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Review

Evolution of monitoring for *Giardia* and *Cryptosporidium* in water

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ABSTRACT

This review describes the evolution of monitoring methodology for *Cryptosporidium* and *Giardia* in water since the 1970's. Methods in current use for *Giardia* and *Cryptosporidium* in water are highlighted, though attention is given to all available published methods by country and continent. The review is intended to stimulate research leading to future improvements and further developments in monitoring methodology for *Giardia*, *Cryptosporidium* and other waterborne protozoan parasites in water.

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1. Introduction

Cryptosporidium and *Giardia* are parasitic protozoa that constitute the leading causes of waterborne enteric disease outbreaks worldwide (Karanis et al., 2007a; Baldursson and Karanis, 2011; Efstratiou et al., 2017), and can infect a wide range of vertebrate hosts. Species within these genera cause human cryptosporidiosis and giardiasis, generating significant morbidity and mortality in both the developing and developed world. A brief summary of morbidity from statistics in Europe (European Centre for Disease Prevention and Control, 2010) and the USA (Painter et al., 2015) indicates that incidence of giardiasis in industrialized regions is approximately 6–8/100,000 per year, typically 5 times that of cryptosporidiosis, around 1–2/100,000 per year. Transmission occurs following direct or indirect contact with the transmissive stages of the parasites through the fecal-oral route by a variety of mechanisms, including person-to-person, zoonotic, and consumption of contaminated water and food. Monitoring of surface water to define the characteristics of *Cryptosporidium* and *Giardia* presence has a significant history, with initial efforts for their detection in water being reported as early as the 1970's. This review is intended to trace the evolution of monitoring methodology, as well as relate technological development to fundamental objectives in light of information available through the course of the last 40 years.

1.1. Literature search strategy and selection criteria

The scope of this review of monitoring methodologies for *Cryptosporidium* and *Giardia* was based on a search of the literature databases PubMed and Web of Science. In these databases the terms "water (and) *Cryptosporidium*", "water (and) Cryptosporidia", "water (and) cryptosporidiosis", "water (and) *Giardia*", "water (and) giardiasis", "waterborne (and) parasite(s)", "waterborne (and) parasitic" and "waterborne (and) protozoa(n)" were applied and the listed articles were reviewed. The terms "*Cryptosporidium* (and) concentration", "*Cryptosporidium* (and) filtration", "*Cryptosporidium* (and) purification", "*Cryptosporidium* (and) detection", as well as "*Giardia* (and) concentration", "*Giardia* (and) filtration", "*Giardia* (and) purification" and "*Giardia* (and) detection" were also used to ensure all available methodologies in the current bibliography were included. The obtained literature covers a period of approximately 40 years, from the late 1970's until December 2016, and includes more than 600 relevant papers. For the aim of this review, each paper was examined and the monitoring methodologies described were taken into consideration.

2. Background

2.1. Scope

As presented below, hundreds of efforts have been described in the literature addressing selected components of the problems encountered in monitoring these parasites in water. To provide context for this review, it is crucial that basic objectives and background details are clearly defined. The objective of monitoring any water source is to find and quantify *Cryptosporidium* oocysts and *Giardia* cysts, with secondary objectives related to their viability and human infectivity. The purpose of sampling and analysis is the collection of information permitting quantitative understanding of public health risks in relation to public water supply. The principal uses of this information are to provide a basis for catchment/watershed management, water treatment design and operation, and public water supply advice and regulation. Although closely related and subject to similar principles, sampling and analysis of wastewater and sewage effluent will not be reviewed here.

2.2. Monitoring context and requirements

Both *Cryptosporidium* and *Giardia* are human pathogens of worldwide distribution and significant presence in ambient surface water. They are also common and universally distributed pathogens of virtually all mammalian as well as avian and reptilian species, with animals in large-scale commercial production being of particular interest to public water supply catchments/watersheds. Both *Cryptosporidium* and *Giardia* have many defined species, yet only a minor fraction has been identified as infective of humans (Cacciò and Ryan, 2008; Plutzer and Karanis, 2009; Xiao, 2010). Nevertheless, whether to direct monitoring to quantifying all (oo) cysts or to a refined subset consisting of viable and potentially infective organisms is a matter of debate and requires a management decision. *Cryptosporidium* is ubiquitous and the water authorities are only concerned about the dominant human infective species (*C. parvum* and *C. hominis*), so there is a strong expectation from health regulators for better data on infectivity and species presence, not just total oocyst loads. Some countries may take into account the infectivity in their next revision of drinking water guidelines when implementing treatment targets for managing *Cryptosporidium* risk. Treatment targets will be set to achieve health-based outcomes.

The overall monitoring procedure in use today has been developed for the distinguishing characteristics of the two target organisms. *Cryptosporidium* oocysts and *Giardia* cysts exist among other naturally occurring particles in various sources including tap, surface, and wastewater. Distinctive characteristics are the size of the organisms, 3 × 5 μm for *Cryptosporidium* and 7 × 12-15 μm for *Giardia*, and their presence at low concentrations among a vast array of naturally occurring particles, a large proportion of which are of similar size and specific gravity. Although oocysts and cysts in a water sample may be alive, they cannot reproduce, multiply or be cultured as in the case of bacteria. Furthermore, a preponderance of data shows ambient concentrations are often below the limit of detection, typically ca. 0.2–1/L when using 10 L samples according to the US Environmental Protection Agency (USEPA, 1995) Methods 1622 and 1623 (USEPA, 2005). Even in low turbidity surface water, each liter contains more than 10^6 particles in the 1–25 μm range (Ongerth, 2013a). As will be discussed further below, any ability to recover the target organisms is highly dependent on the quality of the water analyzed.

In a review and evaluation of approaches and procedures for monitoring *Cryptosporidium* and *Giardia,* details of sampling strategy are inevitably involved. Key issues pertaining to generating useful information include the sample volume, the total particle concentration that can be processed, and whether to collect grab or composite samples. At a typical cost of $250-$400 US for processing a single 10 L sample, water quality will have a significant influence on procedure. Other issues that may affect selection of monitoring details include monitoring of raw or treated water, the frequency of sample collection, and the value and interpretation of negative analytical results. In this review, these factors will have a bearing on the suitability and utility of individual sample processing components to produce the required information.

To satisfy the objectives stated above, any monitoring procedure must be capable of identifying the target organisms among assemblages of naturally occurring particles. As turbidity and particle concentrations increase, this task becomes progressively challenging. Criteria for evaluating the effectiveness of method components should thus include the ability to efficiently process volumes of at least 10 L, separate and concentrate the particle assemblage, selectively segregate the target organism incurring