Proceedings

The U.S. EPA's Research on Microorganisms in Drinking Water Workshop

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Stig Regli

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Tom Carpenter

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Cynthia Chappell, P. Okhuysen, R. Langer, S. Tzipori

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Abstract Not Provided
William Blanford

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Evaluating Microbial Indicators and Health Risks Associated With Bank Filtration
Floyd J. Frost

Application of a Multipath Microsphere Tracer Test To Understanding Transport of Bacteria and Protozoa at a Bank Filtration Site
Rick Langford, Dirk Schulze-Makuch, Suresh Pillai

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Joseph N. Ryan, Yumiko Abe, Rula Abu-Dalo, Menachem Elimelech, Garrett Miller, Zachary Kuznar, Ronald W. Harvey, David W. Metge

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Introduction

One of the high-priority research areas identified by the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD) is drinking water. Although the United States has one of the safest water supplies in the world, drinking water quality varies from place to place, depending on the condition of the source water and the treatment it receives. In 1996, Congress amended the Safe Drinking Water Act to emphasize sound science and risk-based standard setting.

Threats to drinking water safety come from the occurrence of chemical contaminants or pathogens in drinking water, and research is needed in a variety of areas to improve the ability to assess and thereby reduce the public health risks from America's public water systems. The continued occurrence of waterborne disease outbreaks demonstrates that the safety of drinking water might be threatened by pathogenic microorganisms if treatment is inadequate or if the quality of water in the distribution system is compromised.

ORD's research on microorganisms that may impact human health through drinking water is conducted across its three national laboratories (National Health and Environmental Effects Research Laboratory, [NHEERL]; National Exposure Research Laboratory [NERL]; and the National Risk Management Research Laboratory [NRMRL]) and through two national centers (National Center for Environmental Assessment [NCEA] and National Center for Environmental Research [NCER]). In addition, EPA's drinking water research program may indirectly benefit from microbial research being conducted through its National Homeland Security Research Center, although no individual research efforts from this center were presented at the workshop. NCER is responsible for implementing the Science To Achieve Results (STAR) competitive grants program; the remaining national laboratories and centers manage intramural research programs.

This workshop was organized by representatives from ORD's laboratories and centers to bring together ORD's intramural and extramural scientists who are researching microorganisms in drinking water, staff from the Office of Water (OW), and regional office representatives. The meeting was open to the public. The workshop provided a forum for the scientists to present their research, for OW to identify the research needs associated with their upcoming regulatory agenda, and for all participants to discuss applications of the research.

The EPA uses meetings like this one to discuss research progress on topics of major scientific interest to the Agency. The research reported is of critical importance to EPA, as it has the potential to strengthen the scientific basis for both assessing the risk from exposure to pathogenic microorganisms and developing appropriate risk-management practices to mitigate their effects.

The meeting had both platform and poster sessions. Presentations were provided by ORD intramural scientists, STAR grantees, and representatives from a regional office and OW. The abstracts in this report are organized by research topic area into platform presentations and poster presentations in the order of the Agenda or poster listing. For the one regional office presentation and the two presentations by OW in which regulatory agendas or research needs were identified, rather than an abstract, the full presentations are provided in Appendix 1, Presentations of Regional Research Needs and Office of Water Regulatory Activities and Research Needs. In addition, one STAR grantee was unable to attend the meeting; the abstracts of her two research grants are provided in Appendix 2, Additional NCER STAR Drinking Water Grant Microbial Research.

Finally, for this meeting EPA arranged for Web-broadcast of the presentations, enabling remote participants to hear and view the plenary presentations and discussions. Hence, you will see a remote participants list included in this proceedings document.

Rather than participate in this meeting, STAR grantees doing riverbank filtration research were invited to present their research at a separate meeting arranged by EPA/ORD/NCER and EPA/OW jointly with the U.S Geological Survey (USGS), The USEPA/USGS Meeting on *Cryptosporidium* Removal by Bank Filtration. This meeting was held on September 9-10, 2003, at the USGS facility in Reston, VA. The meeting consisted of a series of plenary presentations provided by researchers from OW, NCER's STAR program, USGS, U.S. De-

partment of Agriculture, universities, and states. This meeting also was open to the public. No formal proceedings document was prepared; however, the event description, agenda, and abstracts of the STAR research presented are included in Appendix 3, STAR Grant Presentation Abstracts and Agenda From the USEPA/USGS Meeting on *Cryptosporidium* Removal by Bank Filtration, September 9-10, 2003. In addition, pdf versions of the full presentations can be found at: http://es.epa.gov/ncer/publications/meetings/drinking_sept9_03.html.

For more information on ORD's drinking water research program, please contact the Acting National Program Director for Drinking Water, Gregory Sayles, at 513-569-7607 (sayles.gregory@epa.gov). For more information about EPA's STAR drinking water research grants program, you may contact Cynthia Nolt-Helms at 202-343-9693 (nolt-helms.cynthia@epa.gov) or Angela Page at 202-343-9826 (page.angelad@epa.gov). For more information on EPA's ORD, please visit our homepage: http://www.epa.gov/ord.

Overview of the U.S. EPA's Drinking Water Research Program

Fred S. Hauchman
National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC

Presentation Abstract

Drinking water is one of the highest priority research programs of the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD). To address the wide range of issues relating to waterborne contaminants, the U.S. EPA has established an integrated, multidisciplinary drinking water research program that is closely linked to the Office of Water's (OW) regulatory activities and timelines. Drinking water research is conducted or supported by ORD's six national laboratories and centers: the National Exposure Research Laboratory, the National Health and Environmental Effects Research Laboratory, the National Risk Management Research Laboratory, the National Center for Environmental Assessment, the Nationational Center for Environmental Research, and the National Homeland Security Research Center. The research program supports OW decisionmaking and the implementation of EPA rules at the state and local level through the development of new scientific data, innovative methods, and cost-effective technologies for improving the assessment and control of drinking water risks.

ORD's drinking water research program activities and plans for fiscal years 2003–2010 are described in a new Drinking Water Research Multi-Year Plan (MYP). As described in the MYP, the Safe Drinking Water Act (SDWA) provisions with the most significant implications for research on waterborne pathogens include the Microbial/Disinfection Byproduct (M/DBP) set of rules, the Contaminant Candidate List (CCL) of unregulated contaminants, distribution systems, and source water protection. Research to address key uncertainties associated with the Source Water and Ground Water rules (part of the M/DBP cluster of rules) includes efforts to: (1) improve methods to detect *Cryptosporidium* in water matrices; (2) assess risks associated with exposure to protozoa and viruses; and (3) remove *Cryptosporidium*, particularly for small systems. Research on unregulated pathogens is primarily focused on developing new or improved analytical detection methods, and on determining the ability of conventional and advanced treatment to remove or inactivate microorganisms. Studies also are being conducted on innovative molecular approaches to characterize and prioritize pathogens for possible listing on future CCLs. Distribution system studies include research on opportunistic pathogens in biofilms, and on the relationship between *Mycobacterium* disease and isolates of this microorganism in the distribution system. Finally, research on source water assessment methods, tools, and best management practices is being conducted to support both SDWA and Clean Water Act provisions that relate to the protection of drinking water sources. This presentation will provide an overview of current and planned research activities in each of these areas.

Regional Concerns for Microorganisms in Drinking Water

Bruce Macler
U.S. Environmental Protection Agency, Region 9, San Francisco

The full presentation can be found in Appendix 1.

Topic Area 1: Research Supporting Office of Water's Ground Water/Source Water Regulatory Activities

SDWA Requirements & Microbial Research Needs (Surface Water, Ground Water, & Distribution Systems)

Stig Regli
U.S. Environmental Protection Agency, Office of Water/Office of Ground Water and Drinking Water

The full presentation can be found in Appendix 1.

Microbial Dose-Response Modeling: A Predictive Bayesian Approach

James D. Englehardt[1] and Jeff Swartout[2]
[1]University of Miami, Miami, FL; [2]Office of Research and Development, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH

Presentation Abstract

Absolute dose-response assessments have not been possible for low doses of chemicals and microbes, due to the infeasibility of direct testing of low-dose response. The problem may apply to high doses in microbial risk assessment as well, because health effects of microbes may not generally extrapolate from animals to humans as readily as for chemicals. The objective of this project is to develop a robust dose-response model for human population response across all doses following exposure to a pathogen. The goal is to derive an approach that is physically and biologically plausible, and that accounts for variability in human susceptibility, variability in microbial (intra-species) strain virulence, variability in human-pathogen interaction, and variability in the form and quantity of available information.

A predictive Bayesian approach has been selected as the most efficient means of integrating all the desired factors into a framework that is independent of any arbitrarily chosen confidence limit. Recently, a predictive Bayesian method for absolute dose-response assessment from limited information has been proposed. Information types can be diverse, such as epidemiologic results, genetic prevalence data, cell culture data, and medical judgment, as well as conventional dose-response data. All available information is integrated rigorously, and the function narrows in response to information content. Response is measured in terms of believed risk, which is slightly higher than the expected frequency of health response as would be estimated by traditional frequentist methods, even with an allowance for uncertainty.

Predictive Bayesian models based on both the infection and illness endpoints are demonstrated in this project. The exact form (based on the confluent hypergeometric function) of the beta-Poisson dose-response model is used for modeling the infection endpoint. The model for the illness endpoint is derived from a self-organized critical pattern of pathogenic illness severities and is demonstrated numerically. Results indicate that self-organizing characteristics of pathogenesis result in a third parameter of the dose-response function for microbes corresponding to the assumed definition of illness (minimum severity). Information-limited predictive Bayesian dose-response assessments, obtained for *Cryptosporidium parvum* infection and illness endpoints, are compared and contrasted. The dose corresponding to 10^{-4} cases of waterborne cryptosporidiosis per capita-year is estimated to be 0.002 oocysts per exposure. Figure 1 shows the predictive dose-response function for Cryptosporidiosis based on the human response data for three *C. parvum* isolates, which also are shown in the figure.

The findings are significant in that they incorporate varied information, rigorously accounting for limited data in a manner that allows for straightforward updating with new information, without dependence on preset confidence intervals. Follow-on activities include an analysis and integration of data for a new *C. parvum* isolate and application of the model to other pathogens. Investigation of more flexible, but still biologically relevant models for better fitting of the entire dose-response curve is being pursued.

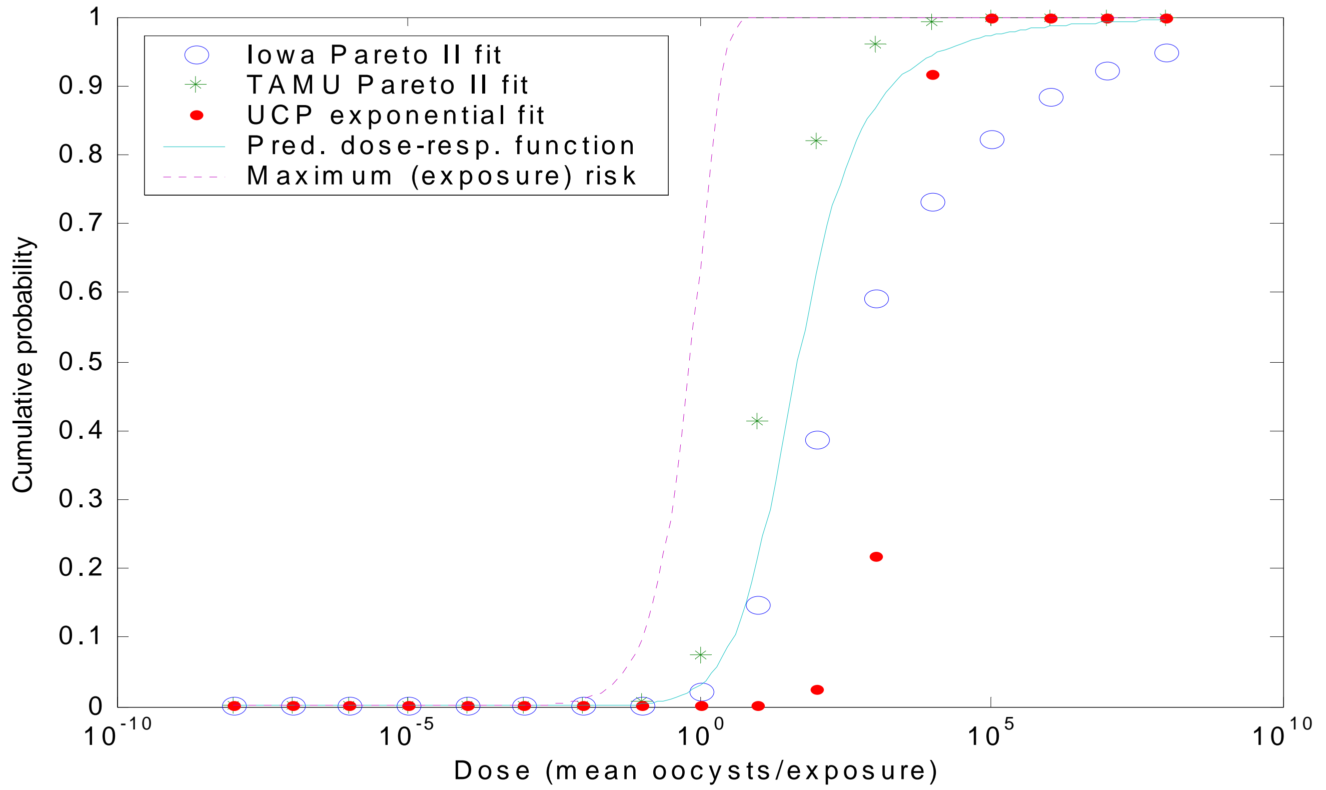

Figure 1. Predictive dose-response function for *C. parvum*: illness endpoint.

Microbial Dose-Response Modeling: A Predictive Bayesian Approach

James D. Englehardt[1] and Jeff Swartout[2]
[1]University of Miami, Miami, FL; [2]Office of Research and Development, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH

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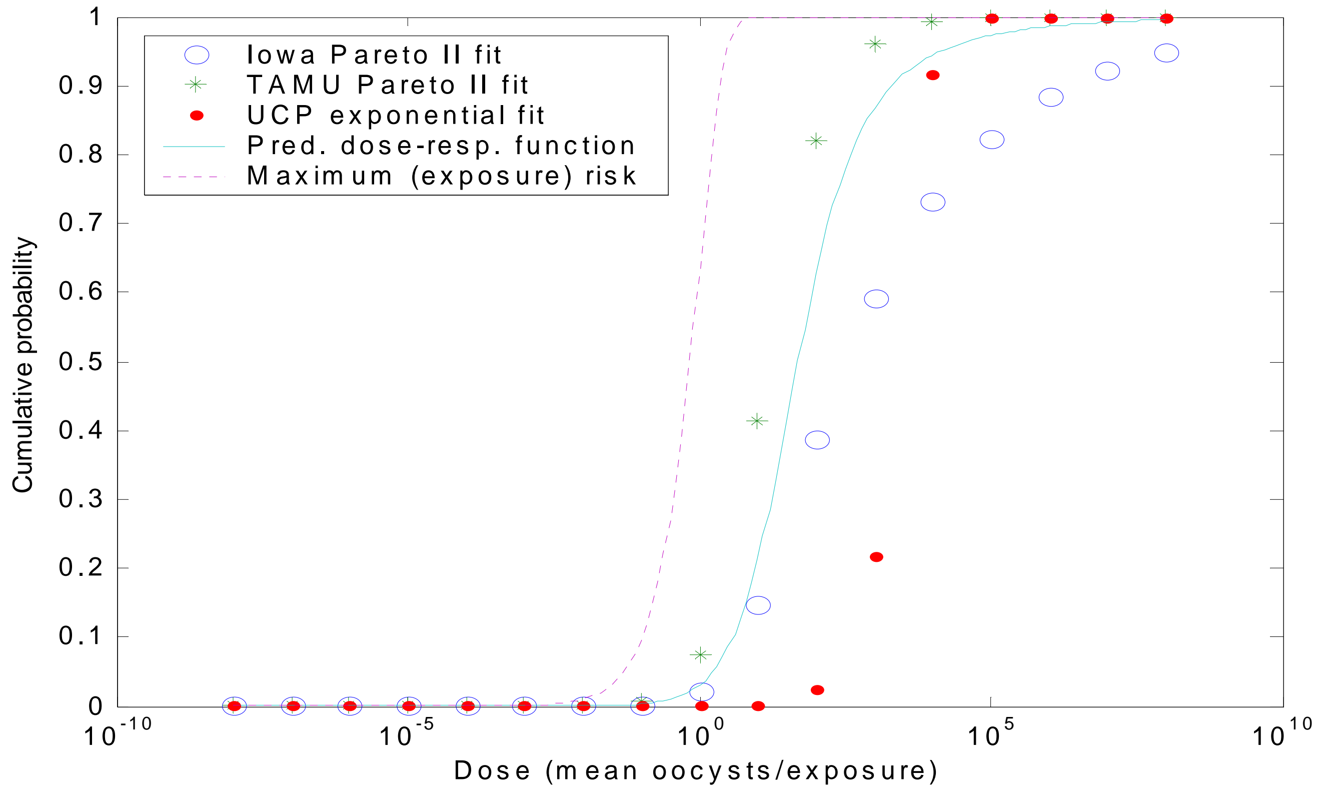

Figure 1. Predictive dose-response function for *C. parvum*: illness endpoint.

The Use of Randomized Trials of In-Home Drinking Water Treatment To Study Endemic Water Borne Disease

Timothy J. Wade[1], Rebecca Calderon[1], and John M. Colford, Jr.[2]
[1]Human Studies Division, U.S. Environmental Protection Agency, Chapel Hill, NC; [2]School of Public Health, University of California–Berkeley, Berkeley, CA

Presentation Abstract

Randomized trials of water treatment have demonstrated the ability of simple water treatments to significantly reduce the incidence of gastrointestinal illnesses in developing countries, where drinking water is of poor quality. Whether or not additional treatment at the tap reduces enteric illness in areas where water is treated to a higher degree has not been fully resolved. Randomized trials of in-home water treatment have been conducted to determine how much enteric illness, if any, is transmitted through a public water system and to determine whether or not current water treatment practices adequately protect public health.

The goal of this project is to review randomized trials of in-home water treatment conducted in developed countries, discuss key study design characteristics and sources of bias, and present preliminary data from several ongoing trials. The advantages and limitations of this type of study design also will be presented. The basic study design is as follows: households are randomly assigned to receive a device that provides additional treatment of pathogenic microorganisms in the household. The control group receives either no device, or receives an identical looking placebo device. Household members record occurrences of gastrointestinal symptoms. Any statistically significant difference between the rate of illness in the control group and the rate of illness in the active group is considered to be the amount of excess illness attribut- able to drinking regularly treated tap water. Clinical specimens also may be collected and tested for common pathogenic microorganisms.

