

Risk assessment of waterborne protozoa: current status and future trends

C. J. GIBSON III^{1*}, C. N. HAAS² and J. B. ROSE¹

¹Department of Marine Science, University of South Florida, St. Petersburg, FL 33701, USA

²School of Environmental Science, Engineering and Policy, Drexel University, Philadelphia, PA 19104, USA

SUMMARY

Throughout the past decade much research has been directed towards identifying the occurrence, epidemiology, and risks associated with waterborne protozoa. While outbreaks are continually documented, sporadic cases of disease associated with exposure to low levels of waterborne protozoa are of increasing concern. Current methodologies may not be sensitive enough to define these low levels of disease. However, risk assessment methods may be utilised to address these low level contamination events. The purpose of this article is to provide an introduction to microbial risk assessment for waterborne protozoa. Risk assessment is a useful tool for evaluating relative risks and can be used for development of policies to decrease risks. Numerous studies have been published on risk assessment methods for pathogenic protozoa including *Cryptosporidium* and *Giardia*. One common notion prevails: microbial risk assessment presents interesting complications to the traditional chemical risk assessment paradigm. Single microbial exposures (non-threshold) are capable of causing symptomatic illness unlike traditional chemical exposures, which require a threshold to be reached. Due to the lack of efficient recovery and detection methods for protozoa, we may be underestimating the occurrence, concentration and distribution of these pathogenic micro-organisms. To better utilize the tool of microbial risk assessment for risk management practices, future research should focus in the area of exposure assessment.

Key words: *Cryptosporidium*, *Giardia*, risk assessment, protozoa.

INTRODUCTION

Risk assessment methods have been used to prioritise risks and hazards and to set policy for 30 years. The process of risk assessment became an organized activity by the United States federal government in the 1970s and focused primarily on chemical exposures, historically applied to hazards such as pesticides, food additives, and industrial toxins (i.e. acetic acid, benzene, cyclohexanol, formaldehyde, and toluene). The resulting risks/endpoints were embodied within 2 subgroups: cancerous or non-cancerous. Recent advances in the scientific community have improved detection methodologies associated with low level exposure and health outcome assessments and in turn refined risk assessment outputs. The recognized value of risk assessment has led to frameworks for addressing a wider variety of stressors effecting both ecosystems and human health. In particular, the development of quantitative microbial risk assessment has begun to address enteric viruses (Hepatitis A, Norwalk virus, rotavirus, SRSVs), parasites (*Ascaris*, *Eimeria*, *Cyclospora*, *Toxoplasma*) and bacteria (*E. coli*, *Shigella*, *Salmonella*, *Vibrio cholera*) (Haas, Rose & Gerba, 1999).

Risks associated with waterborne protozoa have been recognized for over a decade and much research

has been directed toward identifying the occurrence and epidemiology of these organisms in various water types worldwide (Craun, 1986; CDC, 1993, 1996; Smith, Robertson & Ongerth, 1995; Smith & Rose, 1998). In particular, *Cryptosporidium* and *Giardia* are enteric protozoan parasites which commonly occur in similar environments and have emerged as prevalent and widespread intestinal parasites. The mode of transmission for both agents is the faecal-oral route and both have caused waterborne illness. Each produce environmentally robust oocyst and cyst stages that are resistant to standard disinfection methods. While outbreaks are continually documented, exposure to low concentrations of micro-organisms in water may be associated with endemic levels of disease. Epidemiological methods may not be sensitive enough to define these low risks. Sporadic cases of disease associated with occasional contamination of drinking water may be a risk that has gone unrecognized. Only with risk assessment methods can these low contamination events be evaluated.

Risk assessment may be defined as a systematic process for qualitative or quantitative characterization of adverse effects (risks) associated with hazardous substances, processes, actions, and/or events (NRC, 1983; Covello & Merkhofer, 1993). Risk assessment is part of a larger operation entitled risk analysis, which is a process involving risk assessment, risk management and risk communication.

* Corresponding author.

E-mail: cjgibson3@seas.marine.usf.edu

In 1983 (NRC, 1983), the National Academy of Sciences–National Research Council stated that uniform guidelines for risk assessment must be established for both cancerous and non-cancerous effects. Four major steps in the process were defined: (1) *Hazard Identification*: Traditionally describes the acute and/or chronic human health effects (developmental, mutagenic, and carcinogenic) associated with a particular chemical exposure. For microorganisms, hazard identification is the defining of the pathogenic protozoan, virus, or bacteria and the illness/symptoms associated. (2) *Dose-Response Assessment*: A characterization (associated with various models) of the relationship between various doses administered and the response. Microbial dose-response quantifies the probability of infection (colonization) in relation to the exposure/ingestion of bacteria (colony forming units), viruses (plaque forming units), and protozoa (cysts or oocyst counts). (3) *Exposure Assessment*: This determines the routes of exposure (inhalation, ingestion, and dermal absorption), the duration of exposure, and the level of exposure. (4) *Risk Characterization*: This step integrates the preceding steps and evaluates the probability of adverse consequences and discusses the variability and uncertainty in the assessment.

