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Use of Pathogen-Specific Antibody Biomarkers to Estimate Waterborne Infections in Population-Based Settings

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Abstract

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Compliance with Ethical Standards

Conflict of Interest Douglas A. Granger is the founder and chief scientific and strategy advisor at Salimetrics LLC and SalivaBio LLC, and these relationships are managed by the policies of the committees on conflict of interest at the Johns Hopkins University School of Medicine and the University of California at Irvine. All other authors declare no conflict of interest.

Human and Animal Rights and Informed Consent This is a review article which does not report new results of human or animal subjects performed by the authors.

Purpose of review—This review discusses the utility of pathogen-specific antibody biomarkers for improving estimates of the population burden of waterborne infections, assessing the fraction of infections that can be prevented by specific water treatments, and understanding transmission routes and the natural history and ecology of disease in different populations (including asymptomatic infection rates).

Recent findings—We review recent literature on the application of pathogen-specific antibody response data to estimate incidence and prevalence of acute infections and their utility to assess the contributions of waterborne transmission pathways. Advantages and technical challenges associated with the use of serum versus minimally invasive salivary antibody biomarkers in cross-sectional and prospective surveys are discussed.

Summary—We highlight recent advances and challenges and outline future directions for research, development, and application of antibody-based and other immunological biomarkers of waterborne infections.

Keywords

Waterborne infections; Pathogens; Biomarkers; Antibodies; Serology; Immunoconversion; Saliva

Introduction

Waterborne infections cause an estimated two million deaths and four billion episodes of diarrheal illness per year worldwide [1]. Waterborne diseases will continue to be of broad public health importance as peri-urban populations rapidly expand at a pace that exceeds developing countries' abilities to invest in infrastructure [2]. While most of these illnesses occur in developing countries, industrialized countries also bear a substantial burden of waterborne diseases [3]. For high-income countries, if investments in water supply and sewer systems do not enable proper maintenance and timely replacement of aging infrastructure, the risk of waterborne infections is likely to increase [4].

Waterborne disease outbreaks are defined as two or more persons experiencing a similar illness after exposure to water where epidemiologic evidence implicates water as the probable source of the outbreak [5]. Waterborne pathogens that result in human infections include bacteria (e.g., *Campylobacter* spp., *Shigella* spp.), viruses (e.g., norovirus, rotavirus), and protozoa (e.g., *Cryptosporidium* spp., *Giardia* spp.), and these pathogens may be conveyed to humans via drinking and/or recreational water transmission routes [6]. The health outcome most commonly associated with exposure to waterborne pathogens is acute gastrointestinal illness (AGI). AGI is defined in various ways, and definitions used in epidemiological research range widely [7]. One commonly used definition is as follows: diarrhea (three or more loose stools in a 24-h period), vomiting, nausea, stomach ache, fever, and/or interference with regular activities (missed time from work or school or missed regular activities as a result of illness) [8–10]. Other illnesses caused by waterborne pathogens include viral hepatitis (hepatitis A and E viruses [11]), skin and soft tissue infections and sepsis (*Vibrio* spp., *Staphylococcus aureus* [12]), primary amoebic meningoencephalitis (*Naegleria fowleri* [13]), and pneumonia (*Legionella pneumophila* [14]).

In this review, we summarize the latest evidence on use of pathogen-specific antibodies as biomarkers (defined as "any substance, structure, or process that can be measured in the body or its products and can influence or predict the incidence of outcome or disease" [15]) of infection for the waterborne pathogens that cause the greatest population burden of AGI in the USA (norovirus, Shiga toxin-producing *E. coli*, and *Cryptosporidium* spp.) [16] and in developing countries globally (rotavirus, *Cryptosporidium* spp., *Shigella, Giardia* spp., *Vibrio cholerae*, and *Campylobacter* spp.) [17, 18]. We also include hepatitis A and E viruses because these pathogens are the most common causes of feces-transmitted acute viral hepatitis worldwide (Table 1) [19, 20]. Such pathogen-specific antibody biomarkers represent promising tools to identify causative agents in population-based studies of AGI, including waterborne disease outbreak investigations, surveillance studies, and observational and randomized intervention studies to test hypotheses related to transmission routes, water treatments, and disease ecology. Because not all individuals who become infected with waterborne pathogens will experience symptoms of AGI—i.e., a waterborne infection may be asymptomatic (without clinical disease) or symptomatic (clinical disease observable) [21]—biomarkers of host immunological response can be used to identify a causative pathogenic agent and estimate symptomatic and/or asymptomatic waterborne disease burden. Knowledge of the waterborne pathogens responsible for asymptomatic infections can improve estimates of waterborne infections in source populations and advance understanding of upstream risk factors and transmission routes. Not knowing these can hinder the development of effective prevention strategies to reduce waterborne outbreaks and/or contamination events (e.g., via infrastructure improvements or other interventions prior to onset of symptoms).