Features to consider when designing or interpreting the results include: source water quality, distribution system water quality, study location, study population, sample size, device design, use of a placebo, recruitment methods, blinding of participants and investigators, randomization procedures, tracking water consumption, collection of clinical specimens, collection of water samples, statistical analyses, and outcome measurement and definition.

Four trials have been completed and published, two in Canada, one in Australia, and one small pilot study in the United States (Walnut Creek, California). The two trials in Canada identified a significant excess risk of illness in those receiving regular tap water. A blinded study in Australia, however, found no such increase in illness and, unlike the Canadian studies, included a placebo device and a blinded control group. Several major trials are ongoing or nearly completed in the United States. These include the full-scale followup to the pilot study being conducted in Davenport Iowa. Results from a second trial in an HIV infected population also will be published soon, and a large multiyear study in an elderly cohort is nearing completion. An additional study is being conducted in a community with a groundwater drinking water supply.

Results of these ongoing trials may provide a more complete picture of the adequacy of current microbial drinking water regulations. It is unlikely that trial data alone can provide a complete picture of the risks associated with tap water consumption. The applicability of trials in restricted geographic locations to the entire U.S. population is questionable. Moreover, the interpretation of null results must be fully considered, and alternative hypotheses must be developed to explain such findings.

Screening Models To Predict Probability of Contamination by Pathogenic Viruses to Drinking Water Aquifers

Bart Faulkner
Office of Research and Development, National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Ada, OK

Presentation Abstract

The purpose of this research is to develop simple, screening level models to predict the overall ability of groundwater systems to attenuate viable viruses introduced to them. Compartmental modeling approaches are used because of their simplicity. Stakeholders and decisionmakers often seek cursory screening level models to identify at-risk drinking water supplies (see Figure 1). When applied under appropriate circumstances, compartmental models can allow prioritization of groundwater systems for further investigation, especially if such models employ probabilistic methods in their development that can capture much of the uncertainty in input parameters. The goals of this research are to develop such screening models and to perform sensitivity analyses of the models, and to develop prior distributions that correspond to actual uncertainties in the domain of the inputs.

The approach used is to identify components of groundwater systems that can be considered to behave as compartments, contributing to a final catchment scale subsurface flow model. After these flow models are developed, appropriate attenuation functions are derived and applied. In most cases, these are based on physical, deterministic governing differential equations. By invoking certain assumptions such as steady state, or well-mixed reactors, the equations can be solved to yield algebraic expressions. Uncertainty is captured in final model estimates of attenuation by (1) error propagation methods, or (2) Monte Carlo methods. Bayesian approaches will be used to specify prior distributions for parameters to allow for sparse data and robustness, and to quantify uncertainty.

At present, a complete probabilistic modeling approach has been developed for homogeneous unsaturated soils. Assuming gravity flow, a suite of prior distributions has been developed for each of the 12 U.S. Department of Agriculture soil categories. Probabilistic outputs show that for one-half meter thick soils, most exhibit probabilities of failure to attenuate most viruses to the 99.99 percent reduction demanded in many regulatory compliance settings. Sensitivity analyses show that mean $\log_{10}$ of saturated hydraulic conductivity and the water-to-air mass transfer affected virus fate and transport about three times more than any other parameter, including inactivation rate of percolating viruses. A user-friendly computer model with a graphical user interface has been developed and released.

The sensitivity analyses suggest extreme infiltration events may play a predominant role in leaching of viruses in soils, as such events could impact hydraulic conductivity. The water-air interface also plays a large role. The current research issue of accurate estimation of the air-water interfacial area is an important one, not only for modeling transport of contaminants subject to hydrophobicity effects, but also for unsaturated-zone virus transport modeling.

Developing additional components of an overall catchment-scale groundwater model, including residence time in the saturated zone, remains as an important next step. Challenges include developing appropriate assumptions to make residence time prediction for spatially explicit virus loadings tractable, and yet still reasonable for useful prediction.

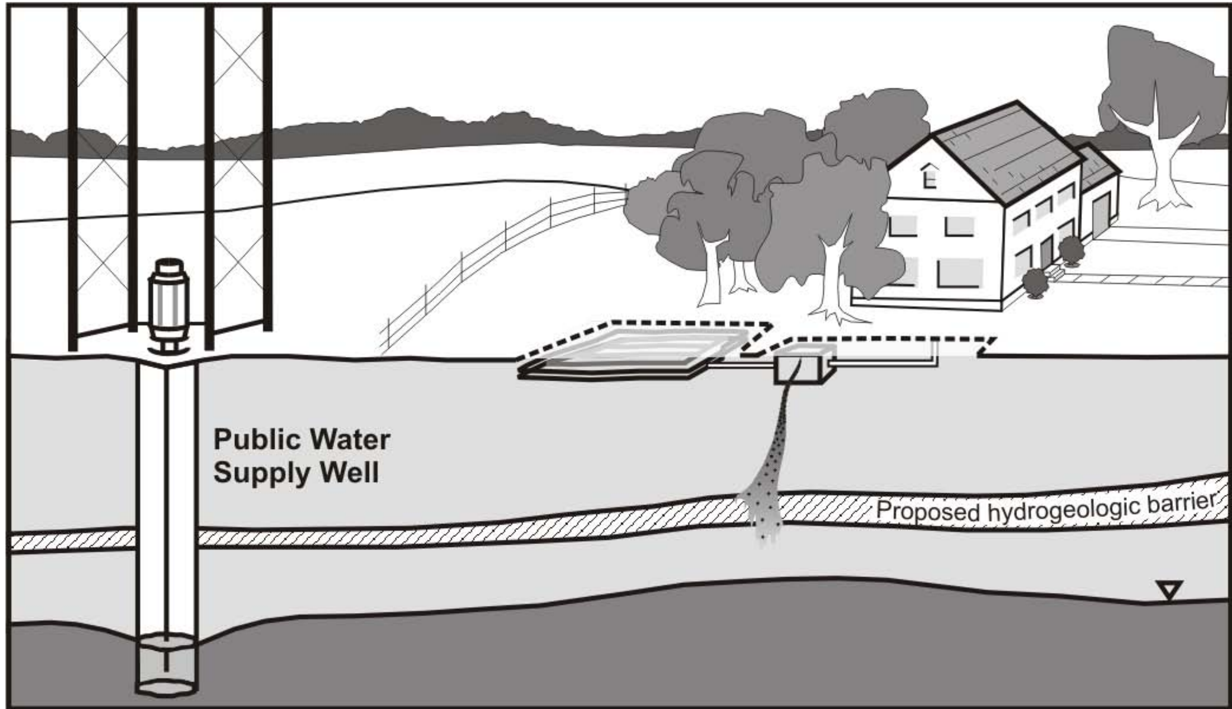

Figure 1. A user-friendly Monte Carlo-based model (Virulo) has been developed by the U.S. EPA. It can be used to predict the probability of failure of a proposed hydrogeologic barrier to attenuate viruses to a given level.

Integrated Approach for the Control of *Cryptosporidium parvum* Oocysts and Disinfection By-Products in Drinking Water Treated With Ozone and Chloramines

Jason L. Rennecker, Amy (Driedger) Samuelson, Benito Corona-Vasquez, Jaehong Kim, Hongxia Lei, Roger A. Minear, and Benito J. Mariñas
Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, IL

Presentation Abstract

The overall goal of this project was to develop process design recommendations for the simultaneous control of *Cryptosporidium parvum* oocysts and disinfection by-products (DBPs) during ozone/chloramines sequential disinfection of natural waters. Because the main objective of the study was to develop an integral control strategy, the scope of work included investigating a limited number of DBPs (bromate, formaldehyde, and cyanogen halides) associated with the ozone/chloramines sequential disinfection process. This presentation will emphasize experimental findings on the inactivation of *C. parvum* oocyst with sequential ozone/chloramines (as well as ozone/free chlorine) schemes. The DBP work of the study will be presented at a future NCER STAR meeting focusing on DBP aspects.

C. parvum oocysts have emerged as the microbial water contaminant with greatest resistance to chemical disinfectants. There is particular concern because both the free and combined forms of chlorine, most commonly used as primary inactivation agents, are considered practically ineffective in controlling *C. parvum* oocysts under typical drinking water conditions. In contrast, ozone and chlorine dioxide are both considered viable chemical disinfectants, but there is concern about potentially high disinfection requirements. A promising alternative for more efficient control of *C. parvum* oocysts is the sequential application of certain combinations of disinfectants. The efficiency of *C. parvum* oocyst inactivation by combined as well as free chlorine can be increased significantly after limited exposure to ozone.

There are various factors that could affect the overall efficiency of sequential ozone/monochloramine as well as ozone/free chlorine disinfection processes. These include pH, temperature, ozone pretreatment level, and oocyst resistance variability. Experimental results showing the role of these various parameters will be presented.

Prevalence and Distribution of Genotypes of *Cryptosporidium parvum* in United States Feedlot Cattle

Robert Atwill
University of California at Davis, Davis, CA

Presentation Abstract

The overall goal for this research project is to characterize the ability of feedlot cattle in the United States to load the environment with the protozoal parasite, *Cryptosporidium parvum*. Specific objectives are to establish the prevalence, intensity, and distribution of genotypes of *C. parvum* in populations of feedlot cattle and to identify risk factors that are associated with feedlot cattle shedding *C. parvum*.

Fecal samples were collected from 5,274 cattle, whereby approximately 240 cattle from 22 different feedlots located in California, Washington, Texas, Oklahoma, Colorado, South Dakota, and Nebraska were sampled. Within each feedlot, fecal samples were typically collected from sets of cattle who had just arrived, had been at the feedlot 4 to 8 weeks, and from cattle several weeks prior to slaughter to generate a comprehensive survey of fecal shedding of *C. parvum*.

Out of 5,274 fecal samples, only 9 (0.2%) had detectable levels of oocysts as measured by our standard diagnostic test, direct immunofluorescent microscopy (see Table 1). This assay can reliably detect oocyst concentrations down to about 600 oocysts per gram of feces. To determine if a percentage of cattle were shedding small numbers of oocysts, 10 negative cattle were retested from each feedlot using immunomagnetic separation of oocysts followed by direct immunofluorescence (IMS-DFA). Our IMS-DFA method can detect as few as 1 oocyst per gram of bovine fecal material, arguably the most sensitive method published to date for detecting *C. parvum* in bovine feces. Using this highly sensitive method, 2 out of 220 (0.9%) fecal samples contained low levels of oocysts, indicating that false negatives were relatively uncommon in our data. This dataset is being statistically modeled to estimate the environmental loading rate using methods developed in our laboratory.[1] Using a nested polymerase chain reaction technique associated with restriction fragment length polymorphism developed by Xiao et al. (1999) of the Centers for Disease Control and Prevention that targets the 18S rRNA gene, the genotype for these isolates was the bovine genotype A.

Although we are still in the process of calculating the environmental loading rate of *C. parvum* bovine genotype A from feedlot cattle, these preliminary data suggest that feedlot cattle are not heavily infected with *C. parvum* in middle and western United States. This lack of substantial shedding of *C. parvum* among feedlot cattle is a positive finding, given the fact that feedlots are located throughout the United States, feedlots can produce large amounts of fecal material on a per acre basis, and given the infectious potential of bovine *C. parvum* for susceptible humans.

Reference

1. Atwill, E.R., et al. 2003. Improved quantitative estimates of low environmental loading and sporadic periparturient shedding of *Cryptosporidium parvum* in adult beef cattle. *Applied Environmental Microbiology* 69:4604-4610.

Table 1. Prevalence of Fecal Shedding of *Cryptosporidium parvum* in Feedlot Cattle in the United States.

State			
California	4	0/960	0/40
Washington	1	2/240 (0.8%)	0/10
Texas	3	0/711	0/30
Oklahoma	3	0/722	1/30
Colorado	4	6/957 (0.6%)	0/40
South Dakota	4	1/964 (0.1%)	1/40
Nebraska	3	0/720	0/30
TOTAL	22	9/5,274 (0.2%)	2/220 (0.9%)

[1] Diagnostic method was direct immunofluorescent assay.
[2] Diagnostic method was immunomagnetic separation of oocysts coupled with a direct immunofluorescent assay, capable of detecting one oocyst per gram of feces.

Microbial Drinking Water Contaminants: Endemic and Epidemic Waterborne Gastrointestinal Disease Risks in the United States

Rebecca L. Calderon[1] and Gunther Craun[2]
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[2]Gunther F. Craun Associates, Staunton, VA

Poster Abstract

In the United States, drinking water treatment is rightfully considered a major public health achievement of the 20th century. However, a residual number of waterborne outbreaks continue to be reported when water treatment systems fail or are poorly operated, and distribution systems or sources become contaminated. In some reported outbreaks, current drinking water standards had not been exceeded. Recent epidemiologic studies also have suggested that cases of mild, unreported gastroenteritis may be associated with consumption of tap water from systems where no outbreak was reported. The U.S. EPA has collaborated with the Centers for Disease Control and Prevention for more than 30 years in the surveillance and investigation of waterborne disease outbreaks. The periodic analysis of waterborne outbreak statistics from 1971 to 2000 has assisted the U.S. EPA in developing a research program and promulgating regulations for safe drinking water (e.g., the Surface Water Treatment Rule, Ground Water Treatment Rule, Total Coliform Rule). The nature and magnitude of endemic waterborne disease, however, is not well characterized in the United States. Recent studies conducted in Canada suggest that in some communities that meet all current drinking water regulations, drinking water could be a significant cause of gastrointestinal (GI) disease. Because persons with GI illnesses rarely visit a physician for treatment, the causes of these illnesses in developed countries are difficult to study by traditional observational epidemiologic designs.

EPA's Surface Water Treatment Rule (SWTR) of 1989 requires all communities that use surface water as a source of their drinking water to filter their water, unless special criteria are met. The promulgation of this rule provided an opportunity to use a natural experiment and a quasi-experimental epidemiologic design to evaluate endemic waterborne illness risks. After surveying water utilities affected by the SWTR, 21 utilities were found that would be good candidates for an epidemiologic study of waterborne disease. A pilot epidemiologic study was conducted in one community to evaluate the endemic waterborne gastrointestinal illnesses risks and obtain information for use in designing additional studies. This community intervention study collected information about daily GI symptoms from families before and after filtration was added to the drinking water treatment process Information was obtained during July through December in 1996 and 1997. As a requirement for participation, families had to include one or more children in the study. Analyses of the pilot study community indicate a significant reduction occurred in the rate of credible-gastrointestinal (CGI) illness after filtration of drinking water. The relative risk of CGI before filtration was almost double that after filtration (RR=1.8, 95% CI=1.5-2.1). The attributable risk of CGI associated with unfiltered drinking water is estimated to be 34 percent. We concluded that this is an efficient study design and have moved forward with two other community intervention studies. The second and third communities will include an assessment of CGI in families in a control community to control for community variability in the incidence of GI illness and help interpret the observed waterborne contribution to illness risks. The second study in Washington State has finished data collection, and the third study in southwest Texas is in the middle of data collection.

Evaluating Microbial Indicators and Health Risks Associated With Bank Filtration

Floyd J. Frost
Lovelace Clinic Foundation, Albuquerque, NM

Poster Abstract

The purpose of the proposed project is to compare serological responses to *Cryptosporidium* antigens in users of bank-filtered water (one community with only bank filtration and disinfection and one community with bank filtration, conventional filtration, and disinfection) with the responses of similar people residing in an area that uses disinfected but unfiltered high-quality groundwater. The hypothesis is that, if bank filtration completely removes *Cryptosporidium* oocysts, the serological responses of the three populations should be similar. The specific goals of the study are to: (1) identify approaches to collecting sera from similar populations in different geographic locations so that rates of serological responses can be compared; (2) pilot test the approach in three different geographical locations by collecting sera from cities that use bank filtration and nearby cities that use high-quality groundwater for a drinking water source; (3) analyze the sera for serological responses to *Cryptosporidium* and *Giardia* antigens and compare the frequency and intensity of responses between the bank filtration cities and the groundwater cities; and (4) compare serological responses in the same cities at times when bank filtration efficacy is likely to be optimal and when it is likely to be least effective.

Sera from 50 people from each of three communities (users of bank filtered and chlorinated, bank filtered plus direct filtration plus ozonation, and chlorinated groundwater) will be collected at baseline and at five followup blood draws. A questionnaire on risk factors will be collected at each blood draw. Sera will be tested for the presence of antibody responses to two *Cryptosporidium* antigens (15/17-kDa and 27-kDa) and for serological changes (seroconversion). The baseline level of serological responses as well as the rates of seroconversion will be compared for each population (50 baseline and 250 periods for estimating rates of seroconversion). Comparisons will adjust for collected risk factor data from each individual. For purposes of extrapolating these results to other locations, a series of source and finished water quality indicators will be measured for each water source.

No results are available at this time. Analysis of sera will take place once all sera are collected. Then, all sera from a subject will be run on the same Western blot to reduce variations between blots. Blood draws will continue every 4 months. Data entry protocols will be developed and implemented for data entry of the questionnaires. Sample analysis will commence once all of the samples are collected, because the analysis of each subject's samples will be on the same Western blot. The distribution systems analysis also will commence.

A Prospective Epidemiological Study of Gastrointestinal Health Effects Associated With Consumption of Conventionally Treated Groundwater

Christine Moe[1], Stuart Hooper[1], Deborah Moll[2], Debi Huffman[3], Ricardo Izurieta[2,3], Renea Doughton-Johnson[1], Tatiana Ochoa[1], Jim Uber[4], Dominic Boccelli[2,4], Joan Rose[5], and Pierre Payment[6]
[1]Rollins School of Public Health, Emory University, Atlanta, GA; [2]Centers for Disease Control and Prevention, Atlanta, GA; [3]University of South Florida, St. Petersburg, FL; [4]University of Cincinnati, Cincinnati, OH; [5]Michigan State University, Lansing, MI; [6]Université du Québec, Québec, Canada

Poster Abstract

The overall goal of this study is to estimate the risks of endemic gastrointestinal (GI) illness associated with the consumption of conventionally treated groundwater in the United States and determine the relative contributions of source water quality, treatment efficacy, and distribution system vulnerability to endemic waterborne disease.