The field of risk assessment has developed over the last decade from diverse disciplines and has traditionally focused on exposures associated with harmful substances (chemical and physical agents) in the environment. Only recently, has effort been directed toward risk assessment methodologies for defining and characterizing pathogenic microorganisms such as bacteria, viruses, and protozoa (Haas, 1983; Regli *et al.* 1991; Rose, Haas & Regli, 1991; Haas *et al.* 1993, 1996; Teunis *et al.* 1997).

Risk assessment is a valuable tool that can be used to evaluate relative risks (i.e. waterborne disease associated with contamination of potable versus recreational waters) and, as previously mentioned, can be used to address low levels of contamination for development of policies to decrease risks. Moreover, the risk assessment permits integration of science with social and political considerations, engineering/technological solutions, and economic feasibility (cost-benefit ratios).

MICROBIAL RISK ASSESSMENT

Over the past several years, much has been written on risk assessment methods for pathogenic protozoa (*Cryptosporidium* and *Giardia*) and various viruses (rotavirus) (Regli *et al.* 1991; Rose *et al.* 1991; Gerba *et al.*, 1996; Haas *et al.* 1996; Teunis *et al.* 1997). One common notion prevails: microbial risk assessment presents interesting complications to the traditional chemical risk assessment paradigm.

Microbial pathogens are able to replicate in the host, causing a wide variety of endpoints ranging

from asymptomatic infection, to disease (symptomatic infection), to death (mortality). In addition, single microbial exposures (non-threshold) are capable of causing symptomatic illness unlike traditional threshold chemical exposures. Epidemiological data on waterborne disease surveillance are limited and underestimate the burden of disease (Craun, Calderon & Frost, 1996; Frost, Craun & Calderon, 1996). This review will summarise the National Academy Risk Assessment process for *Cryptosporidium* and *Giardia*, and will address data requirements for the future of microbial risk assessment.

HAZARD IDENTIFICATION

The enteric pathogenic protozoa, *Cryptosporidium* and *Giardia*, cause gastrointestinal disease: particularly diarrhoea, abdominal cramps, nausea, malabsorption, weight loss, and vomiting (MacKenzie *et al.* 1994). Both *Cryptosporidium* and *Giardia* are self-limiting in most immunocompetent individuals. *Cryptosporidium* infections have an incubation period (the time interval between infection and the first appearance of symptoms) of 1 to 12 days with an average of 7 days (Benenson, 1995) with the duration of disease lasting 1 to 2 weeks (Fayer, 1997). *Giardia* infections have an incubation period of 3 to 25 days with a median of 7 to 10 days with the duration of disease ranging from 1 week to 4 months (Benenson, 1995). The mode of transmission for both organisms is the faecal-oral route via an environmentally robust oocyst (*Cryptosporidium*) or cyst (*Giardia*) stage excreted in the faeces of an infected host. Therefore, ingestion of faecally contaminated waters or foods is a major source of disease transmission.

In the United States, *Giardia* was the most identified waterborne disease causing agent from 1976 to 1980 (Craun, 1986). From 1971 to 1994, *Giardia* has been responsible for 127 outbreaks associated with 27 241 cases of illness (Craun, 1986; CDC, 1993, 1996). Moreover, from 1991 to 1994 there have been 8 outbreaks (175 cases) associated with recreational water (Table 1) (CDC, 1993, 1996). Foodborne outbreaks of giardiasis have been reported, but do not appear to be as common as waterborne outbreaks. These outbreaks have been associated with contamination by food handlers and include foods such as salmon, fruit salad, raw vegetables, lettuce, onions, and tomatoes (Table 1).

Cryptosporidium gained importance as a waterborne pathogen as an increasing number of large outbreaks were experienced; Carroll County, Georgia (1987), USA (13 000 affected individuals), Swindon/Oxfordshire (1989), UK (> 515 affected individuals), N. Humberside (1990), UK (447 affected individuals) and Milwaukee, Wisconsin (1993), USA (403 000 affected individuals)

Table 1. Some waterborne and foodborne outbreaks of cryptosporidiosis and giardiasis in the United States.

| Etiological Agent | Type of outbreak | Number of outbreaks | Number of cases | Year(s) |
|------------------------|---------------------------|---------------------|-----------------|------------|
| <i>C. parvum</i> | Drinking water | 8 | 406 822 | 1991–1994 |
| <i>G. lamblia</i> | Drinking water | 9 | 508 | 1991–1994 |
| <i>C. parvum</i> | Recreational water | 8 | 1218 | 1991–1994 |
| <i>G. lamblia</i> | Recreational water | 8 | 175 | 1991–1994 |
| <i>Cryptosporidium</i> | Foodborne (apple cider) | 2 | 185 | 1993, 1996 |
| <i>Cryptosporidium</i> | Foodborne (chicken salad) | 1 | 15 | 1995 |
| <i>Giardia</i> | Foodborne (salmon) | 1 | 29 | 1979 |
| <i>Giardia</i> | Foodborne (noodle salad) | 1 | 13 | 1985 |
| <i>Giardia</i> | Foodborne (fruit salad) | 1 | 10 | 1986 |
| <i>Giardia</i> | Foodborne (sandwiches) | 1 | 88 | 1986 |
| <i>Giardia</i> | Foodborne (vegetables) | 2 | 48 | 1989, 1990 |
| <i>Giardia</i> | Foodborne (ice) | 1 | 27 | 1990 |

References: Osterhom *et al.* (1981); Peterson *et al.* (1988); White *et al.* (1989); Porter *et al.* (1990); Quick *et al.* (1992); Mintz *et al.* (1993); Smith, 1993; CDC, 1993, 1996; CDC, 1996, 1997.