We review the challenges in measuring population burdens of infection that can be attributed to waterborne versus other transmission routes (contaminated food, hygiene, sanitation, person-to-person and animal-to-person contact). Antibodies as biomarkers of waterborne infections are then discussed to highlight their current and future utility in population-based settings. Antibody responses to specific pathogens are described as they relate to measuring immunoconversions (defined as a change from antibody negative to antibody positive in serial samples or a four-fold increase in antibody titer in serial samples), rates, and time intervals of infection. The use of antibody biomarkers in serum is presented, followed by the discussion of novel salivary antibody biomarkers and their potential to improve upon estimates of waterborne infections. The utility of antibody biomarkers for detection of acute and chronic infections in population-based settings is discussed, including how estimates of the incidence of acute short-term infections can be obtained within the context of both cross-sectional and prospective study designs. Finally, the technical challenges involved with using minimally invasive saliva samples as a matrix for the detection of pathogen-specific antibodies are presented along with future directions for salivary immunoassay work.

Challenges with Epidemiologic Estimates of Waterborne AGI in Population-Based Settings

The outcome most commonly employed in epidemiologic studies of waterborne disease is self-reported AGI symptoms. Because most AGI symptoms are self-limited, only a small proportion of the individuals who experience AGI actually seek medical care and have a stool sample submitted for testing. Furthermore, clinical diagnostic laboratories are not

always able to identify a pathogenic agent responsible for AGI symptoms [22]. Thus, only a small proportion of AGI disease will be captured by studies of, or reporting systems involving, patient populations seeking a clinical diagnosis (Fig. 1). AGI symptoms are also non-specific, with numerous pathogens and transmission routes that must be investigated in order to determine the etiologic agent. These features of AGI symptoms mean that epidemiologic studies that rely upon AGI as a primary outcome may not provide an accurate estimate of the population burden of disease. The ability to determine a host's immunologic response to specific pathogens that are responsible for waterborne infections could improve the specificity and decrease the misclassification of AGI in epidemiologic studies. Biomarkers of pathogen-specific host immunologic response could improve studies of the effects of improved water treatment and/or source water protection as well as advance understanding of pathogen exposure (e.g., spatial and temporal distribution) and modifiable factors that are associated with progression from asymptomatic to symptomatic states of infection (e.g., natural history and ecology of disease) in populations. For example, objective biomarkers of asymptomatic waterborne infections have helped identify low water pressure at the faucet as an important risk factor for self-reported diarrhea in the control group of a case-control study of sporadic cryptosporidiosis [23].

Most evidence of waterborne transmission in developed countries comes from outbreaks of infectious diseases. In the USA, the Centers for Disease Control and Prevention (CDC) as well as state and local authorities investigate outbreaks and attempt to identify the source. CDC publishes the biannual Morbidity and Mortality Weekly Report on outbreaks associated with drinking and recreational water sources. For example, in 2011–2012 for drinking water, a total of 32 outbreaks were reported and associated with 431 illnesses, 102 hospitalizations, and 14 deaths [16]. For recreational water in 2011–2012, there were 90 outbreaks that resulted in at least 1788 cases, 95 hospitalizations, and one death [24].

Knowledge of the pathogen-specific etiology of waterborne infections would help identify different risk factors and transmission routes, which can improve the evidence base for decision making about management and prevention strategies. A classic example of this is the massive waterborne outbreak of cryptosporidiosis in Milwaukee in 1993 when the chlorine-based disinfectant used had little effect on *Cryptosporidium parvum* oocysts and the drinking water treatment plants consequently had to investigate alternative disinfectants such as UV light [25]. Another example is a study of the presence of enteric viruses in non-disinfected drinking water from municipal wells and their relation with community incidence of AGI [26]. In this study, the authors noted a positive association between norovirus genogroup I (GI) and AGI. But, the associations between the presence of other enteric viruses—adenovirus and echovirus serotypes—and AGI were not statistically significant. This lack of association could be due to misclassification and/or the non-specificity of AGI as an outcome in epidemiologic studies (e.g., potential influence of measurement error due to participant self-reporting of AGI symptoms).

Waterborne outbreaks usually occur from causative factors such as weather events, wastes from animals, agriculture, or humans, and failures in water treatment [27]. Drinking water-associated outbreaks are often caused by contaminated source waters, inadequacies in treatment, or contamination occurring within the distribution system [28]. Whereas,

recreational water-associated outbreaks have been attributed to swimming in waters impacted by inadequate chlorination or other disinfection (swimming pools) [29], fecal contamination shed by swimmers (swimming pools and natural waters) [30], runoff from publicly owned treatment work (POTW) wastewater effluents, sanitary and combined sewer overflows of untreated sewage, private on-site septic systems, agricultural production, and wildlife [31].