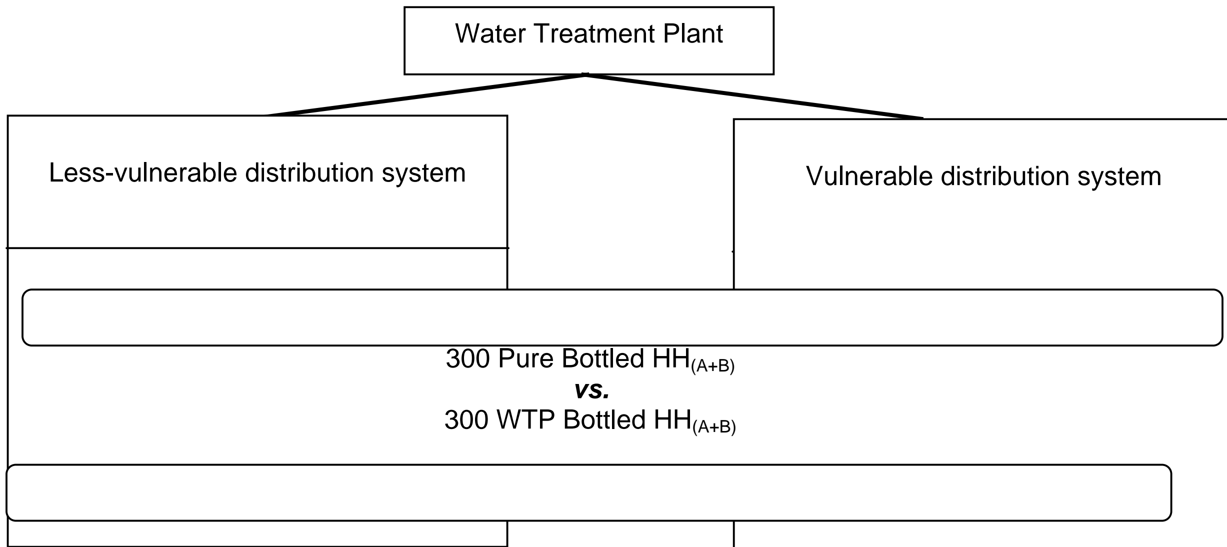
In the United States, nearly 89 million people depend on community groundwater systems for drinking water. Epidemiology studies in communities using surface water sources have suggested that 10-40 percent of GI illness may be associated with drinking water. Recent national groundwater surveys have found significant occurrence of microbial contamination in groundwater sources, and there is no information about the endemic illness that may be associated with consumption of treated groundwater. Also, there is uncertainty about the relative magnitudes of risks from problems with distribution systems and risks from treatment deficiencies. The specific aims of this study are to: (1) compare GI illness rates in individuals drinking highly purified bottled water to GI illness rates in individuals drinking conventionally treated groundwater bottled at the treatment plant to determine the risk of GI illness associated with source water quality and treatment; and (2) compare GI illness rates in individuals drinking bottled treatment plant water to GI illness rates in individuals drinking tap water from selected areas of the distribution system to determine the risk of GI illness associated with distribution system vulnerability.

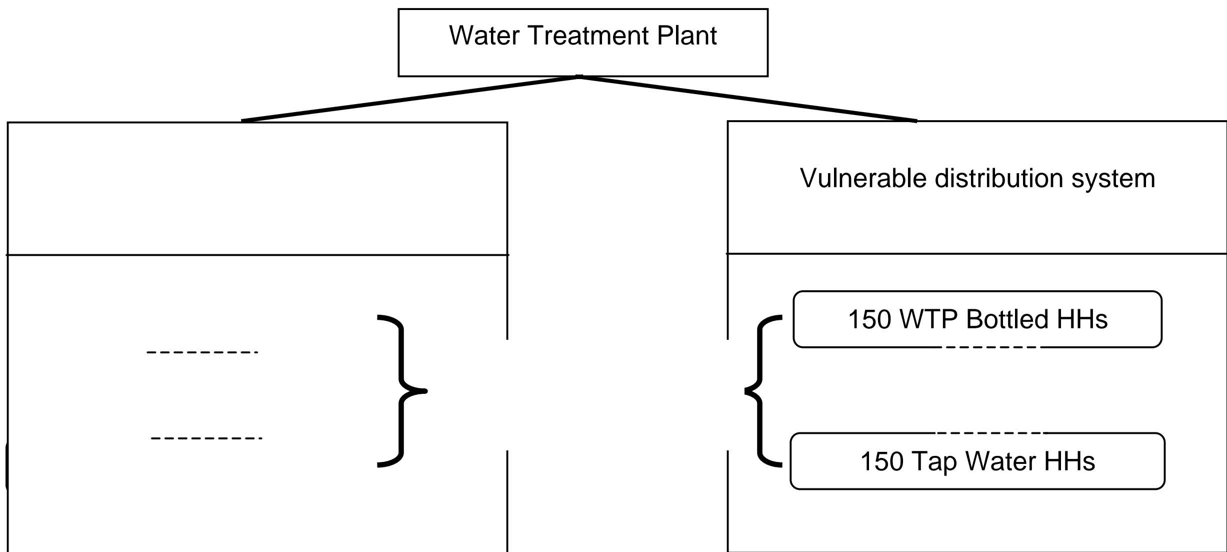
This study is a 12-month, double-blinded, randomized intervention trial of 900 households in a large metropolitan area in the southeastern United States with a community groundwater system that uses conventional treatment, meets current water quality standards, and has a well-characterized distribution system with areas of vulnerability. Study households will be randomly divided into three groups of 300 households: Group 1 households will drink bottled water that has been treated with ozonation and reverse osmosis (O_3-RO bottled water), Group 2 households will drink bottled water collected at the water treatment plant after treatment (WTP bottled water), and Group 3 households will drink tap water from their home. Groups 1 and 2 will be blinded to their group assignment. One-half of the households within each group will be recruited from vulnerable areas in the distribution system to examine the health risks from distribution system intrusion. A summary of the experimental design is shown in Figure 1. Study participants will report GI illness symptoms and selected risk factors in a weekly health diary and biweekly telephone interview. Samples will be routinely collected from raw source water, treated water, distribution system water, and bottled water and analyzed for microbial indicators of fecal contamination and intrusion. Data analyses will compare GI illness rates and water quality among the three study groups and among study households in different parts of the distribution system.

Work to date has involved developing survey materials and obtaining Institutional Review Board approval, coordinating activities with the local utility, obtaining and analyzing historic and current water quality data, and investigating areas of the distribution system that may be more vulnerable to intrusion. The vulner-

ability assessment has been performed using hydraulic simulations of the distribution system, water quality data, operations data (main breaks and repairs, customer complaints), and expert opinion provided by plant and distribution system personnel.

This is the first study to measure the risk of GI illness associated with the consumption of conventionally treated groundwater and to distinguish between the risk from source water and treatment vs. the risk from the distribution system. The results of this study will provide valuable information on the magnitude of endemic GI illness associated with drinking water in the United States. The next steps in this study will be to recruit and enroll households and begin the intervention and data collection.


Water Treatment Plant
Less-vulnerable distribution system
Vulnerable distribution system
300 Pure Bottled HH(A+B)
vs.
300 WTP Bottled HH(A+B)


Water Treatment Plant
Vulnerable distribution system
150 WTP Bottled HHs
150 Tap Water HHs

Using Neural Networks To Create New Indices and Classification Schemes

Gail Brion and Srini Lingireddy
Department of Civil Engineering, University of Kentucky, Lexington, KY

Poster Abstract

The hypothesis for this project is that shifts in indicator and indigenous bacterial populations can be reliably and mathematically related by neural network models to the presence, concentration, age, and source of microbial pathogens in river water. To test this hypothesis in the Kentucky River, tools must be developed that provide early warning of potentially "risky" conditions in source waters for Water Treatment Plants. Specifically, the objectives are to: (1) collect and analyze surface water samples for a host of surrogate indices and probable human pathogens over a multiyear period; (2) apply traditional modeling techniques and statistical analysis to the database to find relationships between indices and pathogen presence; (3) define the correlation between atypical coliform colonies and presence/ concentration of other surrogate indicators and pathogens; (4) confirm the relationship between the ratio of atypical coliform colonies to total coliforms (AC/TC) with time; and (5) apply neural network modeling (ANN) for identification of indices and combinations of indices related to pathogen presence, concentration, and probable source as well as to gain insight into guidelines for the application of ANNs for surface water quality modeling.

A multiparameter database will be created locally, while other databases are accumulated internationally. ANNs will be applied to predict the presence of pathogens (enteric viruses, protozoa) from other water quality parameters (i.e., turbidity, pH, alkalinity, indicator bacteria, indicator bacteriophage, fecal sterols, bacterial ratios). ANN modeling has been applied to a dataset provided by multiple investigators from Europe for the prediction of enteric viruses from shellfish samples. It has been shown that the ANN approach is superior to that of logistic regression. Also, it has been demonstrated how ANNs can be used to determine which of the variables are of significance. A case study related to the research objectives of this grant has shown that the AC/TC ratio can find sources of human pollution in a creek where other proposed indicators cannot. Along the waterway studied, levels of fecal coliforms and enterococci were statistically indeterminate. However, the AC/TC ratio dropped whenever the waterway passed through inadequately sewered towns with statistical significance.

Initial sampling of the Kentucky River has shown a trend between drops in the AC/TC ratio and the presence of fecal sterols, FRNA phage, protozoa, and enteric viruses. However, many more samples are required before this noted trend can be statistically verified. ANN modeling has again shown itself to be superior to other regression-based modeling methods for the precise prediction of pathogen presence. This provides the basis for new monitoring and control models. If the AC/TC ratio is upheld as a valid means of assessing the presence of fresh fecal contamination, these new indices could be applied for initial watershed sampling to find "hot spots" for applying remediation. The ratio might provide the basis for new types of TMDL models that consider the age and source of fecal material as well as load.

The next steps are to continue sampling on a weekly to biweekly basis and analyzing the in-house databases accumulated, and to develop an LC/MS based methodology for fecal sterols.

Topic Area 2: Research Supporting Office of Water's Contaminant Candidate List (CCL)

The Contaminant Candidate List: Determining the Need for Future Drinking Water Standards

Tom Carpenter
U.S. Environmental Protection Agency, Office of Water/Office of Ground Water and Drinking Water

The full presentation can be found in Appendix 1.

The Roles of Pathogen Risk Assessment in the Contaminant Candidate List Process

Glenn Rice, Michael Wright, Brenda Boutin, Jeff Swartout, Michael Broder, Patricia Murphy, Jon Reid, and Lynn Papa
National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH

Presentation Abstract

Under the 1996 Amendments to the Safe Drinking Water Act, the U.S. EPA is required to make regular determinations of whether or not to regulate contaminants on the Contaminant Candidate List (CCL). The provision of safe public drinking water includes ongoing monitoring for contaminants with the potential to cause adverse health effects in humans through tap water exposures. The CCL includes 10 bacterial and viral pathogens. The U.S. EPA's Office of Research and Development (ORD) and Office of Water (OW) have published a draft research strategy to identify and prioritize research for contaminants listed on the CCL. The CCL research strategy highlights the need to conduct risk assessments at two different time points. The initial assessment (see Figure 1) identifies and integrates all of the available information needed to estimate the risk posed by a contaminant, and identifies and prioritizes the information gaps so that the most appropriate research can be conducted. After the data identified in the initial assessment are collected, a second risk assessment is conducted to provide a more accurate estimate of the risk posed by the contaminant than was determined by the initial assessment. The OW can use these assessments to decide whether or not a contaminant should be regulated. EPA's National Center for Environmental Assessment (NCEA) is developing pathogen assessments to address this initial assessment phase. A recently completed NCEA assessment of exposure to *Mycobacterium avium* Complex (MAC) via tap water ingestion will be presented as an example of this process. After evaluating the available information, the infection risk from MAC-contaminated tap water appears to be limited to a few populations including people with AIDS, transplant recipients and others patients receiving immunosuppressive therapies, individuals with compromised pulmonary systems, and children. $CD4^+$ cell counts are a strong predictor of MAC infection risk in the AIDS population; therefore, the MAC assessment targeted the fraction of the U.S. AIDS population having less than 100 $CD4^+$ cells/mm^3, the severely immunocompromised fraction of the AIDS population. Members of this population would be expected to experience the most severe responses to MAC infections. Two exposure assessments were developed to address MAC ingestion exposures in this population (see Figures 2 and 3). Epidemiologic research designed to examine the association between MAC infections in the severely immunocompromised fraction of the AIDS population and water-related activities is needed. If water-related activities are risk factors for MAC infection, then research assessing pathogen exposure-response relationships in the AIDS population and in other susceptible populations also is needed.

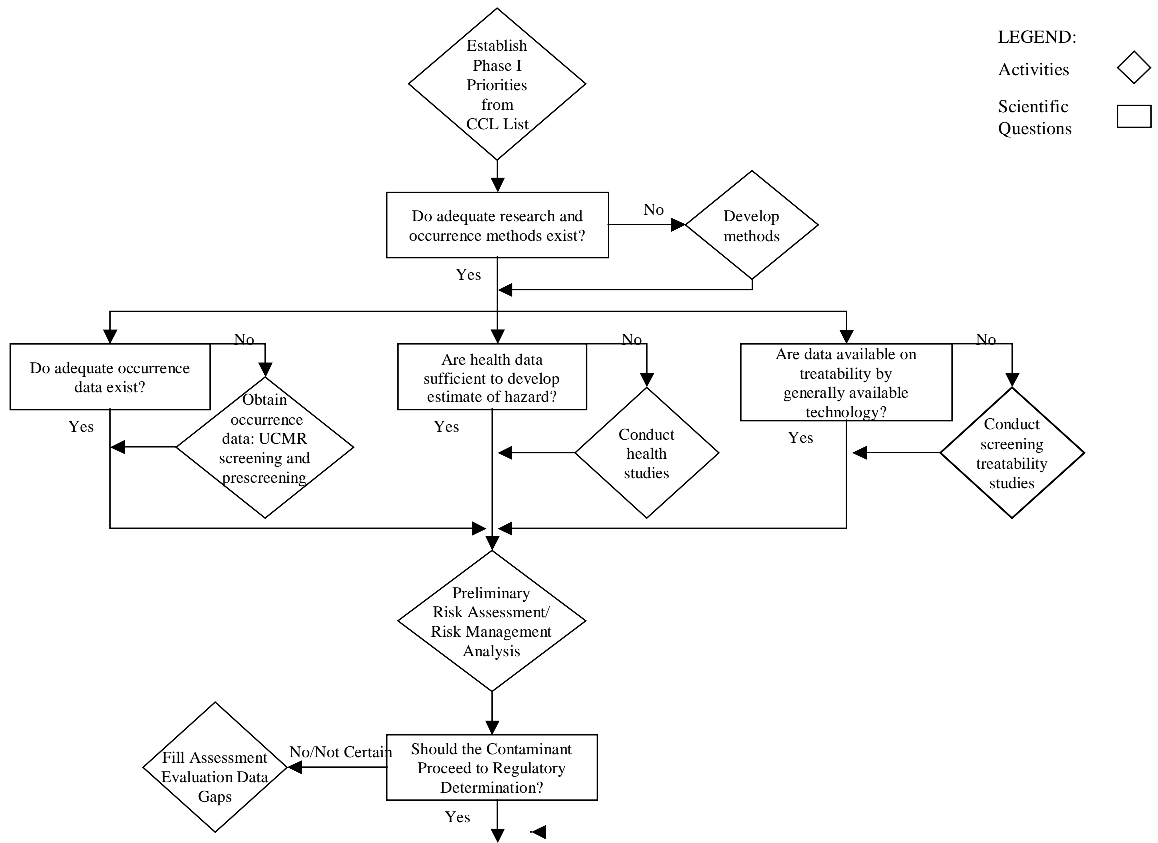


Figure 1. CCL Phase I decision making process.

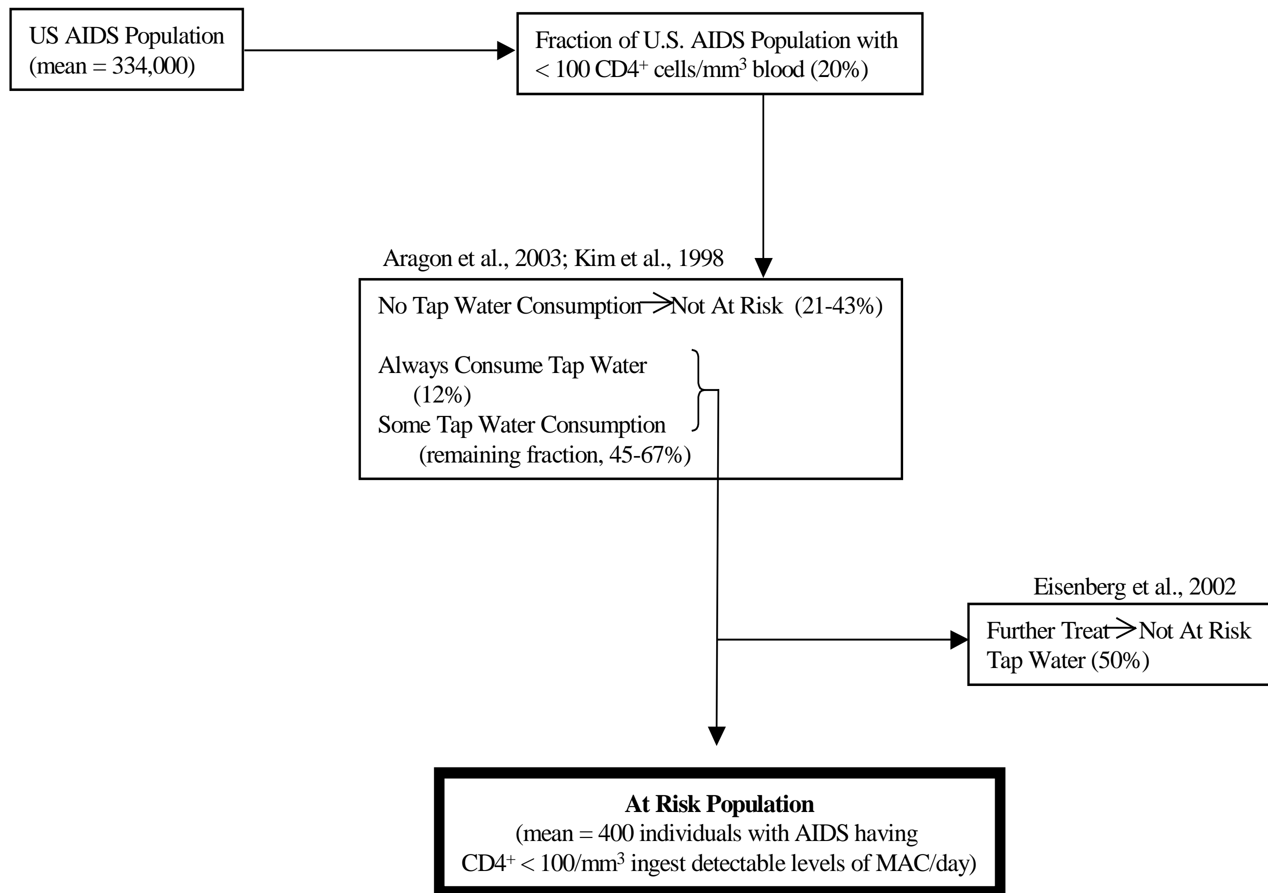


Figure 2. Model 1: Severely immunocompromised AIDS population exposed to MAC via tap water ingestion.