(MacKenzie *et al.* 1994; Smith & Rose, 1998). A total of 19 outbreaks affecting over 427 000 individuals have been reported in the United States and United Kingdom from 1984 to 1996 (Smith & Rose, 1998). *Cryptosporidium* has also been responsible for a few foodborne outbreaks. The first foodborne outbreak of cryptosporidiosis in the US was reported in apple cider in 1993 (Millard *et al.* 1994). Other foodborne outbreaks of cryptosporidiosis were associated with chicken salad and again with apple cider (Table 1).

Cryptosporidium and *Giardia* do not appear to be major aetiological agents of foodborne disease. However, other enteric protozoa, *Toxoplasma* and *Cyclospora*, have a greater association with foodborne disease. Bacterial contamination, such as *Escherichia coli* O157:H7, of foodstuffs is also a major source of foodborne disease outbreaks (Tauxe, 1998).

Not only does contaminated food and water intended for consumption pose a problem, but contaminated recreational water is also a threat to the public. During 1991–1992, 6 (55%) of the 11 outbreaks associated with recreational water with identifiable aetiological agents were attributed to either *Giardia lamblia* (4) or *Cryptosporidium parvum* (2) (CDC, 1993). In comparison, during 1993–1994, 10 (71.4%) of the 14 outbreaks of swimming-associated (unintentional ingestion) gastroenteritis were caused by either *C. parvum* (6) or *G. lamblia* (4) (CDC, 1996). The first outbreak of cryptosporidiosis reported from recreational water in the United States occurred in New Jersey during the summer of 1994. *C. parvum* was detected in the lake water samples 5

weeks after the end of the outbreak period (CDC, 1996). The 4 recreational outbreaks of *G. lamblia* were associated with 2 lakes, a river, and a community swimming/wading pool (CDC, 1996). Swimming and other recreational activities where unintentional ingestion of water occur are known to increase the risk of gastrointestinal illness, even in non-outbreak settings (CDC, 1996). Table 1 shows the number of outbreaks and cases of *Cryptosporidium* and *Giardia* associated with recreational water.

Healthy immunocompetent adults are less affected by these pathogenic protozoa than are other portions of the population. Gerba, Rose & Haas (1996) reviewed the existing literature and identified those populations at greatest risk to waterborne and foodborne gastrointestinal disease. The most sensitive populations to enteric micro-organisms include the young, elderly, malnourished, disease impaired (diabetes), and a broad category of immunocompromised individuals (AIDS patients, transplant recipients and those on chemotherapy). The aforementioned group of individuals represents 20% of the current population in the US and is predicted to increase in the years to come (Rose, 1997).

DOSE-RESPONSE

Dose-Response models have been developed from a number of studies conducted on human volunteers in a laboratory setting (Rose *et al.* 1991; DuPont *et al.* 1995; Haas *et al.* 1996). Although these studies are limited, they remove the uncertainty and contro-

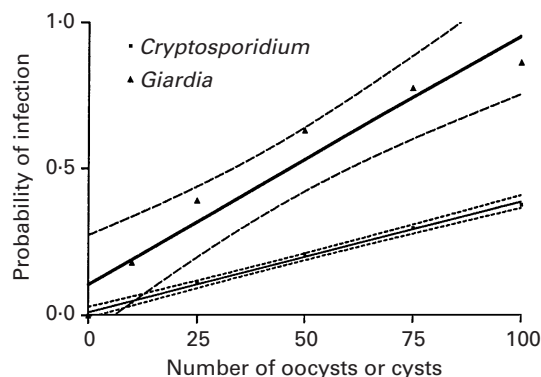


Fig. 1. Comparison of the dose-response relationship of *Cryptosporidium* and *Giardia*. Dashed Lines are the 95% confidence intervals.

versy surrounding the extrapolation of data from laboratory animal studies to humans. These studies also define a non-threshold limit for infectivity. In theory, one viable *C. parvum* oocyst or *G. lamblia* cyst could cause infection. In healthy immunocompetent adult human volunteer studies, 30 oocysts caused a 20% infection rate while 10 cysts caused a 100% infection rate (Rendtorff, 1979; DuPont *et al.* 1995).

The risk equation developed for pathogenic protozoa dose-response studies is an exponential model. If 1 viable micro-organism is capable of causing an infection and if the host-micro-organism interactions are constant, then the probability of an infection (P_i) resulting from ingestion of a single volume of water containing (i.e. 2 l used for tap water) an average number of organisms (N) may be defined by a simple exponential equation: (Haas, 1983; Rose *et al.* 1991; Haas *et al.* 1996):

$$P_i = 1 - e^{(-rN)},$$

where r is the fraction of ingested micro-organisms that survive to initiate an host-specific infection. For *Cryptosporidium*, $r = 0.00467$ (95% C.I., 0.00195–0.0097) (Haas *et al.* 1996). For *Giardia*, $r = 0.01982$ (95% C.I., 0.009798–0.03582) (Rose *et al.* 1991).