Most cases of waterborne infections are sporadic or diffuse, low-level outbreaks. Ingestion of waterborne pathogens can also result in a completely asymptomatic infection depending on the interplay of pathogen-specific and host-specific factors, such as a pathogen's virulence and a host's immune response [32]. They may be caused by deficiencies in drinking water treatment, resulting in contamination with waterborne pathogens, and transmission to consumers [33]. Waterborne pathogens that are resistant to chlorination (especially *Cryptosporidium* spp.) [34] or physical removal (especially viruses) can pass through the water treatment barrier and contaminate tap water even when water quality indicators based on surrogate bacteria (total and/or fecal coliforms, *E. coli*) are within the regulatory limits [35]. Viruses, such as noroviruses, can filter through the soil, contaminate shallow groundwater sources and present a health risk in drinking water systems that are groundwater supplied and do not use chemical disinfection [26]. Individual sporadic cases of AGI usually cannot be linked to a specific source in the framework of routine surveillance, contributing to the underestimation of waterborne infections in the population.

Antibody Biomarkers of Waterborne Infection

Specific antibody responses can be used as biomarkers of infection in epidemiological studies to estimate the prevalence and incidence of infections and to assess the contribution of waterborne transmission. Different pathogens result in different temporal distributions of antibody response and infection. Both symptomatic and asymptomatic infections typically cause an antibody response in the host [33]. A preexisting antibody response can be a factor affecting host's susceptibility to re-infection or the probability of developing symptoms if infection occurs [36]. The presence of antibodies specific to the pathogen of interest in biological samples (e.g., serum, saliva, stool, breast milk) is an indication of current or prior infection [33]. The major immunoglobulin isotypes (IgG, IgA, IgM) have different utility as estimates of population disease frequency and burden. Single time-point measurements of pathogen-specific IgG have utility as an estimate of historical/prior exposure or prevalent infection, whereas IgA and/or IgM has utility as an estimate of acute phase or incident infection [37, 38]. Immunoconversion is used to detect incident infections in prospective survey settings. This change from an antibody-negative sample to an antibody-positive sample in a time series of two or more samples, or a fourfold increase in antibody titer in a time series of two or more antibody-positive samples, is used to measure new, acute cases in a defined population over a defined time period [39–41].

Serologic Antibody Response

Serum is the most accurate and widely used matrix to monitor population immune responses to pathogens. Sera can be collected by sampling populations, or residual blood banks can be used. However, there are significant drawbacks to both since blood collection requires

trained individuals to visit participants [42] and may be cost prohibitive along with low response rates that have been shown in Europe due to the invasive nature of blood collection [43, 44]. Its application in prospective studies and especially in studies involving children is problematic due to high attrition and low compliance [45]. Relying on previously collected samples from sera banks overcomes these issues; however, they are usually anonymous with limited data available on the patient and, importantly, their background as it pertains to water, sanitation, and hygiene-related behaviors and activities [46]. However, a number of studies have successfully used seroepidemiological methods in the context of waterborne disease [47, 48]. Frost et al. found that people who live in cities using surface-derived drinking waters had an increased risk of *Cryptosporidium* infection compared to those using drinking water from municipal groundwater sources [47]. And, in the context where sanitation conditions are poor and clean water supplies are limited, Priest et al. found IgG antibody responses during *Cryptosporidium* infections with *C. parvum, Cryptosporidium felis*, and *Cryptosporidium meleagridis* and with four different subtypes of *Cryptosporidium hominis* [48].

Salivary Antibody Response

The utility of novel salivary antibody biomarkers as a measure of host immune response to specific pathogens has the potential to improve upon estimates of waterborne infections that rely on invasive collection of serum. Saliva collection is minimally invasive and can be self-collected and returned by mail [49••], allowing for a larger sampling of the population than is possible with serum. Saliva is a mixture of secretions from salivary glands. Oral fluid contains saliva (enriched with secre-tory IgA) and crevicular fluid (flows from between the gum margins and teeth) and is enriched with serum antibodies [50]. Some oral fluid sampling techniques are specifically designed to collect samples enriched with crevicular fluid for measurements of systemic antibody responses [51, 52, 53•].

