IDENTIFICATION AND CHARACTERIZATION OF BIOLOGICAL RISKS FOR WATER

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Abstract

Life on this planet is dependent on water but our health is greatly impacted by the quality of that water. The global water crisis is clear, one only needs to quote the statistics on the billions of people without access to safe water, sanitation and the global estimates of the burden of waterborne disease. The greatest sources of biological contaminants associated with this disease burden in water remains human and animal feces. There is a critical need to develop a science-based program to address both water quantity and quality of water, water uses and discharges. Recommendations to achieve better access to scientific information for decision making include: 1) develop watershed approaches for determining the source and the behaviour of water-borne biological contaminants which can be used within Water Safety Plans, 2) utilize new tools and technologies for measuring the hazards and the exposure within a risk assessment framework and 3) develop a global data base and goals for biological contaminant loading for achieving safe water.

Keywords: Waterborne disease/parasites/viruses/monitoring/microbial risk assessment.

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1. Introduction

The terrifying tsunami and the subsequent events which unfolded in Asia at the end of 2004 exemplify many of the challenges facing the management of water resources for achieving water safety and public health protection. Displacement of people, disruption of services with the destruction or lack of infrastructure, inadequate water treatment, flooding (storms) and thus widespread fecal contamination, all resulting in the fear of massive waterborne disease particularly impacting those individuals who are most susceptible, the children and those with weakened systems due to stress, poor nutrition, (other diseases such as AIDS) and lack of medical care. The assurance of safe water and adequate control of fecal contamination of water continues to be a common struggle for millions and these same issues which have been extolled for the tsunami victims are those that the global health and water science and technology community must address to meet the Millennium Development Goals for water and sanitation (UN, 2000; 2002). Yet unfortunately, water quality problems and massive fecal contamination remain invisible, with little water quality data available for those who need to know and make decisions and thus it takes a massive and dramatic crisis, before even minimal attention is given to those dealing with these challenges on a daily basis. The World Health Organization has estimated that 40% of the world's people lack sanitation (2.4 billion people) and 1.1 billion people lack access to safe water (WHO, 2000). Up to 2.2 million children will die from diarrhea annually. Significant issues will need to be addressed regarding the biological agents found in water and the infectious diseases associated with these agents in order to make progress without slipping further and further behind in achieving the MDG. Fecal contamination and the loading of pathogens to water need to be determined and then strategies and goals for reducing this burden can be implemented. New tools can be used to build a global water quality data base on fecal contamination of waters, identifying biological agents in a risk-based scientific framework and thus will enhance the implementation of Water Safety Plans providing a true basis for measuring and achieving safe water (WHO, 2004).

2. Emerging and persistent biological contaminants

Studies of emerging infectious diseases (Taylor et al. 2001; Cleaveland et al. 2001; Ewald 1996) in human and animal populations have revealed the following:

- Viruses, prions, bacteria, and protozoa are more likely than fungi or helminths to be associated with emerging infections.
- Zoonotic pathogens comprise 75% of emerging infectious diseases.
- Pathogens which are subject to relatively frequent mutation or genomic reassortment events (e.g. RNA viruses and viruses with segmented genomes) are more likely to emerge.
- Pathogens which infect multiple hosts or pathogens that infect species that can harbour multiply closely related agents providing an opportunity for reassortment or recombination (e.g. SARS in cats) are likely to emerge.
- Agents transmissible by more than one route or by indirect contact, e.g. water, food, environmental contamination, vectors, etc, are likely to emerge.

Hundreds of pathogens are found in the feces of humans and animals all ending up excreted into the environment. Population growth, urbanization, increased animal concentration exacerbated by lack of infrastructure and waste treatment, climate variations (floods and droughts) all influence the occurrence and spread of biological agents of concern in water.

Viruses, due to their small size and structure, their environmental stability and low infectivity, remain biological contaminants which elicit a high probability of infection via ingestion of contaminated water (Haas et al., 1999). Viruses cause a wide-range of significant diseases many which may have chronic outcomes including polyomelitis, meningitis, myocarditis, and hepatitis. Viral agents have been implicated in endemic diarrheal disease due to contaminated groundwater in rural areas in Wisconsin likely associated with septic tanks. Children were shown to be at particularly high risk of illness (Borchardt et al. 2003). The coliform indicator bacteria did not prove to be a sufficient measure of risk. Therefore viral contamination of groundwater needs to be particularly assessed as children are very susceptible to severe disease; bacterial indicators are not indicative of viruses and groundwater is often used without disinfection.