Fig. 1 compares these models for *Cryptosporidium* and *Giardia* and shows the 95% confidence intervals surrounding the model. Although the confidence intervals surrounding the *Giardia* model are wider, a greater risk is estimated as compared to *Cryptosporidium*. This may be a result of limited volunteers, doses, and/or study design in the 1954 *Giardia* experiments. The *Cryptosporidium* study had 8 doses, 29 volunteers and was designed to examine dose-response modelling (DuPont *et al.* 1995).

More recently a repeat of the dose-response experiments was undertaken for *Cryptosporidium* (Okhuysen *et al.* 1998). In this study, Okhuysen *et al.* (1998) investigated if *C. parvum* exposure could produce resistance to re-exposure. Nineteen healthy immunocompetent adults were re-challenged, 1 year

after primary exposure (30–10⁶ oocysts) with a second dose of 500 *C. parvum* oocysts. The results show comparable rates of diarrhoea between the primary and secondary exposures, fewer oocysts were shed after secondary exposure, and clinical severity (measured by number of unformed stools passed) was lower after re-exposure (Okhuysen *et al.* 1998). Prior to conducting the study, the authors determined by interim analysis that the ID₁₀₀ (the dose that would infect 100% of those exposed) would be 500 oocysts. However, after primary challenges were completed, 500 oocysts represented an ID₈₆, which is in close agreement with the study results of the first infectivity investigation (DuPont *et al.* 1995) where 500 oocysts represented an ID₈₃. Okhuysen *et al.* (1998) concluded that initial exposure was not sufficient to protect against re-exposure and/or clinical illness one year later.

EXPOSURE ASSESSMENT

Exposure assessment requires an understanding of the occurrence of oocysts and cysts associated with faecal sources, runoff or discharge into waterways, transport and survival, and reduction through treatment.

The Information Collection Rule (ICR) promulgated by the United States Environmental Protection Agency (USEPA) requires that all drinking water utilities serving a population over 100 000 monitor for the occurrence of protozoan pathogens, viruses, and faecal indicators in the nation's source waters. This set of data, along with risk assessment methods, will allow regulators, directors of municipalities, health officials, environmental microbiologists, engineers, and water quality managers to communicate and develop treatment standards to better protect the local population from epidemic outbreaks and endemic waterborne disease. The United Kingdom preceded the US by establishing a Group of Experts to complete a comprehensive review of the knowledge on the occurrence and importance of *Cryptosporidium* as an aetiological agent in waterborne disease (Smith & Rose, 1998). The report was presented in 1990 and a National Research Program was initiated to further the knowledge on *Cryptosporidium* as a waterborne pathogen and determine for efficient technologies for eliminating/controlling *Cryptosporidium* in the potable water systems (Smith & Rose, 1998).

Table 2 shows the occurrence of *Cryptosporidium* and *Giardia* in various water types. Most surface waters will have some level of contamination. Recent studies have shown that groundwater also contains oocysts and cysts (Hancock, Rose & Callahan, 1998). Wastewater and storm water can contribute high concentrations of oocysts and cysts to surface waters utilised for potable water. Survival studies suggest

Table 2. The occurrence of *Cryptosporidium* and *Giardia* in various waters.

| Type of Water | <i>Cryptosporidium</i> | | <i>Giardia</i> | |
|---|------------------------|---------------------------|------------------|---------------------------|
| | Percent positive | Concentration (oocysts/l) | Percent positive | Concentration (oocysts/l) |
| Untreated wastewater | 67 | 1–120 | 100 | 642–3375 |
| Activated sludge effluent | 42 | 0.025–11 | 83 | 0.14–23 |
| Filtered secondarily treated Wastewater | 42 | 0.01–0.13 | 75 | < 1–0.18 |
| Surface water | 51.5 | 0.065–65.1 | 45 | 0.02–43.8 |
| Groundwater | 11 | 0.002–5.28 | 6 | 0.001–1.2 |
| Treated drinking water | 13.4 | 0.0029–0.57 | 4.6 | 0.0098–0.09 |
| Combined sewer overflows | 100 | 2.5–400 | 100 | 90–2830 |

References: Sykora *et al.* (1988, 1991); LeChevallier & Norton (1995); Rose *et al.* (1996); Rose (1997); Hancock *et al.* (1998); Gibson III *et al.* (1998).

that these protozoa are quite rugged and will survive for months in the environment (DeRegnier *et al.* 1989; Smith *et al.* 1995). Routine wastewater treatment reduces *Cryptosporidium* and *Giardia* levels by 92.8% and 93.0%, respectively, and only with filtration are the levels reduced by > 99% (Rose *et al.* 1996). Although optimal wastewater treatment can remove > 99% of oocysts and cysts, a large number may still be discharged and detected (42–75% positive) post-filtration (Rose *et al.* 1996).