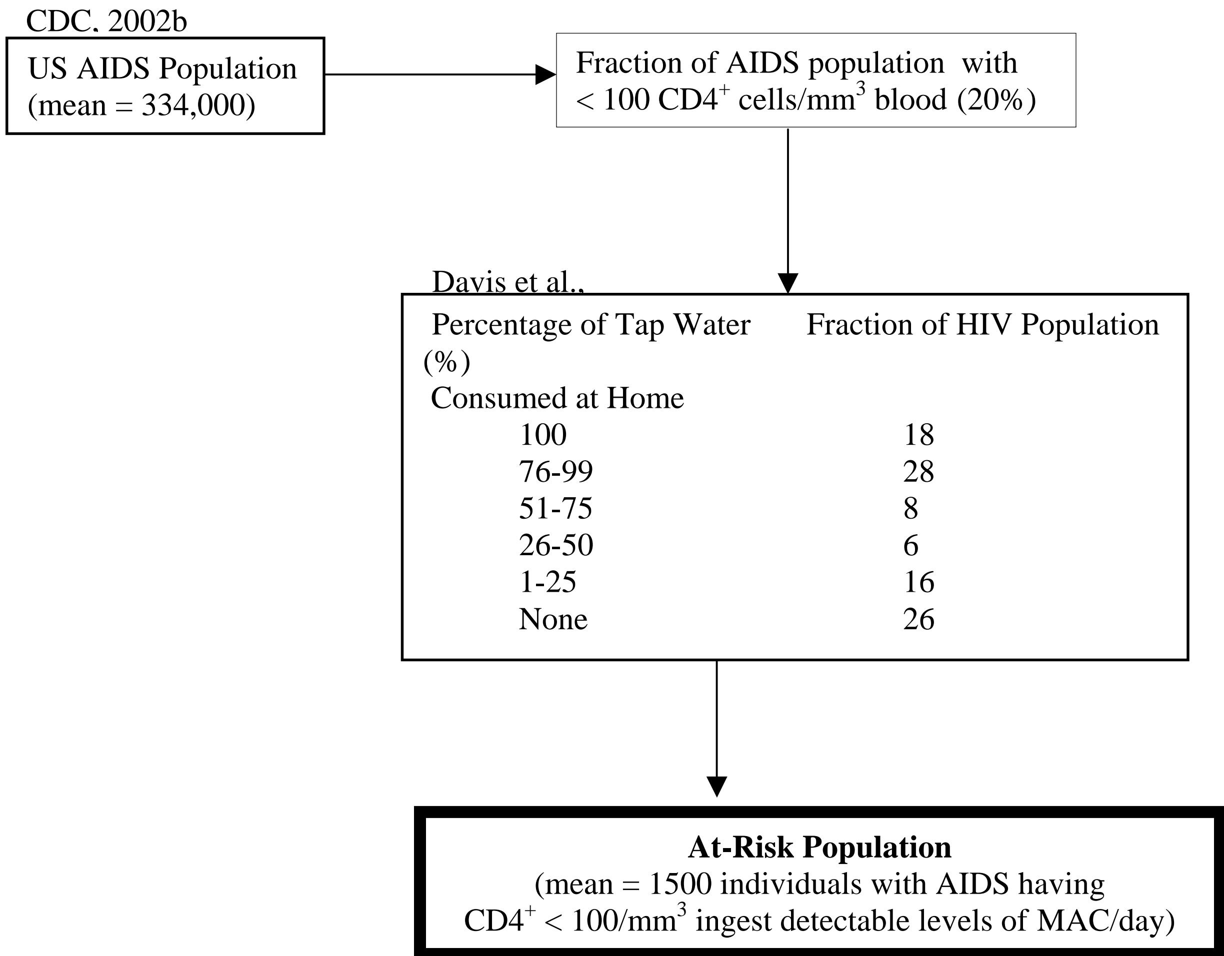


Figure 3. Model 2: Severely immunocompromised AIDS population exposed to MAC via tap water ingestion.

Overview: CCL Pathogens Research at NRMRL

Donald J. Reasoner
Microbial Contaminants Control Branch, Water Supply and Water Resources Division,
National Risk Management Research Laboratory, U.S. Environmental Protection Agency,
Cincinnati, OH

Presentation Abstract

The Microbial Contaminants Control Branch (MCCB), Water Supply and Water Resources Division, National Risk Management Research Laboratory (NRMRL), conducts research on microbiological problems associated with source water quality, treatment processes, distribution, and storage of drinking water. MCCB's work on Contaminant Candidate List (CCL) microorganisms was formally initiated in 1998 coincident with the publication of the CCL. The CCL included 10 microorganisms: *Acanthamoeba* (Guidance), Adenoviruses, *Aeromonas hydrophila*, Caliciviruses, Coxsackieviruses, Cyanobacteria (blue-green algae), Echoviruses, *Helicobacter pylori*, Microsporidia, and *Mycobacterium avium intracellulare*. MCCB has completed planned disinfection studies on *A. hydrophila* and will complete similar studies with *H. pylori* next year. Disinfection studies with Adenovirus were poised to begin shortly after September 11, 2001. Those studies were set aside to conduct disinfection studies on surrogates for bioterrorism agents. When disinfection studies on Adenovirus begin, they will be done as a collaborative effort between NRMRL and the National Environmental Research Laboratory. In collaboration with the University of Arizona, limited studies on the disinfection of microsporidia have been completed using chlorine and chloramine. As a result of the events of September 11, disinfection studies on Coxsackievirus, Echovirus, and *M. avium intracellulare* will be delayed for 2 to 3 years. Work on inactivation of cyanobacteria toxins by drinking water disinfection treatment is ongoing with the University of Wisconsin and the Wisconsin State Health Department.

Topic Area 2.1: CCL Protozoa

Detection of *Cyclospora cayetanensis* and Microsporidial Species Using Quantitative Fluorogenic 5' Nuclease PCR Assays

Frank W. Schaefer, III, Jeff D. Hester, Manju Varma, Michael W. Ware, and Harley D.A. Lindquist
National Exposure Research Laboratory, Cincinnati, OH

Presentation Abstract

Both *Cyclospora cayetanensis*, a coccidian parasite, and *Encephalitozoon* spp., a microsporidian parasite, have a fecal-oral life cycle. These parasites can be transmitted as contaminants of either food or water. Traditional microscopic methods for detecting and identifying these organisms in water are tedious, time consuming, and not always accurate. In the case of microsporidians, detection and identification of spores (see Figures 1 and 2) is usually done by transmission electron microscopy, which, although accurate, is not feasible for rapid analysis of water samples. To address these shortcomings, we have developed molecular assays for these organisms using 5' nuclease PCR that incorporate both a primer set and a dual labeled fluorogenic probe. For *C. cayetanensis*, both a species-specific primer set and fluorescent labeled probe were designed based on the uniqueness of the 18S ribosomal gene sequence of this parasite. For *Encephalitozoon* spp. (*E. hellem*, *E. intestinalis*, and *E. cuniculi*), species-specific primer sets and a genus-specific fluorogenic probe designed to anneal within the *Encepahalitozoon* 16S rRNA gene were used. Oocysts and spores were counted accurately on a fluorescence activated cell sorter to determine the sensitivity of these assays. Results were that as few as 1 *C. cayetanensis* oocyst and 1 *Encephalitozoon* spp. spore could be detected per 5 µL reaction volume. Utilizing standard curves, the quantity of the parasites detected can be estimated with these assays. Specificity of these molecular assays were tested against DNA isolated from numerous other related and unrelated protozoa, fresh water algae, and bacteria. In no case were any cross-reactions detected. These assays, although sensitive and specific, will not determine the viability or infectivity of the detected parasite. In addition, these are assays and are not complete methods suitable for routine use with water samples. A complete method for analysis of water samples will require insertion of both suitable water concentration steps as well as parasite purification steps before the 5' nuclease PCR assay is utilized.

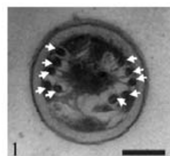


Figure 1. Transmission electron micrograph of an *Encephalitozoon* spp. spore with its polar tube extended.

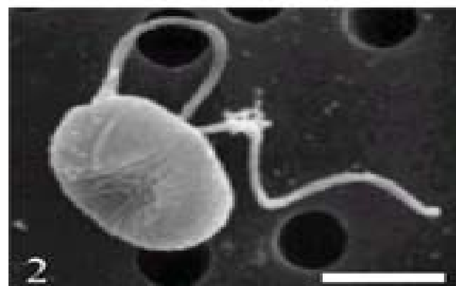


Figure 2. Scanning electron micrograph of an *Encephalitozoon* spp.

Development of Detection and Viability Methods for Waterborne *Microsporidia* Species Known To Infect Humans

***Rebecca Hoffman*[1], *Marilyn Marshall*[2], *and Mark Borchardt*[3]**
[1]*WI State Laboratory of Hygiene, University of Wisconsin—Madison, Madison WI;* [2]*Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ;* [3]*Marshfield Medical Research Foundation, Marshfield, WI*

Presentation Abstract

Microsporidia are obligate intracellular parasites capable of initiating disease in a host of vertebrate and invertebrate species following ingestion of a small, environmentally resilient spore. Waterborne transmission of this organism is suspected. However, methods to detect *Microsporidia* in the aquatic environment are developmental at best. The objective of this project is to develop a robust strategy for detection of waterborne *Microsporidia* using seeded model and natural waters, and ultimately perform method validation in unseeded natural waters.

Flow cytometry with cell sorting (FCCS) was used to generate precisely enumerated *Encephalitozoon intestinalis* seeding standards. Standards were spiked into 10 L of filtered tap water at a concentration of either 10 or 100 spores/L and concentrated using a modified continuous flow centrifuge (CFC). Retentate and rinse volumes were further concentrated by centrifugation, dried on well slides, and examined microscopically for the presence of spores. Recoveries ranged from 39 to 76 percent (n=24).

Sample purification studies included the evaluation of several direct and indirect immunomagnetic separation (IMS) products. In reagent water samples, indirect methods using beads directed against rabbit IgG were shown most promising with recoveries of spores ranging from 73 to 95 percent. Flow cytometry with cell sorting also was evaluated for the ability to isolate *E. intestinalis* spores from water concentrates. Approximately 80 percent of spores seeded into reagent water were recovered using FCCS with subsequent microscopic detection. Further experiments combined CFC concentration of seeded filtered tap water samples with isolation by FCCS and microscopic detection. Spore recoveries ranging from 31 to 77 percent (n=23) were achieved using this approach.

Molecular methods including polymerase chain reaction (PCR) and reverse transcription-PCR (RT-PCR) following cell culture were assessed for detection of *Microsporidia* and viability determination. Several primer sets, including those directed against beta-tubulin, 16S rRNA, and hsp70, were shown capable of detecting *Microsporidia*; however, their usefulness as viability indicators differed. More recently, real-time PCR using primers directed against 16S rRNA has been shown promising for detecting low levels of spores seeded into reagent and source water. Future studies will evaluate recoveries of spores seeded into natural water samples, concentrated using the CFC, and detected using real-time PCR.

Development and Evaluation of Procedures for Detection of Infectious *Microsporidia* in Source Waters

Paul A. Rochelle
Metropolitan Water District of Southern California, La Verne, CA

Presentation Abstract

The *Microsporidia* group of protozoa, particularly *Enterocytozoon bieneusi* and *Encephalitozoon* spp., are responsible for a substantial human disease burden. Many animals can carry *Microsporidia*, so it is possible that source waters may be contaminated and consequently serve as a route of transmission to humans. However, there are no routine methods for detection of *Microsporidia* in water and very little is known about their occurrence. There is a critical need to determine the role that drinking water plays in the epidemiology of this group of parasites. The overall objectives of this study are to develop methods to recover *Microsporidia* from water, determine the viability and infectivity of detected spores, and use the methods to assess the occurrence of *Microsporidia* in untreated source waters.

The project involved: (1) evaluation of filtration methods for recovery of *Microsporidia* spores from environmental water samples; (2) development and assessment of an immunomagnetic separation (IMS) procedure for purification and further concentration of spores; (3) evaluation of molecular and microscopic methods for detecting spores; and (4) development of an infectivity assay combining in vitro cell culture with a molecular detection assay.

Recovery efficiencies for capsule filters ranged from an average of 13.2 percent for 0.8–1 µm porosity filters to 38 percent for 0.55 µm porosity filters. Initial trials with centrifugal filters, consisting of a modified nylon membrane in a centrifuge tube, resulted in recovery efficiencies of 52 percent, 22 percent, and 36 percent with porosities of 0.2 µm, 0.3 µm, and 0.45 µm, respectively. A prototype ultrafiltration apparatus achieved up to 40 percent recovery using 0.05 µm hollow fiber filters. Most antibody preparations demonstrated considerable background staining, particularly with environmental samples. In addition, a significant number of spores did not stain. Non-antibody based stains, such as a modified trichrome method and Calcofluor, were found to be effective only in nonenvironmental samples. A quantitative molecular detection assay was developed for *E. intestinalis* with a sensitivity of 10 spores. A cell culture-based infectivity assay also was developed for *E. intestinalis* spores. Infection in RK13 cells was rapid and led to effective spore propagation. A confluent monolayer of RK13 cells in a 75-cm^2 flask produced more than 1 x 10^8 spores within 1 week of inoculation with a low dose of *E. intestinalis* spores. The 50 percent infectious dose for this assay was 36 spores (see Figure 1). The infectivity assay was used to measure the efficacy of ultraviolet (UV) disinfection; at least 90 percent inactivation of *E. intestinalis* spores was obtained with a UV dosage of 3.3 mJ/cm^2.

This research project has made considerable progress in the development and evaluation of methods for detecting environmental *Microsporidia* spores and measuring their infectivity. These techniques (once optimized) can be used to assess the extent of *Microsporidia* contamination in water, which will allow the water industry and public health officials to determine whether water represents a significant route of transmission for these parasites.

An efficient recovery procedure will need effective antibodies with high avidity and specificity that can be used for both IMS purification and immunofluorescent detection of spores. Consequently, a wider array of antibodies should be evaluated. In addition, a wider range of polymerase chain reaction primers needs to be screened for improved specificity. The cell culture-based infectivity assay can be used to assess spore survival in the environment and the efficacy of disinfectants such as ultraviolet light, ozone, and chlorine dioxide for inactivation of *Microsporidia* spores.

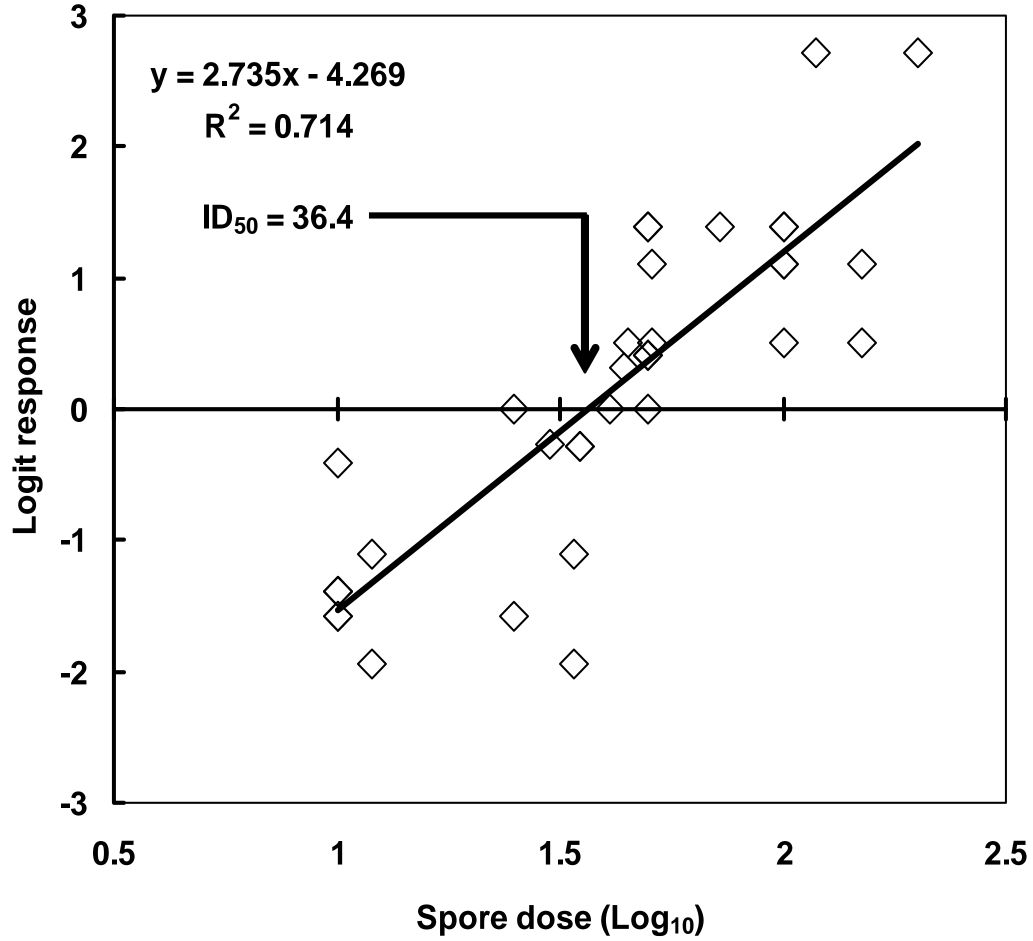


Figure 1. Dose response curve for *E. intestinalis* in RK-13 cell culture.

Development and Evaluation of Methods for the Concentration, Separation, Detection, and Viability/Infectivity of Three Protozoa From Large Volume of Water

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Presentation Abstract

The objective of this project was to evaluate and optimize a modified continuous flow centrifugation (CFC) method for recovery of *Cryptosporidium giardia* and *microsporidia* from turbid and large volumes of water. The CFC method allows for concentration of oocysts, cysts, and spores from large volumes of water, and for continuous monitoring of their presence in water, as opposed to one-time sampling of existing methods. This method is efficient, portable, rapid, and easy to operate. The third phase of this project included further optimization of the CFC method for recovery of *microsporidia* from 10 to 50 L of water, simultaneous recovery of all three pathogens from volumes of 10, 50, and 1,000 L of water, and viability testing after recovery to ascertain that the CFC method does not lead to parasite inactivation.