Salivary assays have been used to identify various viral, bacterial, and parasitic infections [54] (see Table 2). Measuring antibodies in saliva is appropriate for both children and adults and is suitable for population-based surveillance settings [40]. Salivary immunoassays have been developed for pathogens such as Helicobacter pylori, Toxoplasma gondii, Cryptosporidium, and noroviruses [52••]. Griffin et al. (2011) applied the Luminex xMAP microsphere-based technology (Luminex Corp., Austin, TX) assay to measure antibodies to multiple pathogens within a single saliva sample volume [52••]. The Norwalk virus assay developed in Griffin et al. (2011) was subsequently validated using samples from a human volunteer challenge study [53•]. A similar salivary immunoassay is being applied to measure the incidence of norovirus infections following recreational water exposures at beaches in Puerto Rico, Iowa, and Wisconsin where saliva has been collected as part of the Environmental Protection Agency's National Epidemiologic and Environmental Assessment of Recreational Water Study [55].

An important challenge in using saliva to measure immunologic responses is the greater inter- and intra-individual variability in saliva composition and immunoglobulin levels. While saliva contains a high level of secretory IgA (SIgA) antibodies, there can be significant diurnal, age, and oral health-related variability [56], making these factors

important to consider in community-based field studies. The salivary concentrations of IgG and IgM isotypes are lower than in serum. Thus, a salivary antibody assay targeting IgG has to be sensitive enough to quantify low-intensity antibody responses. Typically, it is necessary to assay saliva at relatively low dilutions, where matrix effects (e.g., inhibition, high background signal) can be pronounced in some pathogen-specific antibody assays [57]. For each pathogen-specific antibody target, it is critical to optimize the conditions that may influence assay performance and sensitivity and specificity [53•].

There is scant evidence on the temporal patterns of salivary antibody responses to infection with a specific pathogen (peak levels and rates of decline for different antibody isotypes). Our current understanding of generalized trajectories (Fig. 2) comes from prospective studies using serum or saliva from individuals with confirmed infections, such as volunteer challenge studies for norovirus [40, 58, 59], *Cryptosporidium* [60], *Giardia lamblia* [61, 62], and *Shigella* [63]. The pattern of antibody isotypes may be used in diagnostic and research settings to provide information on the infection state (acute versus convalescent) and to assess the timing of infection [33]. Typically, the IgA and/or IgM response to a waterborne pathogen ramps up before the IgG response [36, 58, 59]. The generalized trajectories of different antibody isotype levels during a transient acute infection from a waterborne pathogen are depicted in Fig. 2. After the convalescent stage, IgG pathogen-specific antibodies may remain detectable for weeks to years, depending on the causative agent, and may remain elevated above preinfection levels [36, 64]. There can be vast differences in these temporal patterns of antibody responses depending on the pathogen causing the infection. Thus, an area of future work is to develop population-based antibody infection curves for specific waterborne pathogens.

Platforms and Assay Types

Various immunoassay platforms have different costs, quantitation levels, dynamic ranges, and multiplexing potentials [65]. The most basic of these platforms is the indirect enzyme immunoassay; however, the low through-put and high sample volume requirements make it less desirable for population-based analyses where multiple pathogens are being analyzed and sample volume is limited. Multiplex immunoassays, such as those based on the Luminex (Luminex Corp., Austin, TX) microbead suspension fluorescence immunoassay platform, require a low sample volume to analyze multiple pathogen-specific antibody analytes simultaneously. They are also less labor intensive because more data are generated per test/analyte and thus are more cost-effective [52••, 53•, 66–69]. Another immunoassay platform that is used and allows multiplexing is the Meso Scale Discovery (MSD; Rockville, M D) electrochemiluminescence (ECL) platform. Platforms that facilitate multiplexing can be used to expand the range of available options for testing the signal of pathogen-specific antibody responses as well as background signals. The adjustment of the pathogen-specific antibody signal for background signals, such as those produced by total IgA or total IgG or by antigen tags such as glutathione-S-transferase (used during antigen purification), can improve the performance of antibody assays [52••, 53•]. Multiplexing of these target signals can also reduce excess use of biospecimen sample volume because all signals can be measured in one sample volume in a single reaction well. Thus, multiplexing testing platforms can facilitate a broader application of antibody testing of serum and/or saliva

biospecimens in population-based epidemiologic investigations of diverse waterborne pathogens.

Applications of Pathogen-Specific Antibody Biomarkers in Population-Based Studies of Waterborne Infections

To improve current epidemiologic estimates of AGI from waterborne pathogens in population-based settings, pathogen-specific antibody biomarkers can be used. For chronic infections, antibody responses can be positive or negative and can be validated against diagnostic tests. The proportion of IgG-positive results in serum or saliva can serve as a direct measure of infection prevalence in the population [70, 71]. In contrast, for acute short-term infections, such as noroviruses and *Cryptosporidium*, the presence of pathogen-specific antibodies in serum or saliva may indicate an ongoing infection or more commonly a past infection with or without symptoms. Thus, the concept of “positive” antibody response to an acute short-term infection or seroprevalence of positive responses often reflects the proportion of results above an arbitrary threshold, such as a detection limit of the method or by standardizing response intensities to the response of a reference sample of positive control sera [72–75] or saliva.