In full-scale seeded experiments, conventional filtration used in drinking water treatment plants reduced oocysts concentrations by an average of 2.25 log₁₀ and cysts by an average of 3.26 log₁₀. Direct filtration of these organisms showed slightly improved performance reducing oocyst levels by an average of 2.79 log₁₀ and cysts by an average of 3.87 log₁₀ (Niemiński & Ongerth, 1995). During a 2 year study evaluating source, occurrence, and treatment for removal of these pathogenic protozoa in river water, States *et al.* (1997) observed *Cryptosporidium* 21% of the time in finished water with a range of 0.4–0.6 oocyst 100 l⁻¹ while *Giardia* was not detected. LeChevallier & Norton (1995) have reported detection of *Cryptosporidium* in 13% of finished water samples with a range of 0.29–57 oocysts 100 l⁻¹ and *Giardia* was detected 17.1% of the time with a range of 0.29–64 cysts 100 l⁻¹. Rose (1997) has summarized the range of percent positive samples (3.8–40%) and the range of concentrations (0.1–72 oocysts 100 l⁻¹) for *Cryptosporidium* oocysts in finished drinking water. These levels may represent the everyday exposure that humans encounter from tap water.

Unfortunately, there is still much that we as a scientific community do not know and understand about these organisms. We know little of their fate and transport in the environment. Currently, there are limited monitoring data due to low recovery efficiencies for current methodologies. Methodologies are further compromised by differing water

qualities. The scientific community is only now developing techniques to determine viability and speciation. These factors have led to either overestimating or underestimating the true exposures. Underestimation occurs because of methodology limitations (i.e. low recoveries), overestimation occurs with assumptions that the organisms observed are viable due to a lack of a feasible viability method.

Better methods are now being utilized. Polymerase chain reaction (PCR) with greater specificity will allow for better hazard identification (Abbaszadegan *et al.* 1993; Filkorn, Wiedenmann & Botzenhart, 1994; Johnson *et al.* 1995; Wagner-Wiening & Kimmig, 1995; Jakubowski *et al.* 1996). Cell culture methods will provide information on infectivity and exposure that is of biological significance (Upton, Tilley & Brillhart, 1994; Jakubowski *et al.* 1996; Slifko *et al.* 1996, 1997). New filtration and concentration methods (such as immunomagnetsable separation) will provide more accurate measurements of the dose.

Disinfection has traditionally been the major barrier for control of microbiological organisms in drinking water (Fayer, 1997). However, routine water chlorination is insufficient to protect our potable water supply from *Cryptosporidium* and *Giardia*. A combination of filtration and disinfection is necessary. The onset of new and emerging technologies may enhance the capabilities of the drinking water industry to remove or inactivate these pathogenic protozoa, thus reducing exposure and risk.

Jacangelo, Adham & Laine (1995) evaluated six commercially available membranes (3 microfiltration (MF) membranes and 3 ultrafiltration (UF) membranes) at bench scale, pilot scale or both to determine removal efficacy and to provide a more in depth look into the mechanism of removal of *Cryptosporidium*, *Giardia* and bacteriophage. The authors found that while the MF membranes remained intact complete removal of *Crypto-*

sporidium and *Giardia* could be obtained. The primary mechanism of action was size exclusion. The largest pore sizes for the MF membranes were an order of magnitude smaller than the size of *Cryptosporidium* and *Giardia* (Jacangelo *et al.* 1995). The MF membranes were able to remove greater than 99.999% of the protozoa, essentially eliminating exposure.

COMPARING THE RISK ASSESSMENT MODEL WITH EPIDEMIOLOGICAL DATA FROM A FOODBORNE OUTBREAK

The epidemiological data from an outbreak of cryptosporidiosis in apple cider, occurring at a school agricultural fair (Millard *et al.* 1994), was compared to the risk assessment model for *Cryptosporidium*. Oocysts were detected in the leftover apple cider and from surface swabs of the portable cider press. Utilizing a sucrose gradient oocysts were detected at levels of 375–750 l in the cider. In addition, a concentration of 500 oocysts/l was detected using ethyl acetate sedimentation. Individuals who consumed 1 cup (112 ml) or less, 2 cups, or more than 2 cups had attack rates of 49%, 62% and 67%, respectively.

The probability of infection was evaluated for exposures of 56, 112 and 140 oocysts (in 1, 2 and 2.5 cups of cider) using the risk assessment model and the best estimate for r (0.00467) with an average of 500 oocysts/l. The risk estimates were also evaluated for the upper 95% confidence limit for r (0.0097) at the 750 oocyst l level. The observed attack rates best matched the risk assessment model when using the upper 95th percentile confidence limit surrounding the r value in the model (P_i of 42%, 66% and 74% for the 1, 2 and 2.5 cups of cider, respectively). The risks for the 750 oocysts/l predicted by the model were 32%, 54%, 62% while the risks for 500 oocysts/l (using the average r value in the model) were 23%, 41%, and 48%, respectively. This suggests that the exposure was underestimated and that the model may under-predict the risk based on the monitoring data.

The risk assessment model was able to predict the attack rates from the apple cider outbreak, perhaps, due to the fact that the oocysts were fairly young and fresh. It appears reasonable that if the age and species of oocysts in water can be assessed then the risk assessment model may have better applicability to drinking water contamination. It is also interesting to note that the apple cider outbreak had a higher hospitalisation (severity) ratio than previous waterborne outbreaks, 7% compared to 1.8% for Milwaukee, and a documented secondary transmission rate of 15% compared to 5% for Milwaukee (secondary transmission within a household) (MacKenzie *et al.* 1994). These types of investi-

gations can further refine the use of the model and the broader implications of community spread after an initial environmental contamination event.