A large number of spiked experiments were conducted over the period of support, and the table below illustrates the rate of recovery of the three protozoa from 50 L of simultaneously spiked tap water.

Table 1. Percent recoveries by CFC when 50 L of tap water are spiked with three parasites (*Giardia, C. parvum*, *E. intestinalis*).

1	1,500	75	2,500	37.6	500	78.4
2	1,500	71	2,500	82.6	500	82.4
3	500	84	2,500	58.2	500	54
4	500	37.4	2,500	10.6	500	N.A.
Average% ± S.D		57.5±38.7		44.6±35		53.6±46.5

Recoveries for *C. parvum, Giardia lamblia*, and *E. intestinalis* spiked various volumes of water and turbidity were better than expected. The recoveries of all three pathogens were particularly impressive after numerous repeated spiking experiments. The reproducibility and the consistency of this system also were very impressive. In spiked experiments performed in parallel, the CFC outperformed other currently used filtration methods in terms of efficiency of recovery, speed, and simplicity. The CFC method is currently undergoing validation by the U.S. EPA Office of Water in several water utilities. The results will be presented at the meeting.

Topic Area 2.2: CCL Viruses

Norwalk Virus Dose Response and Host Susceptibility

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Presentation Abstract

Human caliciviruses are a leading cause of epidemic and endemic acute gastroenteritis and are responsible for numerous waterborne outbreaks. The overall objective of this research project is to develop our understanding of the risks associated with exposure to waterborne human caliciviruses as a function of dose and host susceptibility. The dose-response was examined for two important human caliciviruses (HuCVs), a prototype Genogroup I virus (Norwalk virus (NV)) and a prototype Genogroup II viruses (Snow Mountain Agent (SMA)) to: (1) identify the dose range of NV and SMA (ID_{10}, ID_{50}, and ID_{90}) in human volunteers; (2) examine the immune response (serum and secretory antibodies) and determine the characteristics of volunteers that are susceptible to infection; and (3) evaluate the fit of several mathematical models of dose-infectivity to our data. This presentation focuses on the dose-response results of the NV study.

A double-blinded human challenge study was conducted to determine the dose-infectivity relationship for NV. Subjects were given various doses of a suspension of NV, monitored in a clinical setting for gastrointestinal symptoms for 5 days and returned for Day 8, 14, and 21 followup visits. Stool specimens were assayed for NV by reverse transcription-polymerase chain reaction (RT-PCR). NV serum and salivary antibodies were measured by enzyme immunoassay. Saliva samples were tested for secretor status presence of H type-1 antigen and *FUT2* gene as a marker of susceptibility. Infection was defined as excretion of NV or seroconversion.

A total of 75 subjects were challenged with NV, and 22 became infected. Twenty-two of the 75 subjects were secretor negative, and none became infected. NV doses ranged from 1×10^{-1} to 1×10^{7} PCR detectable units (PDU). Approximately 68 percent of the infected subjects had gastrointestinal symptoms. Most subjects shed the virus for at least 8 days postchallenge, and several continued to shed the virus for 18-23 days post-challenge. Subjects with an early (< 5 days post-challenge) anti-NV salivary IgA response appeared to have protective immunity to NV infection as compared to subjects with a late anti-NV salivary IgA response.

The infectivity response appeared not to be consistent with a simple dose-response model, indicating considerable heterogeneity in susceptibility to infection among subjects. Susceptibility to infection with Norwalk virus appeared to depend on a genetic marker (*FUT2*) for a probable virus receptor (H type-1 antigen) on host epithelial cells in the gastrointestinal tract. The 22 subjects who did not have this gene appeared to be completely resistant to infection, regardless of virus dose. When the analysis was restricted to the susceptible fraction of the challenged volunteers (n=53), a dose-response effect was seen, although considerable heterogeneity still remained. At low doses there was a considerable probability of infection, but it takes very high doses to reach an infection probability near 1.

There seemed to be no indication of a dose response for illness among infected subjects. When pre-challenge anti-NV serum IgG was used as an indicator of prior NV infection (assuming higher levels indicated more recent infection or infection with a virus more closely related to NV), there appeared to be weak evidence of a protective effect against infection among those with higher levels of anti-NV IgG.

When illness conditional on infection was considered, higher baseline IgG seemed to be associated with slightly increased risk. This suggests that a subject with a high baseline anti-NV IgG level needs a higher dose to become infected, but then has a slightly elevated risk of becoming ill.

NV is highly infectious. The low infectious dose, mild illness or asymptomatic infections, and prolonged shedding facilitate waterborne and secondary transmission of this virus. A genetic marker of host susceptibility was identified that suggests that 80 percent of the general population is susceptible to NV infection. However, some persons exhibit an early, protective, salivary IgA response. Quantitative models that describe the interaction between virus inoculation and growth and observable immune variables may improve understanding of the infection process and consequently improve risk predictions. The results of these studies are valuable for estimating the risk of HuCV infection and gastroenteritis associated with exposure to contaminated water and to establish safe exposure limits for HuCVs in water to reduce waterborne disease.

The next steps of the project include completion of the dose-response analyses for the SMA challenge study and completion of the examination of the role of T-cell mediated immunity in NV and SMA challenge and infection.

Development of a Rapid, Quantitative Method for the Detection of Infective Coxsackie and Echo Viruses in Drinking Water

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Presentation Abstract

The objective of this research was to improve on the current analytical methods for quantitative detection of infective nonpolio enteroviruses (NPEV) in drinking water. The specific objectives of this research were to: (1) develop a molecular-beacon-based (MB) RT-PCR method to detect NPEV; (2) establish a potential correlation between IMS-MB-RT-PCR detection and cell culture detection for infective viruses; (3) using the molecular beacon, develop and evaluate real-time monitoring of virus replication in cell culture; and (4) evaluate the above methods to quantify the presence of infective NPEV in concentrated drinking water samples. A set of primers was designed to amplify a 155-base pair section of RNA of Echovirus 11 (Echo 11), the virus chosen to represent the enterovirus group. An antisense-MB also was designed to specifically recognize a 25-base pair sequence within the 5 noncoding region of Echo 11. Viral RNA was reverse-transcribed and subsequently amplified by polymerase chain reaction (PCR). Using the MB-based reverse transcription-PCR (RT-PCR) assay, detection and quantification of the virus was achieved. A detection limit of 0.1 plaque-forming units (PFU) was obtained for Echo 11. Specificity testing positively identified other members of the enterovirus group (Coxsackieviruses B1, B3, and B6; Echoviruses 11, 17, and 19; Poliovirus 1); nonenteroviruses (Parechovirus 1; Adenoviruses 2 and 15; Rotavitus WA; hepatitis A virus; MS2 and phiX174 bacteriophages; *E. coli* O157:H7 and *Salmonella typhimurium*) were not detected.

After development of the MB-based assay, IMS was added to the process to minimize the potential for the amplification of noninfective viruses. Using the IMS-MB-RT-PCR assay, the detection limit for Echovirus 11 was 3 pfu (see Figure 1). The method was then tested using surface and groundwater concentrates that were spiked with echoviruses. The method was able to detect the viruses at a concentration of 3 pfu in the surface water and less than 1 pfu in groundwater. In parallel, the spontaneous hybridization between molecular beacons and their target sequences was exploited as a means for real-time detection of virus replication in situ. Newly synthesized viral RNA was used as an indicator for viral infection. A molecular beacon targeting a specific region of the enterovirus RNA was used for the initial demonstration. Buffalo green monkey kidney cell cultures with or without infection with Echo 11 were collected at various time points, fixed, and permeablized. After introduction of molecular beacons, whole fluorescence was monitored with a fluorescence microscopy. The results demonstrated that only cells infected with Echo 11 were brightly fluorescent, and noninfected cells were not fluorescent. These results could be achieved within a few hours post-infection. This result suggests the possibility to provide real-time and quantitative determination of infective viruses without the need to perform a conventional plaque assay, which takes days to complete.

Detection of echovirus 11 in spiked surface water sample using IMS-MB-RT-PCR

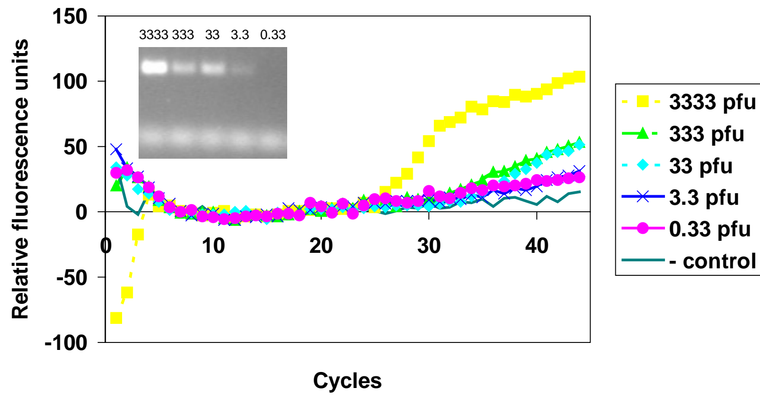

Figure 1. Detection of echovirus 11 in spiked surface water sample using IMS-MB-RT-PCR.

Dose-Response Assessments for NLV and Coxsackievirus in Drinking Water

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Poster Abstract

This poster presents the choices made for surrogate pathogen dose-response selection and the outcome of those choices. The objective of both the calicivirus and coxsackievirus assessments is to determine an estimate of the dose-response based on data available for selected surrogate pathogens.

Dose-response data are limited or nonexistent for coxsackievirus and calicivirus. Drinking water exposure is the only route presented in this poster. Potential surrogate pathogens that had available dose-response data were considered for the coxsackievirus and calicivirus risk assessments. There is no formalized process or criteria for selection of representative surrogates for Contaminant Candidate List pathogens. For the most part, family/genus, relative infectivity, and similarity of disease endpoints may be the most data available, but these data may not substantiate the selection of a surrogate. The National Center for Environmental Assessment (NCEA) is now developing a white paper to recommend criteria and a selection process for surrogates.

Coxsackievirus and echovirus-12 are both single-stranded, non-enveloped RNA viruses in the Picornaviridae family, Enterovirus. The probability of infection by coxsackievirus was based on a dose-response relationship developed from echovirus-12 enterovirus because no data associated with the ingestion of coxsackievirus were available from the published literature. Both viruses replicate in the gastrointestinal tract; infection is mostly asymptomatic. However, disease endpoints for both viruses can range from mild unspecified febrile illness to fatal central nervous system complications.[1]

The dose-response model for calicivirus was developed based on data for rotavirus because dose-response data are not available for Norwalk Like Virus (NLV) or calicivirus. Calicivirus belongs to the Caliciviridae family, of which human calicivirus/NLV are members. Rotavirus is an unrelated (family Reoviridae) but may have similar infectivity, and serves as a conservative surrogate for the infectious calicivirus (attack rates in drinking water outbreaks range from 31 to 87%).[2] The use of the rotavirus dose-response data may represent a conservative assessment for NLV infectivity in humans. All members of the population are at risk of infections from exposure to either calicivirus or coxsackievirus.

A number of models are used in the literature to describe microbial dose-response data. This assessment considers only the physically and biologically relevant dose-response models; these include the exponential and beta-Poisson models. The Pareto II also is considered, as it closely approximates the beta-Poisson over a wide range of parameter values and is much more analytically tractable than the beta-Poisson. The parameter values of these models fitted to the rotavirus data are presented in Table 1.

In addition, the results of fitting these models to the echovirus-12 human-infectivity data, used as a surrogate for coxsackievirus, are shown in Table 1. The table shows the fitted model parameters and selected infectious dose estimates. Uncertainty in low-dose response was estimated by bootstrapping the data set based on the initial beta-Poisson fit. The results of the bootstrap simulation indicate about a five-fold span in the 95 percent confidence interval on risk of infection at low doses (< 0.01 pfu). The upper 95 percent bound on low-dose risk for echovirus-12 is still 260 times less than the maximum possible risk (where exposure to 1 pfu always results in infection). In contrast, a similar bootstrap simulation performed on the rotavirus data, used as a surrogate for NLV, yields an upper 95 percent confidence bound on low-dose risk that is only 20 percent lower than maximum possible risk.

The next steps include recommendations for research on exposure and dose response for calicivirus, coxsackievirus, and NLV. NCEA is working to develop surrogate selection criteria and a selection process, as well as secondary transmission models for person-to-person transmission of infectious virus such as calicivirus.

References

1. Embrey M. Coxsackievirus in drinking water (Literature Summary). Final Report. The George Washington University School of Public Health and Health Services, Department of Environmental and Occupational Health, 1999.

2. Embrey M., et al. *Caliciviridae* in drinking water. In: Handbook of CCL Microbes in Drinking Water. American Water Works Association, 2002.

Table 1. Maximum likelihood parameter estimates, predicted ID_s, and goodness-of-fit statistics for each model (Infectivity data, Echovirus 12).

Exponential	r = 0.000583 ID_{50} = 1187 ID_{01} = 17.2 ID_{0001} = 0.17	D=22.3 (df=3) (p = 0.0002) (significant lack of fit)
Pareto II	α = 1.06 β = 994 ID_{50} = 918 ID_{01} = 9.5 ID_{0001} = 0.094	D=3.21 (df=2) (p = 0.20)
Beta-Poisson	α = 1.06 β = 994 ID_{50} = 918 ID_{01} = 9.5 ID_{0001} = 0.094	D=3.21 (df=2) (p = 0.20)

[a] Subscript indicates cumulative response percentile at which the ID is calculated (based on MLE parameters).
[b] Plaque-forming units.
[c] D = deviance (-2 x maximum log-likelihood); df = degrees of freedom for X^2 statistic; p = significance of fit, where $p < 0.05$ indicates lack of fit.

Methods Used To Analyze a Norovirus Outbreak

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Poster Abstract

The goals and objectives of this project are to: (1) isolate and identify the viral agents in well water samples associated with two outbreaks of acute gastroenteritis reported to the Wyoming Department of Health in February 2001 and October 2001; (2) isolate and identify the viral agents in patient stool samples; and (3) determine the link between water consumption and illness.

The project had a three-way approach: (1) an epidemiological investigation was performed to identify any common routes of exposure among those afflicted with gastroenteritis; (2) an environmental survey was done of the premises involved in each outbreak to determine possible sources of contamination; and (3) laboratory analysis was performed on well water samples for coliform and viral detection using reverse transcription polymerase chain reaction (RT-PCR) and DNA sequencing. Stool samples also were analyzed for the presence of noroviruses.

Epidemiological studies revealed a close association between water consumption and illness. Environmental surveys in both outbreaks determined that the water supply was vulnerable to fecal contamination. Well water samples in both cases were positive for coliforms, and RT-PCR and DNA sequencing revealed noroviruses as the causative agents of acute gastroenteritis.

This investigation demonstrates that the U.S. EPA's viral concentration and molecular methods, in conjunction with epidemiological and environmental analysis, are very useful in outbreak studies. The methods used in this study can be performed in most laboratories with trained personnel and appropriate equipment, which would allow for routine monitoring of enteric viruses in drinking water, thus preventing any future outbreaks from occurring.

Development of a Molecular Method To Identify Astrovirus in Water

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Poster Abstract

Astrovirus is a common cause of gastroenteritis that has been determined to be responsible for several outbreaks. Because astrovirus can be waterborne, there is interest in testing environmental water for astrovirus. We have developed a sensitive reverse transcription-polymerase chain reaction (RT-PCR) assay (see Figure 1) that is designed to detect all known astrovirus strains.

The assay was based on a primer set that contained multiple upper and lower primers as well as multiple probes. This would allow for amplification of all of the known strains of astrovirus using a single reaction. When tested, this assay was able to detect strains from all eight serotypes. In addition, an internal control was developed, so that it will be possible to determine if the sample being tested contains PCR inhibitors. Most probable number analysis determined that when amplified with the developed assay, a single DNA molecule of the internal control could be detected if inhibitors were not present. The assay was successfully adapted to real-time PCR, and this method was used for integrated cell culture/RT-PCR detection of infectious virus. The methods were successfully used to detect astrovirus present in clinical samples and spiked water samples.

A simple, sensitive method for detecting all known astrovirus strains has been developed that can be used to detect this virus in water. This assay will be field tested by analyzing environmental water samples.

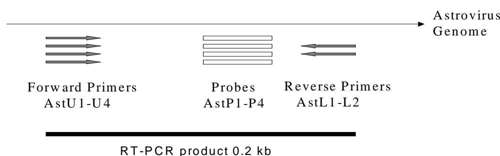

Figure 1. Assay design.

Effectiveness of UV Irradiation for Pathogen Inactivation in Surface Waters

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Poster Abstract

Ultraviolet (UV) irradiation is now recognized as an effective and cost-competitive measure to achieve significant level of inactivation of *Cryptosporidium* while not producing appreciable level of harmful disinfection by-products (DBPs) at practical doses. However, the effectiveness of UV technology against new and emerging pathogens and uncertainties in application of UV disinfection for unfiltered surface waters still needs to be assessed before widespread use of this technology as a primary disinfectant in drinking water treatment processes. The primary objectives of this research project are to evaluate the susceptibility (or resistance) and repair potential of select Contaminant Candidate List (CCL) pathogens and indicator microorganisms to or after UV disinfection from low- and medium-pressure (LP and MP) UV sources, and to investigate the extent of microbial association with particles in unfiltered systems and the effects of this particle association and other water quality parameters on UV disinfection potential.

Preliminary results indicate that both LP and MP UV irradiation are very effective against most of the indicator and emerging microorganisms tested. However, some of the indicator microorganisms like coliphage MS2, bacteriophage PRD-1, and Bacillus subtilis endospores as well as CCL and emerging pathogens like *Mycobacterium terriae* (a substitute for *Mycobacterium avium* complex), adenovirus type 2, and *Toxoplasma gondii* oocysts showed relatively high resistance against both UV irradiation. Although the effectiveness of LP and MP UV appeared to be similar against most of the microorganisms tested, there was some remarkable difference between these two UV technologies in terms of their effectiveness against adenovirus 2. To determine the level of particle association and its effect on UV disinfection, raw surface water samples have been collected from various utilities across the United States, and the waters have been examined for particle associated coliform and aerobic endospores using physical particle disruption techniques such as homogenization and blending. However, the levels of the indigenous microbes in the raw waters were typically low (< 1,000/100 mL), so that it was not feasible to assess the degree of particle association in these waters based on those physical methods.