Virus	Туре	Diseases	Occurrence in Water	Other Key Points
Adeno Viruses	DNA	Respiratory & gastroenteritis	Detected in ground, surface and drinking water	High concentrations in sewage, long-term survival in water.
Coxsackie	RNA	Respiratory, gastrointeritis, meningitis, myocarditis	CB 5 found to be one of the most prevalent viruses in sewage and polluted waters.	Cause of chronic diseases.
HEV	RNA	Hepatitis		Genetically related to that found in Pigs. High mortality in Pregnant women.
Noroviruses	RNA	Gastroenteritis	Sewage, surface and groundwaters	Common cause of recreational and drinking water disease.
Polyoma Viruses	DNA	Brain, colorectal cancers and urinary tract disease	Feces, urine and sewage.	Survives in water but oral transmission uncertain.

TABLE 1. Some Viruses of Emerging Concern.

Hepatitis E virus (HEV) is an enteric RNA virus isolated from humans and causes jaundice and clinical signs similar to hepatitis A virus. HEV is transmitted by the fecal-oral route and has caused devastating waterborne disease outbreaks particularly in tropical and subtropical countries with inadequate sanitation (Aggarwal and Naik 1997; Balayan 1997). In Kanpur, India in 1991, there were 79,000 cases of HEV due to sewage contamination of the drinking water. The earliest confirmed outbreaks occurred in the 1950s in India (Bradley 1992). Children are often asymptomatic and the mortality rate is between 0.1 and 4% (Grabow et al. 1994). In pregnant women in their third trimester, the mortality rate can exceed 20% (Hurst et al. 1996). There has been speculation that HEV is endemic in various parts of the world and subclinical cases may be contributing to the spread of the disease. HEV is

also found in wild and domestic animals. Studies using genetic sequencing have found that human and pig HEV are genetically similar and belong to the same genotype and swine HEV is widespread in U.S. swine herds (Huang et al. 2002; Smith 2001). Thus zoonotic transmission seems quite likely and at least one strain can infect across species barriers (Smith 2001). Recent studies in the U.S. have shown that swine veterinarians are at in increased potential risk of zoonotic HEV infections (Meng et al. 2002). The fecal contamination of water from pigs continues to challenge the human population with potential risks associated with the transmission of these agents. The survival and resistance of HEV to water treatment has not been well studied but is presumed to be very similar to Hepatitis A Virus. As enteric viruses, they are stable in the water environment particularly at lower temperatures. In addition, during water disinfection, the viruses are more resistant to chlorination than bacteria although good inactivation can be achieved with free chlorine. Chlorine disinfection does not inactivate viruses in wastewater as effectively as it does in drinking water because of interference by dissolved organics and suspended particulates; the resulting disinfectant is combined chlorine, which inactivates viruses less effectively than free chlorine does (Mahin and Pancorbo, 1999).

Adenoviruses are double-stranded DNA viruses and they are extremely stable in the environment. (Table 1). Several studies have suggested that adenoviruses outnumbered other enteric viruses in sewage-contaminated waters and may survive longer than other viruses in water (Enriquez et al. 1995; Pina et al. 1998). Adenovirus 40 and 41 are enteric based and are the second most important etiologic agents of childhood gastroenteritis after rotavirus. The number of waterborne outbreaks caused by adenoviruses might have been underestimated because they are not being routinely screened for in outbreak samples. These viruses are commonly detected in coastal systems likely influencing recreational risks and indicative of the sewage discharges to the coast (Griffin et al., 2003) and both respiratory and enteric adenoviruses have been detected in treated drinking water when disinfectant contact times and concentrations were too low for virus inactivation (Lee and Kim, 2002).

Formerly classified as Norwalk-like viruses, the Noroviruses are highly infectious and can cause prolonged asymptomatic shedding in infected individuals for up to two weeks. Noroviruses are extremely stable in the environment, they are stable in less than 10 parts per million (ppm) chlorine and can withstand freezing and heating to 60 °C (Nwachcuku and Gerba 2004). Substantial strain diversity leads to short-lived host immunity to infection permit re-infection and make the development of a vaccine that offers lifelong protection impossible (Glass et al. 2001). The estimated total

number of cases of norovirus infection per year is 23,000,000 in the United States alone (Mead 1999). There has been a world-wide occurrence of norovirus outbreaks in the last few years, causing problems with cruise ships, hotels, drinking water, recreational water and in nursing homes where mortality has been reported. Just in the US. the number of drinking water-related norovirus outbreaks has increased from three during 1999-2000 to five during 2001-2002 (Prevention 2004). Norovirus outbreaks constituted for 9% of waterborne-disease outbreaks of gastroenteritis associated with recreational water during 1993-2002 and 16.7% waterborne-disease outbreaks of gastroenteritis associated with recreational water during 2001-2002.