RISK CHARACTERIZATION

Risk characterization integrates all the aforementioned steps in order to estimate the magnitude of the public health problem, taking into consideration the variability and uncertainty of the hazard. Variability and uncertainty are a part of any risk assessment paradigm. Assumptions and limited data are often used to estimate risks. Risk estimates in the United States based on occurrence and 99% removals by most water treatment plants suggest that acceptable levels of risk and suggested safety goals are not being met on a routine basis for *Cryptosporidium* and would only be met for *Giardia* if disinfection were increased. Regli *et al.* (1991) suggested an annual acceptable risk of 1:10000 (10^{-4}) from waterborne exposure through potable water. Using this acceptable risk and the Surface Water Treatment Rule guidance for accomplishing a removal of $> 3 \log_{10}$, Haas *et al.* (1996) estimated that treatment plants using source waters with average concentrations of less than 2 oocysts 100/l would meet the acceptable low risk level for *Cryptosporidium*. Using the same parameters, Regli *et al.* (1991) estimated that treatment plants using source waters with geometric mean concentrations of less than 0.7 cysts 100/l would meet the acceptable low risk level for *Giardia*.

Teunis *et al.* (1997) undertook a comprehensive risk assessment of *Cryptosporidium* and *Giardia*. The authors used the following parameters (building distributions around each): concentrations of oocysts and cysts (average $< 1/1000$ l), method recovery ($< 2\%$), viability of the recovered oocysts (30%) and cysts (15%), removal of protozoa based on *Clostridium* spores ($2.8 \log_{10}$), daily consumption of tap water (0.15 l/day), and dose-response r values (*Cryptosporidium* = 0.00467, *Giardia* = 0.01982). The cumulative estimate for an annual risk ranged from 10^{-5} to 10^{-4} for *Cryptosporidium* or *Giardia* and from 10^{-4} to 10^{-3} from exposure to both organisms. The data used to develop the parameters utilized by Teunis *et al.* (1997) were specific to the Netherlands with exception to the viability and the dose-response models. Using a similar approach one might expect geographical differences in annual risks worldwide. For example, one would expect the US to have a higher cumulative estimate for the annual risk due to data on occurrence and exposure to these pathogenic protozoa.

FUTURE DIRECTIONS AND COMMENTS

Risk assessment is a useful tool for evaluating relative risks and can be utilized for addressing low levels of microbial contamination. Models and epidemiol-

ogical data are currently available; however, temporal and spatial exposure data are ill defined. Qualitative risk assessment is hazard specific; however, if similar dose-response, occurrence, treatment removal, and health outcomes exist, extrapolations can be made. Problems arise with attempting to fit 1 management option for all micro-organisms, generally the chronic risks are not similar. Moreover, there are geographical and seasonal differences that have not been addressed, particularly worldwide.

To better utilize the tool of microbial risk assessment for risk management practices, future research should be focused in the area of exposure assessment. Reducing exposure to any hazard is the key to risk management: reduce the exposure and reduce the risk involved. Currently, we may be underestimating the occurrence, concentration and distribution of these pathogenic micro-organisms.

Focus on the following areas is required: (1) Better methods for recovery and detection of oocysts and cysts; (2) Determination of both species and viability of recovered oocysts and cysts; (3) Improved database on occurrence, concentration, and distribution of these organisms in various human and animal waste, waters (drinking versus recreational waters) and post drinking water treatment. In order to do this exposure levels and occurrence databases must be developed; (4) Transport studies; (5) Assessment of better treatment controls: filtration using membranes, ultra-violet irradiation, ozone, and their health benefits; (6) Relative risk assessment for foods, recreational, and drinking waters.