Currently, the use of microscopic techniques (nucleic acid staining/probes along with confocal microscopy) are being investigated to determine the level of particle association in those raw waters. Regarding the development of new assay systems for some of the CCL microorganisms, we have been successful in developing a new assay system (Long-template [LT] RT-PCR) for Norwalk virus and a new molecular biology assay (RT-PCR) for adenovirus 40 or 41, which are being and will be used in the current and future inactivation study on these viruses by LP and MP UV. In addition, a method has been established to perform wavelength specific studies using a polychromatic UV light source (MP UV lamps) with a set of UV bandpass filters, and this setup will be utilized to develop wavelength effectiveness information for select CCL microorganisms like adenovirus 2, *M. terriae*, and *T. gondii* oocysts. Finally, protocols have been established to examine repair phenomenon following UV disinfection in various conditions in real water treatment situations, and these protocols will be implemented to evaluate the presence and extent of repair after UV irradiation in the select CCL and emerging microorganisms in the later phases of this research.

Topic Area 2.3: CCL Bacteria

Disinfection of *Helicobacter pylori* and *Aeromonas* Species

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Presentation Abstract

Helicobacter pylori and *Aeromonas hydrophila* are contaminants listed on the U.S. EPA's 1998 Contaminant Candidate List (CCL). The sensitivity of *H. pylori* to chlorine and of *Aeromonas* spp. to inactivation by free chlorine, chloramines, and ultraviolet (UV) was examined. Selective and nonselective monitoring media were evaluated to assess recovery of chlorine or UV-stressed *Aeromonas* spp. Results of experiments using free chlorine showed that the *H. pylori* and *Aeromonas* spp. were readily inactivated under all conditions studied. *H. pylori* showed more than 3.5 orders of magnitude inactivation by 0.5 mg/L chlorine in 80 seconds at 5 °C. The *Aeromonas* spp. were inactivated by more than 5 orders of magnitude within a 1-minute exposure to free chlorine at pH 7 or 8, and at 5 °C or 25 °C. Reductions of the *Aeromonas* spp. with 2.0 mg/L of monochloramine reached approximately 2 orders of magnitude at pH 8.0 for 4 minutes and greater than 5 orders of magnitude inactivation after 8 minutes of exposure. *Aeromonas* spp. were found to be sensitive to UV irradiation, with fluences of less than 7 mj/cm^2, giving between 5-7 log_{10} reductions. For free chlorine, there was no observable difference in recovery of chlorine-stressed *Aeromonas* spp. organisms between selective and nonselective media. However, with UV disinfection, some *Aeromonas* spp. counts on nonselective media were significantly higher than those obtained on selective agar. These findings suggest that selective agars may underestimate the number of viable *Aeromonas* recovered after exposure to UV irradiation.

Genomic and Physiological Diversity of *Mycobacterium avium* Complex

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Presentation Abstract

The *Mycobacterium avium* complex (MAC) is an environmental pathogen of susceptible humans, especially AIDS patients, children, and the elderly. MAC infections are debilitating and very difficult to treat due to intrinsic multi-drug resistance. Infections originating from potable water have been documented; however, the occurrence of MAC in water does not always correlate with high rates of MAC disease. The ability to predict consequences of human exposure to MAC remains an elusive goal. One factor that may contribute to this problem is the genetic and phenotypic heterogeneity of MAC isolates. Strains and colony types are thought to vary with regard to infectivity, susceptibility to antibiotics, and ability to survive in various environments. The goal of this research project is to understand the genomic and physiological bases for these phenotypes. Such an understanding could lead to refined methods for detecting environmental MAC populations that are likely to cause disease in humans.

Microarrays and restriction fragment length polymorphism are being used to quantify the genomic diversity of MAC isolates from clinical and environmental sources. In addition, transposon mutagenesis and disease models are being used to characterize the highly mutable properties of colony type, intrinsic drug resistance, and virulence of MAC. By combining these two lines of investigation, we hope to identify genomic markers that can be used to identify virulent strains of MAC in the environment.

Comparative genomic hybridization to a MAC genomic microarray has revealed extensive strain-to-strain diversity within MAC. There also is extensive heterogeneity within individual isolates, as evident from RFLP analysis and the appearance of multiple colony types on laboratory growth media. A novel colony type switch, termed red-white, has been identified that affects virulence, drug susceptibility, and other phenotypes. To characterize the genetic basis for these phenotypes, a transposon mutagenesis system has been developed with which we have begun to identify MAC genes required for pathogenicity and intrinsic multi-drug resistance (see Figure 1).

Microarray and mutational analysis will be expanded. Moreover, phenotypic and genomic diversity will be examined among environmental isolates. Phenotypic diversity will be measured by using intracellular growth assays, and genomic diversity by microarray analysis. Through this investigation, we hope to: (1) test the hypothesis that some environmental strains of MAC are more virulent than others, and (2) identify genomic markers that may be used to identify virulent strains.

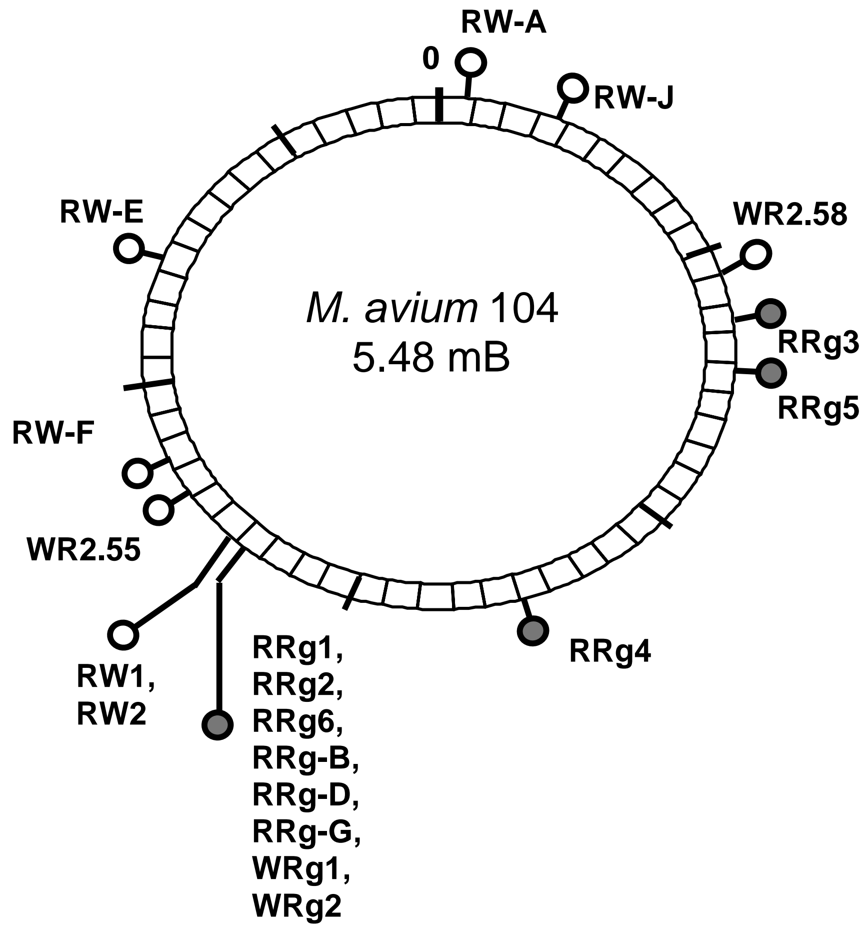

Figure 1. Map of *Mycobacterium avium* strain 104 genome, with positions of transposon insertions that affect colony morphotype and/or multi-drug resistance.

Mycobacterium avium Complex (MAC) in Drinking Water: Detection, Distribution, and Routes of Exposure

Phanida Prommasith[1] and Timothy E. Ford[2]
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Presentation Abstract

The objectives of this project are to: (1) develop techniques and methods for detecting MAC in both drinking water and biofilm samples; (2) investigate the presence and distribution of MAC in water distribution systems in four Massachusetts towns; and (3) examine possible routes of exposure for residential end-users. A method was developed to detect MAC from drinking water, standard filtering techniques have been modified, and percent recovery was compared with DAPI direct counts. The most efficient protocol was compared with selective culture on paraffin slides. Both environmental water samples and MAC-spiked autoclaved water were used in these tests. Environmental biofilm samples were collected from residential sites and from hot water by-pass systems.

Two fluorescent oligonucleotide probes have now been designed and tested. The probes target the 16sRNA genes of *Mycobacterium avium* and *M. intracellulare*. PCR-restriction enzyme pattern analysis (PRA) of the *HSP65* gene also has been used to identify MAC. Identification of species has been confirmed by comparison with the NCBI-GenBank database. To investigate the presence and distribution of MAC, potable water samples have been systematically examined from four communities with different water supplies and distribution characteristics. All samples have been analyzed for pH, temperature, free and total chlorine, alkalinity, ammonia-N, nitrite-N, nitrate-N, total iron, and assimilable organic carbon, MAC, and heterotrophic plate counts.

In addition, to investigate the possible routes of exposure, water samples have been collected from kitchens and showers in the end-users' homes (cold and hot water) and analyzed for the presence of MAC. Currently, biofilms are being grown in end-user toilet cisterns. Any residential sites found to have MAC in water samples have been subject to be more frequent sampling.

Small doses of antibiotics (nalidixic acid, ethambutol, ofloxacin) and anti-fungus (cyclohexamide) added to 7H10 media were found to improve the detection of MAC. It is still inconclusive as to whether the paraffin slide culture is a more sensitive technique. The FISH probe for the *M. avium* showed a positive hybridization signal less than 35 percent FA stringency hybridization conditions on isolated cells. The probe for *M. intracellulare* needs to be redesigned and retested to ensure successful hybridization.

Approximately 20 percent (n=861) of water samples collected to date were positive for *Mycobacteria*, with 8.36 percent positive for MAC. The household results ranged from 0 percent to more than 40 percent positive for MAC; the distribution system results ranged from 0 percent to almost 30 percent positive for MAC. Of the MAC positive plates, 11 percent were found to have *M. avium* (20-450 cfu/L), and 3.5 percent were *M. intracellulare* (4-12 cfu/L). No significant differences were detected in the presence of MAC between kitchen faucets and showerheads, although numbers of MAC were slightly higher in showerhead samples. Approximately 100 percent of *M. intracellulare* were found in cold water (8-17 °C), whereas most *M. avium* (66 percent) were found in hot water (35-46 °C).

The next steps will include: (1) concluding the investigation of the use of paraffin slide cultures; (2) finalizing FISH protocols to directly hybridize to biofilm samples; (3) improving *M. intracellulare* probe and protocols; (4) evaluating biofilms grown in toilet tanks for MAC; and (5) examining relations of MAC colonization to water quality parameters.

Sensitivity of Three *Encephalitozoon* Species to Chlorine and Chloramine Treatment as Detected by an *In Vitro* Microwell Plate Assay

***Cliff H. Johnson*[1]*, Marilyn M. Marshall*[2]*, Jackie Moffet*[2]*, Charles R. Sterling*[2]*, Laura A. DeMaria*[1]*, and Gene W. Rice*[1]**

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Poster Abstract

Microsporidia are obligate intracellular parasites that form environmentally resistant, infectious spores. These parasites are ubiquitous in the environment, infecting members of almost every class of vertebrates and invertebrates. At least 14 microsporidian species are known to infect humans. Of primary concern are the microsporidian species that infect the human gastrointestinal tract, *Entercytozoon bieneusi* and *Encephalitozoon intestinalis*. Spores are typically able to survive and maintain their infectivity for weeks. A modified *in vitro* cell culture assay was performed on three species of Encephalitozoon (*E. intestinalis*, *E. hellum*, and *E. cuniculi*) after exposure to chlorine and chloramine at a concentration of 2 mg/L at 25EC, pH 7, and pH 8, respectively. Spores were harvested from RK-13 cell monolayers and Percoll purified assayed using an *in vitro* microwell plate viability procedure. Ten-fold dilutions of chlorine and chloramine treated spores were inoculated onto RK-13 cell monolayers grown on 15mm sterile Thermanox coverslips in 24 well plates. Five coverslips were inoculated for each spore dilution. After incubation, the coverslips were fixed with methanol, stained with Giemsa stain, and examined with a light microscope. The percentage of infectivity was calculated by dividing the number of positive (infected) wells by the total number of wells inoculated. Most probable number determinations also were determined for each assay. For each treatment and time period, two replicates of 5 coverslips were performed. Varying log reductions were observed for the three Encephalitozoon species.

Inactivation of *Aeromonas* by Chlorine and Monochloramine

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Poster Abstract

The Bacterial genus *Aeromonas* is currently listed on the U.S. EPA's Candidate Contaminant List (CCL). Resistance to chemical disinfection is an essential aspect regarding all microbial groups listed on the CCL. This study was designed to determine the inactivation kinetics of *Aeromonas* spp. for free available chlorine and monochloramine. Three species, *Aeromonas caviae*, *A. hydrophila*, *A. veronii*, which are known to be associated with human infections, were studied in pure culture under oxidant demand-free conditions. Free chlorine experiments were conducted at pH 7 and 8, at 5 °C and 22 °C. Experiments using preformed monochloramine also were conducted at both temperatures and at pH 8. Three media were evaluated for their ability to recover chlorine-stressed organisms [nutrient agar (Difco), ampicillin dextrose agar (Biolife), and Ryan agar (oxoid)]. Experiments using free chlorine indicated that the *Aeromonas* spp. were readily inactivated under all conditions studied. The organisms were inactivated by more than five orders of magnitude within a 1-minute exposure to free chlorine at both temperatures. Inactivation kinetics were similar for other bacterial organisms, with greater inactivation occurring at lower pH values and at higher temperatures. For free chlorine, there was no observable difference in recovery of chlorine-stressed organisms on the three bacteriological media. Two wells containing *Aeromonas* spp. (approximately 103 CFU/mL) have been located in a recent survey. Water from these wells also was used in disinfection experiments.

Mycobacterium paratuberculosis and Nontuberculous Mycobacteria in Potable Water

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Poster Abstract

Nontuberculous mycobacteria (NTM) include *Mycobacterium* species that are not members of the *Mycobacterium tuberculosis* Complex. Members of the NTM group are important causes of disease in birds and mammals. *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium paratuberculosis* are NTM and members of the *Mycobacterium avium* Complex (MAC). These organisms are found in a variety of environments, including soil and water, and are included on the Contaminant Candidate List (CCL). Earlier exploratory occurrence studies suggest that NTM have widespread occurrence in potable water throughout the United States. *M. paratuberculosis* is the causative agent for Johne's disease in cattle. In addition to well-documented evidence of *M. paratuberculosis* as the causative agent of Johne's disease in cattle, there has been evidence linking *M. paratuberculosis* with Crohn's disease, a chronic inflammatory disease of the intestinal tract in humans. Transmission of *M. paratuberculosis* via water contaminated with cattle feces may be one route of infection.

Current NTM research focuses on three areas: (1) development of an improved cultural method for isolation of NTM in drinking water, (2) development of a rapid polymerase chain reaction (PCR) multiplex method for detection of MAC organisms in drinking water, and (3) development of a molecular method for detection of *M. paratuberculosis* in water.

Improved Cultural Method

Current methods for isolating NTM from environmental samples require harsh decontamination techniques to reduce the levels of background organisms often leading to loss of 50-70 percent of the target NTM. The goal of this research is to develop improved selective method(s) that do not use classical decontamination procedures. The use of antibiotics, dyes, detergents, and other growth inhibitors are being examined for their ability to reduce background organisms and permit growth of NTM. A membrane filter method approach has been selected. Screening studies with spiked drinking water samples comparing candidate methods to classical decontamination techniques have been initiated. Candidate methods that permit better recovery of NTM and better reduction of background organisms will be tested with additional recovery studies and analyses of drinking water samples.

Various antibiotics, dyes, and detergents have been examined using a membrane filter cultural method approach. Thus far, an oxidizer has shown promise for better recovery (80 percent) and reduction of background organisms than the standard accepted cultural method. An improved cultural method would lead to better estimates of the occurrence of NTM, better estimates of the numbers of NTM in positive samples, and the possibility of recovering NTM unusually sensitive to decontaminating agents. Future research will entail additional NTM recovery studies, followed by comparison studies with the standard cultural approach and the improved method with distribution samples.

PCR Multiplex Method

Current methods for detection of MAC organisms in drinking water typically take from 3 to 8 weeks for completion of analyses, with additional time for identification of the organisms. The goal of this research is to develop a rapid PCR multiplex method for detection of *M. avium* and *M. intracellulare*. Drinking water samples (500 mL) are membrane filtered, and the filters are placed in modified 7H9 broth for 7-day enrichment.

After enrichment, the cells are centrifuged and lysed to harvest the genomic DNA. The DNA is amplified (PCR) using primers specific for *M. avium* and *M. intracellulare* and all Mycobacteria. The PCR product is visualized by gel electrophoresis. Sixty samples (reservoir and drinking water) have been analyzed by the standard culture method and the multiplex PCR method. Nine samples were positive by both methods, seven were positive only by multiplex PCR, and three were positive only by the cultural method. The use of multiplex PCR significantly decreases the time for analyses for these organisms, and is able to detect MAC organisms not detected by the culture method. The next steps include completion of detection limit studies and additional comparison studies with the standard culture technique using drinking water samples.

Method for Detection of *M. paratuberculosis*

A new project in our laboratory involves the development of a molecular detection and quantification method for *M. paratuberculosis* (MAP) in water. The method will be an important step in determining the significance of exposure to MAP in contaminated water, and may help to establish the link between contaminated water and Crohn's disease. Current methods of detection, which include culture-based methods, are inadequate. A 16- to 20-week incubation time is required to grow the organism, during which other microorganisms overgrow the medium. Harsh decontamination procedures used to reduce background organisms also kill a portion of MAP. This study proposes to develop a rapid molecular method to detect and quantify MAP in environmental samples by targeting a genetic molecule specific to MAP. One potential target is the MAP-specific insertion sequence IS900. The element is found only in MAP, and is present in 14 to 18 copies per cell. Other possible targets include seven recently discovered MAP-specific gene segments. A quantitative PCR-based method would significantly reduce detection times from approximately 16 weeks to a few hours.