There are disturbing new reports regarding the potential for waterborne transmission of the cancer-causing viruses, the polyomaviruses (Bofill-Mas et al., 2001). The polyomaviruses include the JC and BK viruses, which cause brain, colorectal cancers and urinary tract disease. Polyoma viruses are non-enveloped double-stranded DNA viruses with diameter between 38 to 43 nm. JC virus, a polyoma virus, is etiologically linked to progressive mulfocal leukoencephalopathy, a fatal demyelinating disease (Nwachcuku and Gerba 2004). Polyoma viruses can be excreted in urine of infected persons and have been detected in sewage worldwide (Bofill-Mas and Girones 2003). They have been detected at concentrations of 10¹ to 10³ viral particles per ml in sewage in studies in the US, Europe and Africa. The viruses have been shown to be stable with T-90 (time for 90% decrease in numbers) from 26 days for JCV to 53 days for BKV at 20°C. However, very little is known in regard to transport and exposure of these viruses as emerging waterborne agents.

The parasites *Cryptosporidium* and *Giardia* remain a concern in water world-wide and are probably the most common waterborne infection globally (Leclerc et al., 2004). Characteristics that make these parasites a top priority include:

- concentrations in sewage and feces
- zoonotic nature
- extreme resistant to chlorination
- environmental stability
- low infectivity

Cryptosporidium remains a specific problem for those with AIDS, while *Giardia* remains the most wide-spread parasitic infection in the world, leading to chronic infections. Children are most susceptible to infection and severe outcomes. Recurrent diarrheal disease and chronic parasitic infections in

children have been associated with impaired cognitive ability later in childhood (Berkman et al., 2002).

Levels of cysts and oocysts in surface waters can be very high depending on the amount of fecal contamination. These parasites have been detected throughout the world and are now currently monitored for more than any other pathogen in water. Filtration or some type of physical removal is needed, as the organisms are resistant to chlorination, or if economically feasible uv disinfection is possible (Rose et al., 2002; Betancourt and Rose, 2004). Interestingly, the parasites are more susceptible to heat inactivation, therefore solar water pasteurization systems may provide some protection against parasites, however this has yet to be proven.

It is quite clear that the coliform indicator bacteria do not always provide the necessary measure of microbiological safety of water, especially with respect to viral and parasitic pathogens. The spread of waterborne parasitic and viral diseases will not be addressed or contained via a total coliform test and new tools, alternative indicators, pathogen monitoring and molecular characterization of the organisms in the water will be needed in the future. The most widespread alternative indicators in addition to *E.coli* that have had usefulness include coliphage (bacterial viruses that are easily and cheaply monitored for) and Enterococci (enteric bacteria which have been related to groundwater contamination, recreational risk and improved assessment of wastewater disinfection).

3. General Approach to Microbial Risk Assessment

Significant technical advances have now been made in microbiology and molecular biology that have influenced all associated fields including medicine, food safety, microbial ecology, environmental forensics, and water microbiology. Thus while traditional culture-based methods for bacteria have been and will continue to be used in water, antibody-based and genetic-based methods can be used for almost any biological hazard. Polymerase chain reaction (PCR) the method by which specific identifying genes can be amplified and detected allows for specific, sensitive and fairly rapid analysis of any pathogen of interest, whether culture methods exist or not.

These advances have lead to the ability to build data bases that can be used to identify and characterize the biological hazards in water.

3.1. SOURCE TRACKING

Microbial source tracking is an emerging field of investigation, which has attempted to determine the source of the fecal pollution in waterways, whether it be human or animal (Sinton et al., 1998; Scott et al., 2002; Simpson et al., 2002). While it is acknowledged that exposure to animal fecal wastes represent a human health risk often it is human feces, septic effluent and septage, cess pool discharges, and sewage that would signal a greater risk for the presence of human pathogens, particularly human viruses. Different types of methods have been examined and they can be classified into two separate groups,

- library-based methods rely on the building of a watershed specific database most often for *E.coli* or Enterococci from fecal sources (eg. Birds, cows, humans etc) and comparing that to water isolates and includes ribotyping, pulsed-field gel electrophoresis, repetitive element PCR and multiple antibiotic resistance (Simpson et al. 2002). Round robin studies have shown that library based methods have limited accuracy and lead to high false-positive rates (Griffith et al., 2003).
- library-independent methods rely on the identification of host-specific markers in *Bifidobacterium* spp., *Bacteroides fragilis* bacteriophages, F⁺-specific RNA coliphage and Enterococci (Griffith et al., 2003, Scott et al., 2002; 2004).