REFERENCES

- ABBASZADEGAN, M., HUBER, M. S., GERBA, C. P. & PEPPER, I. L. (1993). Detection of viable *Giardia* cysts in water using polymerase chain reaction. *Proceedings of the American Water Works Association*, WQTC, Miami, Florida, USA.
- BENENSON, A. S. (ed) (1995). *Control of Communicable Disease in Man*, 16th ed. Washington, D.C., USA, American Public Health Association.
- CDC. (1993). Surveillance for waterborne disease outbreaks—United States, 1991–1992. *Morbidity Mortality Weekly Report* **42**, 1–22.
- CDC. (1996). Surveillance for waterborne-disease outbreaks—United States, 1993–1994. *Morbidity Mortality Weekly Report* **45**, 1–33.
- COVELLO, V. T. & MERKHOFFER, M. W. (1993). *Risk Assessment Methods: Approaches for Assessing Health and Environmental Risks*. New York, N.Y., USA, Plenum Press, 3.
- CRAUN, G. F. (ed) (1986). *Waterborne Diseases in the United States*. Boca Raton, Florida, USA, CRC Press Inc.
- CRAUN, G. F., CALDERON, R. L. & FROST, F. J. (1996). An introduction to epidemiology. *Journal of the American Water Works Association* **88**, 54–65.
- DEREGNIER, D. P., COLE, L., SCHUPP, D. G. & ERLANDSEN, S. L. (1989). Viability of *Giardia* cysts suspended in lake, river, and tap water. *Applied and Environmental Microbiology* **55**, 1223–1229.
- DUPONT, H. L., CHAPPELL, C. L., STERLING, C. R., OKHUUSEN, P. C., ROSE, J. B. & JAKUBOWSKI, W. (1995). The infectivity of *Cryptosporidium parvum* in health volunteers. *New England Journal of Medicine* **332**, 855–859.
- FAYER, R. (ed) (1997). *Cryptosporidium and Cryptosporidiosis*. Boca Raton, Florida, USA, CRC Press, Inc.
- FILKORN, R., WIEDENMANN, A. & BOTZENHART, K. (1994). Selective detection of viable *Cryptosporidium* oocysts by PCR. *Zentralblatt für Hygiene and Umweltmedizin* **195**, 489–494.
- FROST, F. J., CRAUN, G. F. & CALDERON, R. L. (1996). Waterborne disease surveillance. *Journal of the American Water Works Association* **88**, 66–75.
- GERBA, C. P., ROSE, J. P. & HAAS, C. N. (1996). Sensitive populations: who is at the greatest risk? *International Journal of Food Microbiology* **30**, 113–123.
- GERBA, C. P., ROSE, J. B., HAAS, C. N. & CRABTREE, K. D. (1996). Waterborne rotavirus: a risk assessment. *Water Research* **30**, 2929–2940.
- GIBSON III, C. J., STADTERMAN, K. L., STATES, S. & SYKORA, J. (1998). Combined sewer overflows: a source of *Cryptosporidium* and *Giardia*? *Water Science and Technology* **38**, 67–72.
- HAAS, C. N. (1983). Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *American Journal of Epidemiology* **118**, 573–582.
- HAAS, C. N., CROCKETT, C. S., ROSE, J. B., GERBA, C. P. & FAZIL, A. M. (1996). Assessing the risk posed by oocysts in drinking water. *Journal of the American Water Works Association* **88**, 131–136.
- HAAS, C. N., ROSE, J. B. & GERBA, C. P. (eds) (1999). *Quantitative Microbial Risk Assessment*. New York, N.Y., USA, John Wiley and Sons.
- HAAS, C. N., ROSE, J. B., GERBA, C. & REGLI, S. (1993). Risk assessment of virus in drinking water. *Risk Analysis* **13**, 545–552.
- HANCOCK, C. M., ROSE, J. B. & CALLAHAN, M. (1998). Crypto and *Giardia* in US groundwater. *Journal of the American Water Works Association* **90**, 58–61.
- JACANGELO, J. G., ADHAM, S. S. & LAINE, J. M. (1995). Mechanisms of *Cryptosporidium*, *Giardia*, and MS2 virus removal by MF and UF. *Journal of the American Water Works Association* **87**, 107–121.
- JAKUBOWSKI, W., BOUTROS, S., FABER, W., FAYER, R., GHIORSE, W., LECHEVALLIER, M., ROSE, J., SCHAUB, S., SINGH, A. & STEWART, M. (1996). Environmental methods for *Cryptosporidium*. *Journal of the American Water Works Association* **88**, 107–121.
- JOHNSON, D. W., PIENIAZEK, N. J., GRIFFIN, D. W., MISENER, L. & ROSE, J. B. (1995). Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocysts in water samples. *Applied and Environmental Microbiology* **61**, 3849–3855.
- LECHEVALLIER, M. W. & NORTON, W. D. (1995). *Giardia* and *Cryptosporidium* in raw and finished water. *Journal of the American Water Works Association* **87**, 54–68.