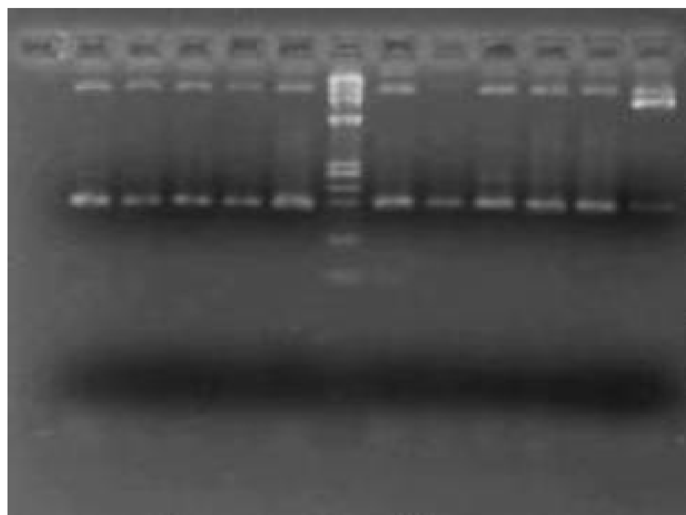

Figure 1. Electrophoretic separation of multiplex PCR products obtained from drinking water isolates. Lanes contain the following: lanes 1, 2, 3, 4, 5, 7, 8, 9, and 10, *M. avium* isolates; lane 11, *M. avium* positive control; lane 12, *M. intracellulare* positive control, and lane 6, φX174 RF DNA/ Hae III molecular weight marker (Gibco-BRL) containing fragments of 1353, 1078, 872, 603, 310, 271, 234, 194, 118, and 72 base pairs.

Detection of *Helicobacter pylori* Using a Highly Variable Locus Upstream of the 16S Ribosomal RNA Gene

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Poster Abstract

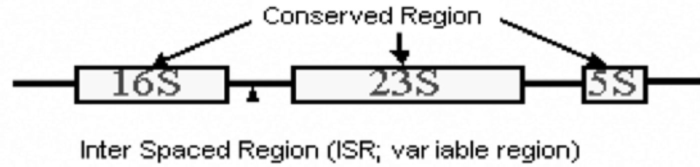
Helicobacter pylori is a highly successful infectious agent, and it is the principal cause of gastritis and peptic ulcer disease. Strong associations also have been found with gastric adenocarcinoma and lymphoma. Although regional rates of infection vary, *H. pylori* is still a major public health concern in many parts of the world. This highly infectious bacterium is able to use multiple routes of transmission to infect a susceptible host. It is thought to be transmitted by the fecal-oral route but also may be transmitted directly through saliva or from the environment; however, the precise mode of transmission is not well understood. *H. pylori* has been demonstrated to change morphologically (see Figure 1) and enters nonculturable stages of survival in the environment and still can become infective. Currently, it is difficult to elucidate the environmental route of *H. pylori*, as there is no reliable method for detecting and differentiating *H. pylori* strains from environmental sources.

To overcome culturability limitations, a molecular approach was utilized consisting of designing primers complementary to intergenic spicer region (ISR) between the 16S and 23S to detect *H. pylori* from water sources. In the *H. pylori* genome, the 16S and 23S rRNA genes are not contiguous (see Figure 2). This study found a highly conserved region followed by an intergenic spacer variable region upstream of the 16S rRNA gene. Primers were designed for this conserved region and to the 5′-end of the 16S rRNA gene to amplify the variable region in between. Each strain of *H. pylori* tested gave a positive amplification by polymerase chain reaction (PCR), using the primers mentioned above to amplify the variable region. A number of other species tested, such as Campylobacter, *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio*, and *Bacillus* species, resulted in no amplification. Tests performed using laboratory constructed mixtures of different bacterial species gave only positive results if *H. pylori* was present. The sensitivity and specificity of this PCR method for direct detection of *H. pylori* in environmental samples has been determined and found to be optimal for water samples.

H. pylori strain differentation is possible using this molecular technique, and future studies will involve terminal restriction fragment length polymorphism analysis to detect and differentiate strains of *H. pylori*. This analysis will provide a rapid and reliable detection tool for *H. pylori* strains in both clinical and environmental samples, deepening our understanding of the source of infection.

Figure 1. *Helicobacter pylori* Strain RSB6 in River water for 140 days.

Typical organization of the ribosomal genes in microorganisms



Relative locations of the ribosomal RNA genes in *H. pylori* genome.

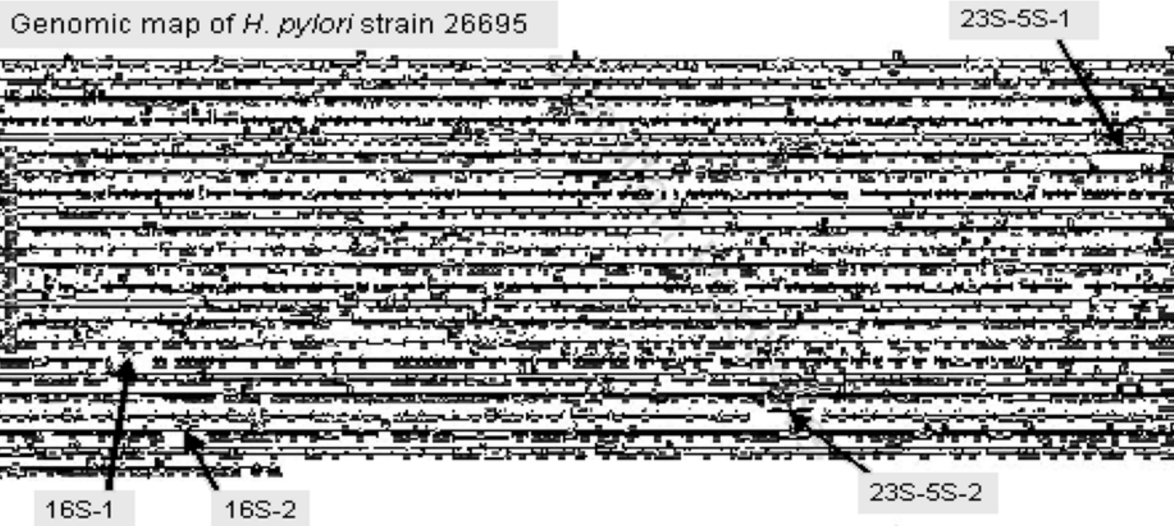


Figure 2. Relative location of ribosomal RNA in *H. pylori* and other bacteria.

Using Real-Time PCR To Detect Toxigenic Strains of *Microcystis aeruginosa*

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Poster Abstract

Both toxigenic and nontoxigenic strains of *Microcystis* exist in nature with a patchy distribution. Only about 50 percent of all *Microcystis* blooms tested for heptotoxicity are positive by the mouse bioassay. The goal of this project is to develop the capability to discern between toxic and nontoxic blooms of *Microcystis*. Polynucleotide sequences were used within the 16S ribosomal RNA gene and the *mcyA* gene in the microcystin synthetase operon to develop primer/probe sets for a multiplex 5'-nuclease polymerase chain reaction (PCR) assay. Presumably the primer/probe set developed for the *mcyA* gene will give positive results only with strains that produce toxins.

Nineteen polynucleotide sequences for the *mcyA* gene and four sequences for the 16S gene were entered into the Primer Express® software. Primer/probe sets were selected using two criteria: (1) the lowest probability of meeting the designated parameters by random chance; and (2) the highest number of *M. aeruginosa* strains that contained the selected sequences as found in GenBank for each primer and probe. Eleven different strains of *M. aeruginosa*, six of which were previously documented to be toxigenic, were obtained from the University of Texas Culture Collection and established in BG-11 media. These strains were originally isolated from samples collected in Canada, Australia, South Africa, and the United States. PCR products for the 16S gene found in the presence or absence of NMT products served as a positive control to show that PCR inhibitors were not present and that analytical procedures were not compromised.

As predicted, only the strains determined to be toxigenic by high performance liquid chromatography and enzyme-linked immunosorbent assay were positive for detection of the NMT region of the *mcyA* gene (see Table 1). In addition, the NMT region of the *mcyA* gene has been detected in 18 toxigenic cultures and 2 nontoxigenic cultures, but not in 17 other nontoxigenic cultures by other investigators. Using real-time PCR to detect toxigenic strains of *M. aeruginosa* was successful and indicates the potential for a highly sensitive and precise assay. Further testing to assess the correlation between the presence of this genetic region and toxigenicity of environmental samples is needed.

Table 1. Multiplex PCR.

B 2662	Yes	18.43	18.88
LB 2664	Yes	18.33	19.17
B 2666	Yes	23.21	23.78
B 2667	Yes	18.27	18.4
B 2669	Yes	19.34	19.93
B 2670	Yes	17.44	17.92
LB 2386	No	18.15	Negative
B 2661	No	18.64	Negative
B 2671	No	17.33	Negative
B 2672	No	21.91	Negative
B 2676	No	18.11	Negative
Negative control		Negative	Negative

*Cycle threshold, Ct, is the first thermocycle in which there is a significant increase in fluorochrome emission from the probe.

Role of Adaptive Response in the Kinetics of *Mycobacterium avium* Inactivation With Monochloramine

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Poster Abstract

Mycobacterium avium is a waterborne opportunistic pathogen commonly detected in drinking water. The persistence of *M. avium* in drinking water distribution systems has been associated to its presence in biofilms, inside which these microorganisms appear to gain protection against disinfectant attack. It is also suspected that *M. avium* detected in tap samples, also containing a measurable level of chlorine residual, survives embedded inside suspended particles detached from biofilms. Because chlorine reacts with biofilm or particle organic matter, the embedded cells get exposed to lower disinfectant concentrations. Furthermore, exposure to low concentrations of the disinfectant hydrogen peroxide has been shown to trigger an adaptive response, referred to as the SOS response, with both *A. hydrophila* and *Escherichia coli*. The SOS response triggers the synthesis of a number of proteins, some of which can repair DNA damage induced by the disinfectants. The main objective of this study is to characterize the effects of disinfectant concentration, temperature, and pH on the inactivation kinetics of *M. avium* (ATCC 15769) with combined chlorine. The occurrence and impact of a biochemical adaptive response during the inactivation of *M. avium* with this disinfectant also has been assessed. Experiments were performed in batch reactors with the temperature controlled at target values in the range of 1-30 ^{o}C. The solution pH is maintained constant at target values in the range of 6-10 with phosphate and borate buffers. Chlorine concentrations range from 0.01 to 10 mg/L as Cl_2.

Resulting inactivation curves were characterized by pseudo-first order kinetics without the occurrence of a lag phase. A single curve was obtained for relatively high concentrations (e.g., 5.0 mg/L and 10 mg/L at 20 ^{o}C and pH 8) when plotting the natural logarithm of survival ratio versus CT. This finding confirmed the validity of the CT concept (a fixed value of the product of the concentration and contact time resulted in a fixed degree of inactivation at a given temperature and pH, independently of the disinfectant concentration used) at relatively high concentrations. In contrast, the inactivation kinetics *M. avium* at low monochloramine concentration (e.g., <1 mg/L as Cl_2 at 20 ^{o}C and pH 8) was only approximately 25 percent of that observed at high concentration at the same temperature and pH. The greater resistance to inactivation observed at the lower monochloramine concentration was consistent with the occurrence of an adaptive response similar to that reported for other bacteria. However, additional work at the molecular level is necessary to assess if the adaptive response mechanism is the SOS response observed with other bacteria.

Topic Area 3: Distribution Systems and Biofilms

The Effect of Chlorine, Chloramine, and Mixed Oxidants on Biofilms in a Simulated Water Distribution System

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Presentation Abstract

Throughout the world there are millions of miles of water distribution pipe lines that provide potable water for use by individuals and industry. Some of these water distribution systems have been in service well over 100 years. Treated water moving through a distribution system comes into contact with a wide range of materials under a variety of conditions, which can affect water quality. Suspended solids in finished water can settle out under low flow conditions and can be resuspended as flows increase. Disinfectants and water additives react with organic and inorganic materials within the distribution system, producing by-product compounds that may be undesirable in the water supply. Oxidant resistant microorganisms can colonize; pipe surfaces, cracks, and crevices produce a complex microenvironment known as "biofilm." These biofilms can be highly resistant to many disinfection methods and techniques. This resistance to disinfection can extend to the entire colony of microbes, which can include microbial indicators of contamination such as coliform bacteria.

The extent of biofilm growth that can occur under conditions of limited nutrients and in the presence of residual oxidizing agents was evaluated using U.S. EPA's water distribution system simulator (DSS). This presentation describes the features of the DSS and how it was used to compare the effectiveness of three disinfecting agents on system biofilms. Results from this work suggest that chloramine and MIOX® were more effective than free chlorine in reducing culturable drinking water biofilms within the DSS.

Molecular Characterization of Drinking Water Microbial Communities

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Presentation Abstract

The objective of this study was to monitor the impact of chlorination and chloramination treatments on heterotrophic bacteria (HB) and ammonia-oxidizing bacteria (AOB) inhabiting a water distribution system simulator (see Figure 1). HB densities decreased while AOB densities increased when chloramine was added. AOB densities decreased below detection limits after the disinfection treatment was switched back to chlorination. The presence of AOB was confirmed using a group-specific 16S rDNA-PCR method. 16S rDNA sequence analysis showed that most bacterial isolates from feed water, discharge water, and biofilm samples were α-Proteo-bacteria or β-Proteobacteria. The latter bacterial groups also were numerically dominant among the sequences recovered from water and biofilm 16S rDNA clone libraries. The relative frequency of each culturable bacterial group was different for each sample examined. Denaturing gradient gel electrophoresis analysis of total community 16S rDNA genes showed notable differences between the microbial community structure of biofilm samples and feed water. The results of this study suggest that disinfection treatments could influence the type of bacterial community inhabiting water distribution systems.

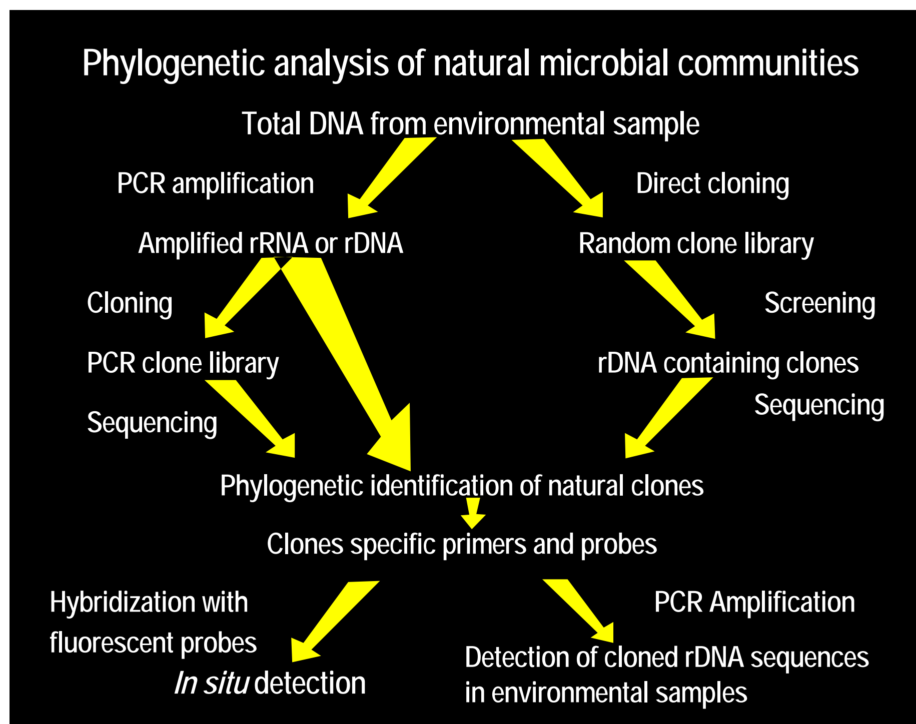

Figure 1. Phylogenetic analysis of natural microbial communities.

Phylogenetic Analysis of Prokaryotic and Eukaryotic Microorganisms in a Drinking Water Distribution System Simulator

Margaret M. Williams[1], Mark C. Meckes[2], Cathy A. Kelty[2], Hildred S. Rochon[3], and Jorge W. Santo Domingo[2]
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Poster Abstract

Within potable water distribution systems, opportunistic pathogens such as *Legionella* species infect protozoa, gaining protection from disinfectant residuals. Analyzing the prokaryotic and eukaryotic populations in distribution system water provides a basis for understanding the interactions between these microorganisms. Samples were obtained from the feed and discharge water of a ductile iron distribution system simulator that receives drinking water containing a 0.5 ppm monochloramine (NH_2Cl) residual. Bacteria were isolated on R2A agar, then identified by polymerase chain reaction amplification of the 16S rRNA gene (rDNA), followed by sequence analysis. To determine a broader range of microbial populations present in the systems, clone libraries of 16S rDNA and 18S rDNA were made from sample water. The majority of the isolates were closely related to bacteria belonging to the alpha proteobacteria, including *Sphingomonas*, *Brevundimonas*, and *Caulobacter* species. Sequence analysis of the clones obtained from the pipe loop discharge water showed a mixture of alpha and beta proteobacteria, as well as the presence of *Nitrospira* sp., which are nitrite oxidizers. Using genus-specific 16S rDNA primers, two *Legionella*-like species were identified: *Tatlockia micdadei* and *Legionella*-Like Amoebal Pathogen 1 (LLAP1). A wide range of eukaryotic microorganisms, including dinoflagellates such as *Gymnodinium* and *Peridinium* spp., have been identified from clones obtained using universal 18S rDNA primers (see Figure 1). Determination of the predominant protozoan species within the distribution water will allow the identification of possible hosts for *Legionella* species and other opportunistic pathogens. Prokaryotic and eukaryotic community analysis is a first step in elucidating the relative activity and survivability of each group of organisms.

Figure 1. Phylogenetic tree demonstrating the relationships among dinoflagellate sequences obtained from the feed (L3F samples) and discharge (L3D samples) of the distribution system simulator (DSS).

Identification and Characterization of *Aeromonas* Isolates From Drinking Water Distribution Systems

***Jennifer Birkenhauer*[1] *and M. Rodgers*[2]**
[1]*Oak Ridge Institute for Science and Education, U.S. Environmental Protection Agency, Cincinnati, OH;* [2]*U.S. Environmental Protection Agency, Cincinnati, OH*

Poster Abstract

Members of the bacterial genus *Aeromonas* are commonly isolated from both fresh and salt waters worldwide, and some are believed to cause infections in humans, including gastroenteritis and wound infections. Currently, aeromonads are on the U.S. EPA's Contaminant Candidate List, and are suspected of contaminating drinking water distribution systems. Identification of aeromonads to the species level is difficult as new species, taxa, and biogroups continue to be proposed. In this study, both metabolic and genomic fingerprinting identification methods were employed to obtain an understanding of the occurrence and types of aeromonads in drinking water distribution systems in the United States.

Water samples were analyzed from 18 drinking water distribution systems across the United States, eight of which were found to contain aeromonads. All colonies were isolated from ADA-V medium and were confirmed to be aeromonads as recommended in EPA Method 1605. Confirmed isolates, 212 in total, were then subjected to both a Restriction Fragment Length Polymorphism (RFLP) analysis (Borrell, *et al.*, 1997) and to a carbon source utilization assay employing the BIOLOG microbial identification system.

