryptosporidium* antigens have been used as an indicator of incident infections in prospective study settings (Priest et al., 2005). Salivary IgG responses (but not salivary IgA responses) to the recombinant immunodominant gp15 antigen of *Cryptosporidium* have been linked with symptoms of gastroenteritis in a community study in Massachusetts (Egorov et al., 2010). Prospective serological studies utilized seroconversion to the same antigen as a biomarker of incident *Cryptosporidium* infections (Kattula et al., 2017; Sarkar et al., 2012). The antigenically identical Cp17 (17 kDa) antigen (Priest et al., 2000) has also been applied in a prospective population-based seroepidemiological study (Priest et al., 2006).

To reduce the risk of waterborne cryptosporidiosis in the US, the Environmental Protection Agency (EPA) promulgated the Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) which requires public water supply systems using surface water sources contaminated with *Cryptosporidium* oocysts to use advanced water treatment methods, such as ultraviolet (UV) light irradiation, to inactivate the *Cryptosporidium* oocysts (EPA, 2006).

Noroviruses are the most common cause of infectious gastroenteritis in the US, accounting for approximately 6% of acute gastroenteritis cases in the general population and 20% of gastroenteritis cases in children (Grytdal et al., 2016; Payne et al., 2013). The presence of diarrhea and vomiting symptoms is typical of norovirus infections (Rockx et al., 2002). Two genogroups cause most human infections: genogroup II (GII) noroviruses cause most outbreaks and account for the winter seasonal peak of norovirus gastroenteritis; genogroup I (GI) noroviruses are less common but their outbreaks are more likely to be associated with water exposure (Bitler et al., 2013; Matthews et al., 2012). There are more than twenty genotypes infecting humans with an even larger number of distinct norovirus variants (Parra et al., 2017). Antibody responses to specific noroviruses exhibit varying levels of cross-reactivity with other norovirus variants, with a greater degree of cross-reactivity within each genogroup (Malm et al., 2015; Parra et al., 2017; van Beek et al., 2016). Noroviruses are not very resistant to conventional chlorine treatment (Shin and Sobsey, 2008). Therefore, outbreaks of noroviruses related to drinking water tend to be associated with untreated ground water supplies or failures of surface water disinfection (Hlavsa et al., 2015; Maunula et al., 2005; Moreira and Bondelind, 2017). Outbreaks of norovirus infection associated with swimming in fresh water bodies have also been reported (Zlot et al., 2015). IgG responses to noroviruses steeply increase within approximately two weeks after infection and then gradually decline to the pre-infection level within several months (Tacket et al., 2003). Salivary antibody immunoconversion, or a steep increase in specific salivary antibody responses between consecutive samples, can be used as an analogue to seroconversion in prospective studies in order to detect incident norovirus infections (Griffin et al., 2015; Moe et al., 2004). It has been shown that salivary IgG immunoconversion is a better indicator of incident norovirus infection than salivary IgA immunoconversion (Griffin et al., 2015).

The main objective of this prospective observational study was to apply a multiplexed salivary antibody assay previously developed by EPA (Augustine et al., 2016; Griffin et al. 2011, 2015) to assess potential beneficial impacts of improving treatment of municipal drinking water in a selected community on waterborne transmission of *Cryptosporidium* and norovirus infections. Secondary objectives were to assess potential associations between recreational water exposures and these infections, and between episodes of gastrointestinal symptoms and these infections.

2. Methods

2.1. Study settings

This prospective cohort study was conducted in the city of Lawrence, Massachusetts (population 75,000), which uses the microbiologically challenged Merrimack River with multiple combined sewer overflow (CSO) discharge sites upstream of its water intake as the sole source of drinking water. Recent studies have suggested a potential association between CSO events and emergency room visits in eastern Massachusetts communities that derive their drinking water from sewage-contaminated rivers (Jagai et al., 2015). Prior to April 2007, the city of Lawrence had an aged (built in the 1930s) and outdated water treatment plant utilizing conventional water treatment methods (primary chlorination, coagulation, filtration and secondary chlorination). The plant could no longer reliably meet the existing drinking water quality regulations and comply with the incoming LT2ESWTR requirements. Therefore, the city built a new water treatment plant designed to comply with LT2ESWTR and to reduce drinking waterborne transmission of *Cryptosporidium*. The treatment regimen at the new plant involved chlorine dioxide disinfection, polymer-aided coagulation, flocculation and clarification in superpulsator clarifiers, granular activated carbon filtration, UV light disinfection, and