Enterococcus faecalis and *Enterococcus faecium* are predominant in human feces and as such have the potential to be excellent indicators of human fecal pollution and a method has been recently developed that can be used with current water sampling approaches to evaluate the presence of human fecal contamination (Scott et al., 2004). The enterococcal surface protein (esp) found in *E. faecium* was the target of the host-associated molecular marker used to develop the human fecal maker. Currently the method is the most robust and sensitive (60 CFU) of all the host-specific approaches and after examination of 65 wastewater and septic samples and 102 animal samples, no false positives were examined and the presence of human fecal material was detected accurately in 97% of the samples, exclusively indicates the presence of human wastes without the need to build a library.

3.2. PATHOGEN MONITORING

An understanding of the ecology of the infectious agents will be crucial in understanding the distribution and dissemination of the environmental forms,

these data will be necessary to address risk and prevention strategies. Thus pathogen monitoring will be a part of the future for characterizing biological hazards in water. Any pathogen of concern can now be monitored for in water. Filtration techniques, followed by clarification methods (e.g. immunomagnetic separation), cultivation, antibody-based detection or molecular detection have been used for detection of bacteria, viruses and parasites. PCR methods can be developed for any microorganism of interest. Most recently the National Academy of Sciences, Organization for Economic & Community Development, and the American Academy of Microbiology have all summarized the methods available for monitoring any pathogen of interest in water (Rose and Grimes, 2001; NRC, 2004; OECD, 1998). Rapid tests are being examined which include antibody-based methods addressing both the traditional and alternative indicators as well as pathogens such as Cryptosporidium. A recent example of the benefits of such approaches has been the use of molecular methods which have been used for monitoring sewage to evaluate the transition of wild type poliovirus infections to the vaccine strain in order to assess the vaccination programs in communities.

No global assessment of any one method has been undertaken for more than 20 years. And while there are continued reports of pathogen levels and indicator levels in waters through out the world, there is no central clearing house for reporting or compiling these data.

Pathogen monitoring is needed if the risk is to be appropriately addressed. The classical risk assessment/risk management paradigm, is widely accepted as the primary basis for regulatory decision making on environmental issues. In this conceptual model, the environmental risk assessment process is initiated by what has been termed "hazard assessment" which can be thought of as identification of an agent capable of causing an adverse health effect. The actual risk that a biological agent poses results from a combination of exposure and infectivity and pathogenicity. The integrity of the risk assessment process thus depends primarily on the base of scientific information related to exposure and disease potential. Both can be exceedingly complex, and rarely is a complete understanding available on how an agent is released, how an agent is transported and transformed in the environment (and also in engineered systems), how exposure occurs, and how exposure relates to disease based at both the individual and population levels.

One can conceptualize a "source-to-disease" structure around water-related disease-causing agents, and if the critical scientific gaps in understanding how these agents behave from the point of release to the resultant health effect are to be addressed then pathogen monitoring is needed.

3.3. QUANTITATIVE MICROBIAL RISK ASSESSMENT QMRA

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The definition of 'microbiologically safe water' is evolving from the utopian concept of pathogen-free water to a more realistic goal of providing drinking water for human consumption (European Union Council Directive 98/83/ EC and WHO guidelines as quoted by Szewzyk et al. 2000) to be, "...free from microorganisms and parasites which, in numbers or concentrations, constitute a potential danger to human health." These statements must be interpreted along with a definition of acceptable risk. The U.S. Environmental Protection Agency defines acceptable risk of potable water to be 1:10000, i.e., 1 case of the disease in 10,000 exposed persons (Szewzyk et al. 2000). These types of guidelines can be used to conduct mathematical modelling of the risk to public health due to the concentrations of various agents in water. However to conduct this risk assessment, key pieces of information are required to be known, or at least predictable, based on databases and applied models. Quantitative microbial risk assessment (QMRA) procedures which address hazard identification, dose-response, exposure assessment and characterization have been defined (Haas et al. 1999), yet this system needs to incorporate both indicator data and treatment strategies.