One approach to estimating incidence of acute infections using antibody data is to use immunoconversion in prospective study settings as a marker of new infections. The sensitivity and specificity of an immunoconversion test are related to its ability to detect infections that occur during the interval between two sampling dates. In prospective studies, biological sampling (serum or saliva) can be combined with symptom diaries to produce information on the association of certain infections with specific types of symptoms and/or the association of exposures with infections or interventions (designed to reduce exposure) with a lack of symptoms [76].

Prior studies have used pathogen-specific antibody markers and demonstrated their ability to identify waterborne infections that were more widespread than previously appreciated. In the massive *Cryptosporidium* outbreak in Milwaukee in April 1993, a retrospective analysis was conducted with banked serum specimens from children that had routine lead level surveillance in blood from March to May of that year and showed a seroprevalence increase from 15–17 % to 82–87 % for levels of IgG antibody against the immunodominant Triton-17 and 27 kDa *C. parvum* antigens [77]. This demonstrated that the outbreak had affected a greater proportion of the population with infection when accounting for both symptomatic and asymptomatic infections than the previous estimate of 26 % that only surveyed the population using the cryptosporidiosis case definition (watery diarrhea) [78]. Teunis et al. applied these approaches in the European Union to estimate seroconversion rates for *Campylobacter* infections and found that they were several orders of magnitude higher than the notification rates, reflecting both detection deficits in the surveillance and the reality that these enteric infections often remain asymptomatic [79]. Frost et al. used serum antibodies to *Cryptosporidium* from a population in Hungary to determine that those using groundwater had significantly lower serological responses than those using conventionally filtered and disinfected surface water and found that riverbank filtration may be an effective alternative treatment to reduce *Cryptosporidium* exposures and infections for individuals using surface

water sources [80]. Tollestrup et al. focused on non-outbreak settings where a low probability of outbreak detection should be expected and found a significant association for residents in the River Valley of New Mexico using onsite wastewater systems combined with private wells to have a strong response to the 27 kDa *Cryptosporidium* antigen [75]. And lastly, in the first postal population-based survey that used saliva, Morris-Cunnington et al. used approximately 5500 self-collected oral fluid samples along with a questionnaire of demographic and social information to successfully demonstrate that antibody prevalence data along with risk factor data can be used to assess the population-based immunity to common viral infections in England and Wales [49••].

Such applications of immunological biomarkers in epidemiologic studies also can improve knowledge of the temporal patterns of antibody responses, which can be used to extrapolate incidence estimates based on cross-sectional data on pathogen-specific antibody responses in the population [79, 81, 82]. Others have expanded this approach using parametric statistical models [67, 83–85] to determine incidence of infection based on pathogen-specific antibody results from a single cross-sectional sampling time. The person-to-person variability in antibody responses to a specific pathogen and limited data on temporal patterns of antibody responses in various populations affect the precision of such estimates. A pattern of antibody responses may also be affected by the number of prior infections and the time interval since the previous infection. This may further limit the applicability of the available antibody pattern data to populations with comparable epidemiological characteristics or to research questions focused on intra-individual variability in antibody responses over time.

In low-income communities where there is less developed drinking water and wastewater infrastructure and individuals may experience repeated exposures to multiple waterborne pathogens, the application of immunological biomarkers can be used as a monitoring and evaluation tool for infrastructure and point-of-use interventions. The multiplex immunoassay methodology targeting salivary IgG and IgA responses to potentially waterborne pathogens [52••] can be applied as a minimally invasive and objective exposure and outcome screening tool to assess the efficacy of interventions designed to reduce pathogen exposure and/or AGI illness within a specified population. Such multiplex pathogen antibody measurements could improve the evaluation and prioritization of a range of water, sanitation, hygiene, and health programs and interventions. Integration of these biomarkers into monitoring activities for the Sustainable Development Goals recently adopted at the 2015 UN Summit (https://sustainabledevelopment.un.org/topics) could improve the evidence base for improved health outcomes related to Goal 6, which is to “by 2030, achieve access to adequate and equitable sanitation and hygiene for all and end open defecation” (Target 6.2) [86].

Biomarkers of pathogen-specific antibody response can also be used to improve monitoring and evaluation of vaccination coverage for specific waterborne infections. Several waterborne pathogen vaccines for which antibody response data can be generated include *Shigella* [87], rotavirus [88], cholera [89, 90], and hepatitis A [91] and E [92], among others. Such pathogen-specific antibody response biomarkers have particular utility in remote, resource-limited population-based settings because they can provide objective measures of vaccination coverage when paper-based records and/or recall of vaccination history is lacking.