- MACKENZIE, W. R., HOXIE, N. J., PROCTOR, M. E., GRADUS, M. S., BLAIR, K. A., PETERSON, D. E., KAZMIERCZAK, J. J., ADDISS, D. G., FOX, K. R., ROSE, J. B. & DAVIS, J. P. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal of Medicine* **331**, 161–167.
- MILLARD, P. S., GENSHEIMER, K. G., ADDIS, D. G., SOSIN, D. M., BECKETT, G. A., HOUCK-JANKOSKI, A. & HUDSON, A. (1994). An outbreak of cryptosporidiosis from fresh-pressed apple cider. *Journal of the American Medical Association* **272**, 1592–1596.
- MINTZ, E. D., HUSON-WRAGG, M., MSHAR, P., CARTTER, M. L. & HADLER, J. L. (1993). Foodborne giardiasis in a corporate office setting. *Journal of Infectious Diseases* **167**, 250–253.
- NIEMINSKI, E. C. & ONGERTH, J. E. (1995). Removing *Giardia* and *Cryptosporidium* by conventional treatment and direct filtration. *Journal of the American Water Works Association* **87**, 96–106.
- NATIONAL RESEARCH COUNCIL (NRC). (1983). *Risk Assessment in the Federal Government: Managing the Process*, NAS-NRC Committee on the Institutional Means for Assessment of Risks to Public Health, Washington, D.C., USA, National Academy Press.
- OKHUUSEN, P. C., CHAPPELL, C. L., STERLING, C. R., JAKUBOWSKI, W. & DUPONT, H. L. (1998). Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infection and Immunity* **66**, 441–443.
- OSTERHOLM, M. T., FORFAN, J. C., RISTINEN, T. L., DEAN, A. G., WASHBURN, J. W., GODES, J. R., RUDE, R. A. & MCCULLOUGH, J. G. (1981). An outbreak of foodborne giardiasis. *New England Journal of Medicine* **304**, 24–28.
- PETERSEN, L. R., CARTTER, M. L. & HANDLER, J. L. (1988). A foodborne outbreak of *Giardia lamblia*. *Journal of Infectious Diseases* **157**, 846–848.
- PORTER, J. D. H., GARNEY, C., HEYMANN, D. & PARKIN, W. (1990). A foodborne outbreak of *Giardia lamblia*. *American Journal of Public Health* **80**, 1259–1260.
- QUICK, R., PAUGH, K., ADDISS, D., KOBAYASHI, J. & BARON, R. (1992). Restaurant-associated outbreak of giardiasis. *Journal of Infectious Diseases* **166**, 673–676.
- REGLI, S., ROSE, J. B., HAAS, C. H. & GERBA, C. P. (1991). Modeling the risk from *Giardia* and viruses in drinking water. *Journal of the American Water Works Association* **83**, 76–84.
- RENTORFF, R. C. (1979). The experimental transmission of *Giardia lamblia* among volunteer subjects. In *Waterborne Transmission of Giardiasis* (ed. Jakubowski, W. & Hoff, J. C.), pp 64–81. U.S. Environmental Protection Agency. Office of Research and Development, Environmental Research Centre, Cincinnati, Ohio 45268, USA, EPA-600/9-79-001.
- ROSE, J. B. (1997). Environmental ecology of *Cryptosporidium* and public health implications. *Annual Reviews in Public Health* **18**, 135–161.
- ROSE, J. B., DICKSON, L. J., FARRAH, S. R. & CARNAHAN, R. P. (1996). Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. *Water Research* **30**, 2785–2797.
- ROSE, J. B., HAAS, C. N. & REGLI, S. (1991). Risk assessment and control of waterborne giardiasis. *American Journal of Public Health* **81**, 709–713.
- SLIFKO, T. R., FRIEDMAN, D., ROSE, J. B. & JAKUBOWSKI, W. (1997). An *in vitro* method for detection of infectious *Cryptosporidium* oocysts using cell culture. *Applied and Environmental Microbiology* **63**, 3669–3675.
- SLIFKO, T. R., FRIEDMAN, D. E., ROSE, J. B., UPTON, S. J. & JAKUBOWSKI, W. (1996). Unique cultural methods used to detect viable *Cryptosporidium parvum* oocysts in environmental samples. *Water Science and Technology* **35**, 363–368.
- SMITH, H. V., ROBERTSON, L. J. & ONGERTH, J. E. (1995). Cryptosporidiosis and giardiasis: the impact of waterborne transmission. *Journal of Water Supply Research and Technology—Aqua* **44**, 258–274.
- SMITH, H. V. & ROSE, J. B. (1998). Waterborne cryptosporidiosis: current status. *Parasitology Today* **14**, 14–21.
- SMITH, J. L. (1993). *Cryptosporidium* and *Giardia* as agents of foodborne diseases. *Journal of Food Protection* **56**, 451–461.
- STATES, S., STADTERMAN, K., AMMON, L., VOGEL, P., BALDIZAR, J., WRIGHT, D., CONLEY, L. & SYKORA, J. (1997). Sources, occurrence, and treatment removal of *Giardia* and *Cryptosporidium* in river water. *Journal of the American Water Works Association* **89**, 74–83.
- SYKORA, J. L., BANCROFT, W. D., STATES, S. J., SHAPIRO, M. A., BOUTROS, S. N., KELETI, G., TURZAI, M. & CONLEY, L. F. (1988). *Giardia* cysts in raw and treated sewage. *Controlling Waterborne Giardiasis* (ed. Logsdon, G. S.). New York, USA, ASCE Press.
- SYKORA, J. L., SORBER, C. A., JAKUBOWSKI, W., CASSON, L. W., GAVAGHAN, P. D., SHAPIRO, M. A. & SCHOTT, M. J. (1991). Distribution of *Giardia* cysts in wastewater. *Water Science and Technology* **24**, 187–192.
- TAUXE, R. V. (1998). New approaches to surveillance and control of emerging foodborne infectious diseases. *Emerging Infectious Diseases* **4**, 455–456.
- TEUNIS, P. F. M., MEDEMA, G. J., KRUIDENIER, L. & HAVELAAR, A. H. (1997). Assessment of the risk of infection by *Cryptosporidium* and *Giardia* in drinking water from a surface water source. *Water Research* **31**, 1333–1346.
- UPTON, S. J., TILLEY, M. & BRILLHART, D. B. (1994). Comparative development of *Cryptosporidium parvum* (Apicomplexa) in 11 continuous host cell lines. *FEMS Microbiological Letters* **118**, 233–236.
- WAGNER-WIENING, C. & KIMMIG, P. (1995). Detection of viable *Cryptosporidium parvum* oocysts by PCR. *Applied and Environmental Microbiology* **61**, 4514–4516.
- WHITE, K. E., HEDBER, C. W., EDMONSON, L. M., JONES, D. B. W., OSTERHOLM, M. T. & MACDONALD, K. L. (1989). An outbreak of giardiasis in a nursing home with evidence for multiple modes of transmission. *Journal of Infectious Diseases* **16**, 298–303.