During water quality monitoring the initial testing is via bacterial indicators which have limitations regarding risk assessment interpretation. Yet by addressing both sources, and pathogen monitoring, transport and fate into the risk assessment framework, exposure and the characterization of the biological contaminants in water and finally, the risk can begin to be elucidated (Figure1). Thus appropriate management strategies can be implemented.

The infectious dose-response of the pathogen of interest; the concentration at which the agent can be found in water, and finally the strategies for prevention of pollution and various water treatment strategies for the reduction the biological contaminants make up the final framework for risk assessment.

Data from both indicators and pathogen occurrence can be used to evaluate the fate and behaviour of a substance in the environment (Exposure Assessment). Other medical data can be used to assess the potential Hazard posed by biological contaminants. The results of the exposure assessment and the hazard assessment are combined to produce an overall risk assessment. Such data are generated from an increasingly comprehensive series of studies termed *higher tier studies*. At each tier a relevant comparison has to take place between the estimated exposure and the estimated hazard and there are thus separate tiers for both exposure and hazard estimation.



Figure 1. Water Quality Monitoring as Part of the Risk Assessment Framework.

4. Need for a Global Water Monitoring Network for Fecal Contamination and Biological Contaminants

Many countries have a health goal of no pathogens in drinking water and require no detectable live (cultivatable) total coliform bacteria per a 100mL sample volume. The WHO drinking water criteria focuses on the absence of cultivatible *E.coli* per 100mL. There are no identifiable goals for ambient water quality for waters used as a potable water supply.

Despite the development of a variety of methods and a global indicator system based on coliform bacteria and *E.coli* very little comprehensive information is available on the quality of the global water supply. The lack of data has been suggested as the main deterrent to developing a vested interest in water systems and the implementation of policies and programs to improve water quality at the local and national levels (Barker, 2004). The United Nations World Water Assessment Programme was set up to address comprehensive water quality monitoring data bases yet, the UN Global Environment Monitoring System for Water (GEMS/Water) created in 1978, has only 104 governments participating and while there are physical and chemical water quality data there are minimal microbial data focused on fecal coliform bacterial levels with poor resolution and no ability to readily compare the data (eg. via mapping). [www.gemswater.org; www.unesco.org/ water/wwap]

In many countries the clinical and medical system is not separate from the public health system. The WHO global Laboratory Network is centred around infectious diseases important to the country. In the US, CDC has established the Global Health Initiatives, more recently to focus on the AIDS issue. While laboratory infrastructure is being assessed, methods and supplies to address the diagnostics are moving forward, there is no reason why the testing of water can not be incorporated into these plans. The building and rebuilding of laboratory capacity is ongoing. As coordination, protocol development, training, technology transfer, assessment and updates move forward, the concept of inclusion of water quality as a key indicator of community health could begin to be developed.

Prioritization of key laboratories to assist in this effort and training of personnel will be needed. This will also require the coordination and communication between Ministries of Health and Ministries of the Environment.

As with other global goals the measuring and improvement of water quality should begin with an action plan. This would address:

- Prioritization of water basins
- Technology to be used and tested
- Development of a tiered testing protocol for addressing risk (Figure 1)
- Development of a common methodology for reporting spatial and temporal data sets.

5. Conclusions

The identification, measurement and control of infectious disease remain a focus of the global health community. It is clear that in most countries some type of system is available for addressing the spread of disease and mobilization of the global and local community medical and public health systems was clearly evident during the recent SARS epidemic; and yet the morbidity and mortality associated with SARS pales in comparison to the numbers of individuals who become ill, are hospitalized and who die as a result of contaminated water.

To really meet the millennium goals, both access to "safe" drinking water and sanitation will be critical. Comprehensive watershed assessment, planning and integrated programs to address both water quality and quantity are likely to be effective at the community level and may be much more costeffective with long-term sustainability in comparison to plans which address temporary access only at the household level through house-hold treatment (Souter et al. 2003). This approach underscores the need to enhance and refine the detection of contaminants in water, monitoring of changes in observable water quality, assessment of exposure and the potential public health impacts and improvements made with the investment in public works.

Development of appropriate databases is necessary. Recommendations to achieve better access to scientific information for decision making include: 1) develop watershed approaches for determining the source and the behaviour of water-borne biological contaminants which can be used within Water Safety Plans, 2) utilize new tools and technologies for measuring the hazards and the exposure within a risk assessment framework and 3) develop a global data base and goals for biological contaminant loading for achieving safe water.

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