Challenges and Perspectives for Future Work

Pathogen-specific antibody assays represent a promising tool for understanding the relative contribution of waterborne versus other pathways to infectious disease burden in population-based settings. However, assays based on invasive serum specimens may fail to capture a majority of cases in population-based field studies. Because saliva swabs can be self-administered and returned by mail [49••], salivary antibody assays may increase participation in surveys of potentially waterborne infections in populations that are difficult to reach, including children, pregnant women, and individuals living in remote, resource-limited settings. This may facilitate a more fine-scale, spatiotemporal study of the ecology and natural history of waterborne disease, including elucidation of optimal points of intervention to prevent waterborne pathogen transmission.

While such minimally invasive pathogen-specific salivary antibody biomarkers are promising, challenges remain in their broad application to diverse pathogen exposures and infections. Not all pathogens elicit a robust systemic or salivary antibody response. Additionally, a majority of waterborne infections may be asymptomatic and not result in adverse health effects. Therefore, the incidence of infections estimated from cross-sectional antibody data may not be representative of disease burden but only reflect recent or historical exposure to a pathogen [93]. Nevertheless, cross-sectional antibody response data can provide an improved estimate of human exposure to specific pathogens and can be used as an epidemiological tool to estimate the contribution of waterborne versus other pathways to the total infection pressure. However, the underlying infection and immune response to the pathogen must be considered in the interpretation of cross-sectional seroprevalence estimates and depend on whether the infection results in lifetime immunity following one exposure or the infection is acute and immunity wanes following exposure.

The detection of cytokines in serum and saliva also presents an opportunity to measure the onset of waterborne infections. However, cytokines are not capable of identifying a specific causative agent; rather, they are more generic biomarkers of infection. The hallmark for a viral infection begins with a wave of cytokine production [94], and their presence can be employed as a marker of infection (Table 2). Cytokine levels in serum of individuals infected with norovirus that were shown to be significantly increased included IFN-gamma, interleukin 6 (IL-6), IL-8, IL-12p70, MCP-1, and TNF-alpha 2 days following exposure [95]. Evidence has shown that the elevation of cytokines in a newborn's salivary gland epithelium promotes secretory immunity [96]. Proinflammatory cytokines can upregulate the polymeric Ig receptor (pIgR), including IL-17, which is particularly abundant at mucosal sites [97]. The extracellular part of pIgR is essential for resistance against proteolytic degradation of the secretory component of IgA (SIgA) found in saliva and the gut mucosa [98]. A challenge in using cytokines in saliva is to determine if there is a serum-saliva association, for which there is currently limited evidence [99]. The most likely hypothesis is that much of the variation in salivary cytokines (e.g., IL-1b, TNF-α, IL-6, IL-8) may be due to inflammatory processes in the mouth caused by poor oral health [100] and/or other disease processes [101–103]. However, there could be specific hyper-inflammatory physiological states (systemic infection/sepsis, burns, etc.) when more of the variance in salivary levels of cytokines could be due to systemic circulating cytokine levels [99]. IL-6,

which has a major role in the regulation of inflammatory processes, was found to be elevated in concentration in both the saliva and serum of inflammatory bowel disease patients when compared to reference persons [104]. An area for future study is identifying if a specific waterborne pathogen generates a unique or predictive cytokine profile that is observable in both saliva and serum.

Conclusion

The ability to estimate waterborne infections via measurements of host immunological response at the population level is improving as technological and analytical advancements are made. Diagnostic advancements are enabling a paradigm shift in how waterborne infections can be measured, not just in clinical settings or outbreak settings but also more widely as tools for population-based screening of incidence and prevalence. The measurement of salivary antibody responses to specific pathogens as biomarkers of waterborne infection holds great potential to expand surveillance to reach larger numbers of people in diverse population-based settings. Future work lies in the development of sensitive and specific multiplexed serum and salivary immunoassays to measure exposures to, and infections with, specific waterborne pathogens.

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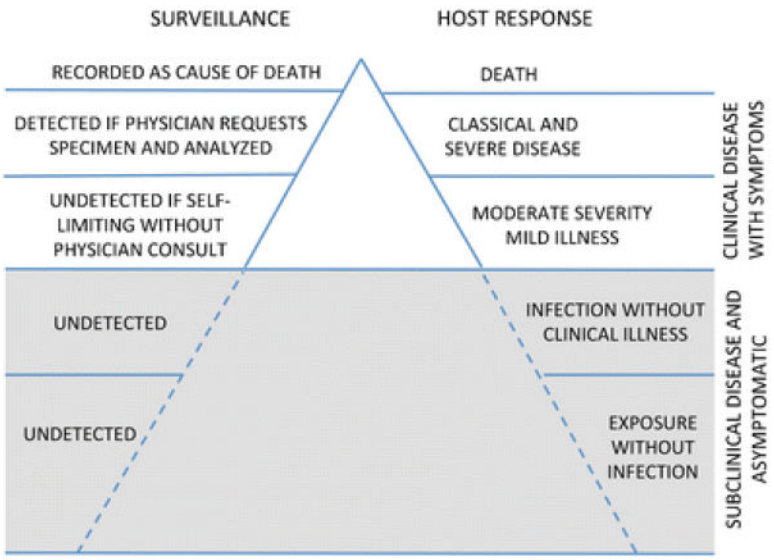

Fig. 1.

The iceberg concept of waterborne infection surveillance. Some of the “host response” information listed in Fig. 1 is adapted from Kaslow et al. [105]

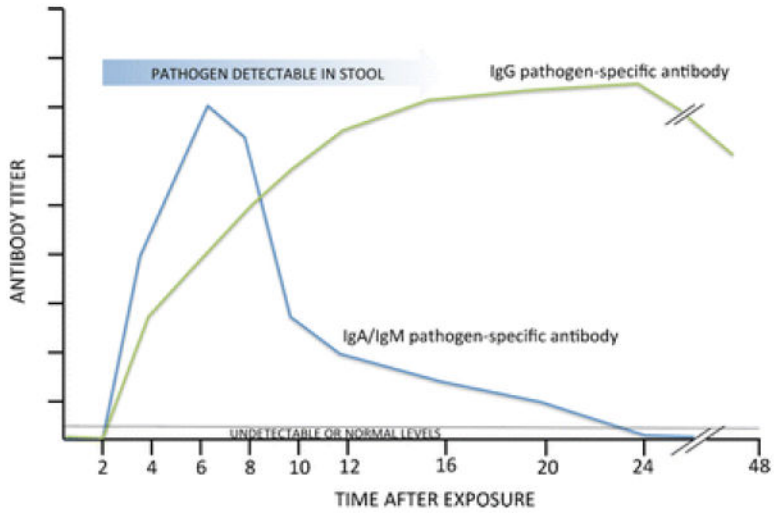

Fig. 2.

Temporal pattern of antibody responses during infection with a waterborne pathogen

Table 1

Studies that measured specific waterborne pathogens and estimated which are responsible for the greatest population burden of waterborne infection

Region	Data source	Top waterborne pathogens identified
USA	CDC Morbidity Mortality Weekly Report (MMWR) Surveillance for waterborne disease outbreaks associated with drinking water, 2011–2012 [16]	Norovirus and Shiga toxin-producing *E. coli*
	CDC MMWR for Outbreaks of illness associated with recreational water, 2011–2012 [24]	*Cryptosporidium* spp.
Developing countries	The Global Enteric Multicenter Study (GEMS)[17]	Rotavirus, *Cryptosporidium* spp., *Shigella, Giardia* spp.,[a] *Campylobacter* spp., *Vibrio cholerae*[b]
	The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) [18]	*Giardia* spp.[c]
	Ishii et al. (2015) [19] and Hoomagle et al. (2012) [20]	Hepatitis A and E virus[d]

[a] In univariate analyses, *Giardia* was identified significantly more frequently in controls than in patients with moderate-to-severe diarrhea aged 12–59 months in ten of the 14 age-site strata [17]

[b] Important in selected sites in GEMS study [17]

[c] *Giardia* spp. were among the top five pathogens in terms of the highest prevalence in diarrheal and non-diarrheal stools for both the 0–11- and the 12–24-month age groups [18]

[d] Hepatitis A and E viruses are the most common causes of feces-transmitted acute viral hepatitis worldwide [19, 20]

Table 2

Immunological biomarkers of infection for waterborne pathogens that are responsible for the greatest global burden of acute gastrointestinal illness (AGI)

Pathogen of interest	Specimen	Immunologic biomarker response	Reference
Cryptosporidium spp.	Serum	IgG antibody	Priest, J. W., et al. [106]; Chappell, C. L., et al. [60]; Crump, J. A., et al. [107]; Sarkar, R., et al. [108]; Becker, D. J., et al. [109]; Checkley, W., et al. [110]
	Saliva	IgG and IgA antibody	Cozon, G., et al. [111]; Moss, D. M., et al. [69]; Egorov, A. I., et al. [112]; Griffin, S. M., et al. [52••];
Campylobacter	Serum	IgG, IgM, and IgA antibodies	Ang, C. W., et al.; [93]; Teunis, P. F., et al. [81]; Rokosz-Chudziak, N. and W. Rastawicki [113].
	Stool	Cytokines (IL-1β, IL-6, IL-8, TNF-α, and IFN-γ), IgA antibodies	Tribble, D. R., et al. [114]; Islam, D., et al. [115];
	Saliva	IgG and IgA antibodies (responses to acid-glycine extracts of *C. jejuni* strain 81116 and an aflagellate mutant, and a whole-cell R2 sonicate)	Cawthraw, S. A., et al. [116]
Giardia intestinalis	Serum	IgG and IgA antibodies	Crump, J. A., et al. [107]; Jiménez, J. C., et al. [117]; Priest, J. W., et al. [66]; Moss, D. M., et al. [68]
	Saliva	sIgA, IgA, and IgG antibody (responses against *G. duodenalis*)	Rodriguez, O. L., et al. [118]; El-Gebaly, N. S., et al. [119]
Hepatitis A virus	Serum	IgM and IgG antibodies	Vitral, C. L., et al. [11]; Hundekar, S., et al. [120]
	Saliva	IgM and IgG antibodies	Laufer, D. S., et al. [121]; Ochnio, J. J., et al. [122]; Morris-Cunnington, M. C., et al. [49••]; Tourinho, R. S., et al. [123]
Hepatitis E virus	Serum	IgG and IgM antibody, cytokines (IL-5, IL-6, IL-8, IL-10, IL-2, IFN-γ,TNF-α,TGF-β1, IL-1β	Adjei, A. A., et al. [124]; Pas, S. D., et al. [125]; Wu, W. C., et al. [38]; Kumar, A., et al. [126]; Gu, G., et al. [127]; Cong, W., et al. [37]; Heaney, C. D., et al. [128], Kmush, B. L., et al. [129]
Norovirus	Serum	IgG and IgA antibodies, cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and IL-12, IFN-β,TNF-α)	Erdman, D. D., et al. [64]; Monroe, S. S., et al. [39]; Moe, C. L., et al. [40]; Lindesmith, L., et al. [58]; Crump, J. A., et al. [107]; Newman, K. L., et al. [95]
	Stool	IgA antibody	Iritani, N., et al. [130]; Ramani, S., et al. [131]
	Saliva	IgA and IgG antibodies	Moe, C. L., et al. [40]; Lindesmith, L., et al. [59]; Lindesmith, L., et al. [58]; Griffin, S. M., et al. [52••]; Griffin, S. M., et al. [53•]
Rotavirus	Serum	IgM, IgA, and IgG antibodies, cytokines (IFN-γ, TNF-α, IL-8, and IL-10)	Grimwood, K., et al. [132]; Azim, T., et al. [133]; Xu, J., et al. [134]; Premkumar, P., et al. [135]; Sindhu, K. N., et al. [136]; Moon, S. S., et al. [137]
	Stool	IgM, IgA, and IgG antibodies	Stals, F., et al. [138]; Grimwood, K., et al. [132]; Azim, T., et al. [133]
	Saliva	IgM, IgA, and IgG antibodies	Stals, F., et al. [138]; Grimwood, K., et al. [132]; Aiyar, J., et al. [139]; Friedman, M. G., et al. [140];
Shiga toxin-producing	Serum	IgG antibodies against 51 O serogroup strains, B subunit of Stx2 and Stx1	Ludwig, K., et al. [141]; Kulkarni, H., et al. [142]; Fernández-Brando, R. J., et al. [143]; Guirro, M., et al. [144]
Escherichia coli	Saliva	IgM and IgA antibodies	Ludwig, K., et al. [145]; Chart, H., et al. [146]
Shigella	Serum	IgA, IgM, and IgG subtypes to *S. sonnei* O-antigen, IgA and IgG antibodies to *S. flexneri* 2a lipopolysaccharide, total IgA antibody-secreting cells (ASC), and anti-LPS IgA ASC, cytokines (IFN-γ,TNF-α,TNF-β, IL-4, IL-6, TGF-β)	Van De Verg, L. L., et al. [147]; Raqib, R., et al. [148]; Rasolofo-Razanamparany, V., et al. [149]; Levine, M. M., et al. [150]; Muhsen, K., et al. [151]; Thompson, C. N., et al. [152]

Pathogen of interest	Specimen	Immunologic biomarker response	Reference
	Stool	Cytokines (TNF-α, IL-6)	Azim, T., et al. [153]
	Saliva	IgA antibody	Schultsz, C., et al. [154];
Vibrio cholerae	Serum	IgA and IgG antibodies, IgG, IgM, and IgA ASC	Chowdhury, F., et al. [155]; Johnson, R. A., et al. [156]; Fujii, Y., et al. [157]; Khan, A. I., et al. [158]
	Stool	IgA antibody	Qadri, F., et al. [159]
	Saliva	IgA antibody	Jertborn, M., et al. [160]