The BIOLOG microbial identification system offers a straightforward approach to identifying environmental microbes. However, we found that only after compiling our own database were we able to gain confidence in the system's ability to correctly identify each isolate. The RFLP analysis, while requiring much more time and technical skill, was able to give a more consistent identification of each isolate, with the exception to certain biotypes.

Based on both the metabolic and genomic fingerprinting of these organisms, we were able to identify several different biotypes, including *A. hydrophila, A. bestiarum,* and *A. salmoncida* from drinking water distribution systems. Because some of the species that were isolated have been implicated in human disease, the results from this study indicate that a more comprehensive survey of drinking water utilities is warranted to determine if aeromonads in drinking water pose a threat to public health.

Pathogenicity of Biofilm Bacteria

Dennis Lye
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Poster Abstract

There is a paucity of information concerning any link between the microorganisms commonly found in biofilms of drinking water systems (see Table 1) and their impacts on human health. For bacteria, culture-based techniques detect only a limited number of the total microorganisms associated with biofilms. The possibility of unknown opportunistic pathogens occurring in potable water and biofilms within drinking water systems still exists, but it is unlikely that pathogenic microorganisms will be found using individual *in vivo* culture-based techniques or by screening large numbers of isolates using the currently available *in vitro* virulence tests. A combination of molecular-based techniques and animal-exposure studies will provide the information necessary to fully characterize the pathogenicity of microorganisms commonly associated with biofilms.

Table 1. Opportunistic microbial pathogens that could be encountered in biofilms of drinking water systems.

Pathogen	Disease
BACTERIA:	
Helicobacter pylori	peptic ulcers
Escherichia coli	gastroenteritis
Mycobacterium avium complex	chronic diarrhea, lung disease
Legionella spp.	Legionnaires disease
Pseudomonas aeruginosa	burn infections
VIRUSES:	
Polio	poliomyelitis
Coxsackie	upper respiratory
Norwalk	gastroenteritis
Hepatitis A	infectious hepatitis
PROTOZOA:	
Cryptosporidium	gastroenteritis
Giardia	gastroenteritis
Entamoeba	amoebic dysentery
Acanthamoeba	eye infection
FUNGI:	
Aspergillus	pulmonary disease

Topic Area 4: Cross-Cutting Research and Emerging Topics

The Application of Mass Spectrometry to the Study of Microorganisms

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Presentation Abstract

The purpose of this research project is to use state-of-the-art mass spectrometric techniques such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS), to provide "protein mass fingerprinting" and protein sequencing information for microorgansims listed on the 1998 Contaminant Candidate List (CCL) that cause waterborne disease. The responsibility of characterizing and investigating microorganisms has traditionally fallen to microbiologists, but recent advances in mass spectrometry have allowed analytical chemists also to enter the realm of microorganisms.

Protein mass fingerprinting libraries will be developed and evaluated to determine whether MS techniques can identify protein fingerprints related to the infectivity/viability of selected microorganisms and whether they can differentiate between species and strains of selected microorganisms. Sequence information for proteins that are found to be specific or unique to species/strain and infectivity/viability also can be obtained with these MS techniques.

This global proteomic project has a number of subtasks for which preliminary results have been obtained on microorganisms such as coxsackievirus, *Cryptosporidium parvum*, and enterococci. Through the use of mass spectrometry, a potential viral biomarker of coxsackievirus has been identified that may indicate whether the virus is infectious. A unique mass spectral peak was observed in an infectious coxsackievirus, but was not observed in a noninfectious coxsackievirus. This unique peak may be responsible for viral infectivity, thus, be a potential biomarker.

In addition to viruses, initial experiments were performed to determine the ability of MALDI to analyze *C. parvum* both in an intact form, as well as oocysts that have been rendered nonviable. MALDI analysis was performed on several different harvests of the intact oocysts, as well as the separated cell walls and sporozoites that make up the oocysts. The analysis of the oocysts walls was inconclusive due to lack of discernable mass spectral peaks, but MALDI analysis of the sporozoites yielded reproducible mass spectra.

Whole enterococci cell protein profiles were evaluated using MALDI as a tool to identify seven different enterococci species. Many mass spectral peaks were shared among the different enterococci species; however, each species showed unique peaks, primarily in the 6,000 to 7,000 m/z region. When environmental isolates were tested, the signature peaks were observed in many of the different isolates, suggesting that these peaks could be used for species identification. Sequence analysis of the environmental isolates by 16S rDNA confirmed the identity of the strains tested, and matched the MALDI identity prediction in 75 percent of the samples. The results from this study indicate that the analysis of whole enterococci cells by MALDI generate unique protein profiles, which can be used for the rapid identification of fecal enterococci environmental isolates.

Although mass spectrometry currently is not sensitive enough to detect single cells in drinking water, the basic proteomic information obtained with these mass spectrometric techniques can be used to develop more sensitive and precise microbiological techniques that focus on these unique proteins in drinking water samples. These conventional microbiological methods can then be used to gather the occurrence data that will be used to create better U.S. EPA regulations for protecting humans from microbiological contaminants in U.S. drinking water supplies.

Cyanobacteria and Their Toxins

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Presentation Abstract

Cyanobacteria and their toxins are listed as microbial contaminants on the Candidate Contaminant List. Increasingly, toxic cyanobacterial blooms are being reported in surface fresh water bodies worldwide. It is believed that both increased occurrence associated with eutrophication and climatic changes, and increased detection due to improvements in scientific knowledge and methods to detect blooms are contributing to this trend. Recent studies suggest that consumers of drinking water derived from surface sources in the United States may be exposed episodically to low concentrations of these toxins. However, there is little documented information about potential human health effects associated with exposure to these contaminants at ambient concentrations. Characteristics of cyanobacteria and their toxins, and recent U.S. EPA activities will be discussed.

Transport of Chemical and Microbial Contaminants From Known Wastewater Discharges: Potential Chemical Indicators of Human Fecal Contamination

Susan T. Glassmeyer[1], Imma Ferrer[2], Edward T. Furlong[2], Jeffrey D. Cahill[2], Steven D. Zaugg[2], Stephen L. Werner[2], Michael T. Meyer[3], Dana W. Kolpin[4], and David D. Kryak[5]
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Presentation Abstract

The quality of drinking and recreational water is currently ascertained using indicator bacteria such as *Escherichia coli* and fecal enterococci. However, the tests to analyze for these bacteria require 24 to 48 hours to complete, and do not discriminate between human and animal fecal material sources. One solution to these problems is to use chemicals that are commonly found in human wastewater as supplementary tracer compounds. The chemicals have the advantage of requiring shorter analysis times (3-4 hours), and a suite of human specific markers can be selected that are unique to human wastewater. For this project, compounds includes those that are produced and excreted by humans (e.g., coprostanol), that are consumed and pass easily through humans (e.g., pharmaceuticals and caffeine), and that are associated with humans and deposited into the combined graywater/blackwater household septic waste stream (e.g., surfactants). At 10 wastewater treatment facilities, a treated effluent sample, as well as surface water samples from upstream and at two successive points downstream from the facility were collected. This longitudinal sampling scheme was used to determine the persistence of the target compounds in streams. Compounds that are quickly removed or degraded may not be persistent enough to serve as tracers; those that are too recalcitrant would similarly not be suitable as they would be present after the pathogens have been eliminated.

To estimate the environmental persistence of pathogens, the water samples were analyzed for *E. coli* and fecal enterococci in addition to the suite of chemicals being measured. For chemical analysis, the water samples were extracted using either solid phase extraction (for the pharmaceuticals) or liquid-liquid extraction (for the other wastewater contaminants) and were analyzed using either high-performance liquid chromatography/mass spectrometry (HPLC/MS; pharmaceuticals) or gas chromatography/mass spectrometry (GC/MS; other wastewater contaminants). The concentration of microbial indicators was determined using modified mTEC (*E. coli*) or mEI (enterococci) media. Of the 114 chemical analytes investigated in this project, more than 80 were found in at least one sample. Although most concentrations were in the range of 0.1 to 1.0 μg/L, in some of the more highly contaminated samples, concentrations were in the range of 5-20 μg/L. The concentrations of the majority of the chemical compounds present in the samples generally followed the expected trend: they were either nonexistent or at only trace levels in the upstream samples, had their maximum values in the wastewater effluent samples, and then declined in the two downstream samples. However, at most locations, there were indicator bacteria in the upstream samples, illustrating some of the difficulty in using bacteria to monitor water quality.

This work indicates that these human wastewater constituents do have utility as tracers of human wastewater discharge. However, until the behavior of these chemical analytes is evaluated in a rigorous epidemiological study, their true potential as chemical indicators of human fecal contamination will not be determined. To begin this assessment, samples are currently being analyzed as part of the National Epidemiological and Environmental Assessment of Recreational Water Study, which should determine if there is a correlation between concentration of any of the chemicals and incidence of illness.

High Throughput DNA-Based Tools To Study Water Microbial Communities

Jorge Santo Domingo, Joyce Simpson, Margaret Williams, and Catherine Kelty
National Risk Management Research Laboratory, Water Supply and Water Resources Division, Microbial Contaminant Control Branch, U.S. Environmental Protection Agency, Cincinnati, OH

Presentation Abstract

The demands for water safe for human consumption and recreational activities have increased rapidly in recent years due to human exponential growth. The impact of this growth has affected the performance of wastewater treatment facilities and changed the biological and chemical stability of watersheds throughout the nation. One of the most significant challenges facing the Nation is to meet the standards established by regulatory agencies, recognizing the shortcomings characteristic of current technologies used to monitor microbiological water quality. One relevant shortcoming is the strict dependence on culturing techniques to determine the presence and estimate densities of indicator bacteria and microbial pathogens. Culture-based methods tend to underestimate the densities and diversity of microorganisms because they can only recover a small number of organisms. Frequently, accurate identification of microorganisms can take a day or two to several weeks. Nucleic acid-based approaches can circumvent many of the shortcomings of the culture-based methods. For instance, the possibility of rapidly and simultaneously monitoring for the presence of hundreds of microorganisms and genes relevant to public health is now becoming a reality in light of the recent advances in microarray technology. This presentation will review recent technological developments in nucleic acid research that can be used to assess the microbiological quality of water systems (see Figure 1). Examples will be provided to illustrate the application of molecular tools to: (1) evaluate microbial changes in water quality of fecally impacted watersheds; and (2) study microbial diversity during treatment, distribution, and storage of drinking water. The importance of constructing comprehensive molecular databases for water distribution systems and watersheds also will be discussed. These databases are necessary to optimize the various methods that will be used in the years to come by environmental microbiologists. In summary, we believe that the use of rapid and high-throughput methods will result in the development of risk management measures that are based on a framework of sound science.

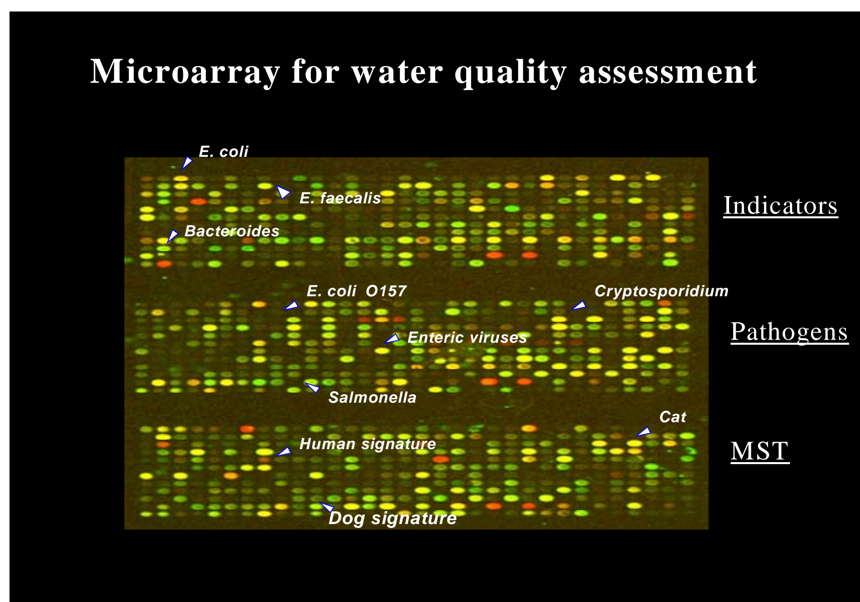

Figure 1. Microarray for water quality assessment.

Detection of Emerging Microbial Contaminants in Source and Finished Drinking Water Using DNA Microarrays

Timothy M. Straub[1], Paul A. Rochelle[2], Ricardo DeLeon[2], and Darrell P. Chandler[3]
[1]Battelle Memorial Institute, Pacific Northwest, Richland, WA; [2]Metropolitan Water District of Southern California, Los Angeles, CA; [3]Argonne National Laboratory, Argonne, IL

Presentation Abstract

DNA microarrays represent a potentially significant technology and analytical technique for the simultaneous detection of multiple pathogens in a single water sample, with the ability to incorporate live/dead discrimination using messenger RNA expression. The objectives of this project are to develop and use DNA arrays for detecting pathogens in natural, turbid, and processed water supplies. *Cryptosporidium parvum*, *Escherichia coli* O157:H7, and *Helicobacter pylori* serve as model organisms.

The genetic sequence differences for certain genes for strains of *C. parvum* and closely related nonpathogenic species are often single nucleotide substitutions at various locations within a gene. An array was designed and tested to investigate these single nucleotide substitutions within the hsp70 (70 kilodalton heat shock protein) gene for two species of *Cryptosporidium*. A similar array was constructed to investigate both single nucleotide substitution and the presence or absence of a virulence gene for *H. pylori*. For *E. coli* O157:H7, certain strains lack either or both shiga-like toxin genes. An array was constructed for this pathogen and tested with various strains of *E. coli* O157:H7. For all arrays, polymerase chain reaction (PCR) using fluorescently labeled primers for each organism was performed and then hybridized to the array. The resulting hybridization patterns on the array were analyzed to determine if differences between species and strains could be elucidated.

For *C. parvum*, differences between two species (*C. hominis* and *C. parvum*) were easily differentiated by both their pattern of hybridization to the array, and statistical discrimination of the data for single nucleotide substitutions. Likewise, single nucleotide substitution also was possible for *H. pylori*. For this organism, the difference between strains also was readily apparent by the absence of a virulence factor gene. For *E. coli* O157:H7, discrimination between strains that lacked either or both shiga-like toxin genes was confirmed. Specificity testing with nontarget organisms revealed extremely low false positive rates. Sensitivity of the arrays was dependent, in part, by the PCR process that was used to generate the fluorescent probes to hybridize to the array, but it was in the general range of 10 to 100 cells.

Microarray analysis of waterborne pathogens allows excellent discrimination between strains and closely related species of organisms. In the context of these findings, the arrays can serve to potentially fingerprint isolates in documented waterborne disease outbreaks. Also, the presence or absence of virulence factor genes in certain isolates may render these organisms more or less pathogenic. In this case, microarray analysis may aid epidemiologists to link mild cases of gastroenteritis due to consumption of contaminated drinking water to less virulent forms of known waterborne pathogens.

The true ability of DNA microarrays for detection of any waterborne pathogen using just one assay method has yet to be realized. This is due, in part, to generation of labeled probes using PCR. To realize this goal, many different PCR primers, to cover known and emerging pathogens, must work within the same reaction vessel. This is an extremely difficult task and will require next generation reagents and bioinformatics software to design PCR primers that will work in a reaction of this type. Currently, this avenue is being investigated as well as an alternate, where messenger RNA is hybridized directly to the arrays. Using the multiplexed PCR approach, a 6-plex PCR has been performed successfully. With the RNA approach (see Figure 1), the presence of 12 different gene sequences representing 8 unique genes for an *E. coli* O157:H7 isolate that lacked its shiga-like toxin 2 gene were detected simultaneously. The principle advantages of the RNA approach are the ease with which multiplexing can be achieved and the potential for live/dead discrimination.

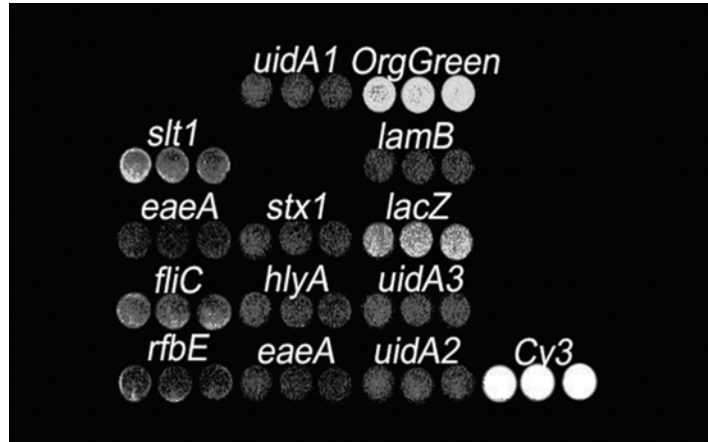

Figure 1. Detection of virulence and marker genes for *E. coli* 0157:H7 ATCC 43890 using the direct mRNA hybridization approach. This strain lacks the shiga-like toxin 2 gene, and this is confirmed in this image.

Mammalian Cell Response to Pathogens

Samuel L. Hayes
U.S. Environmental Protection Agency, Cincinnati, OH

Presentation Abstract

The goal of microbiological water testing is to determine whether or not the consumption of and/or exposure to a water sample will lead to health problems in an exposed population. Water utilities currently rely on the detection of indicator bacteria as a measure of the potential presence of pathogenic microorganisms. Although it is possible to directly analyze for the presence of specific pathogens, it is difficult to design assays that detect the full range of potential human pathogens and assess the virulence of each pathogen detected. A better approach might involve measuring the interaction between waterborne microorganisms (i.e., potential pathogens) and human cells as a measure of exposure assessment.

Microbial pathogens have been shown elicit host responses resulting in shifts in cell metabolism and protein synthesis. These shifts are reflected in the messenger RNAs (mRNAs) produced by the responding cell or tissue. Therefore, it should be possible to determine whether a disease response has occurred by monitoring the expression of mRNA molecules. DNA microarrays are one way to monitor changes in mRNAs. The specific goal of this project is to look at mammalian cellular response to pathogens, specifically assessing gene expression. The focus will be on pathogens that are known (or suspected) to cause disease from exposure to contaminated water. These pathogens typically cause gastrointestinal illness. Thus, experimentation will focus on disease mechanisms associated with intestinal infections. The experimental design is as follows:

Stage 1:

- Establish appropriate animal model for intestinal infection
- Establish corresponding animal intestinal cell line
- Grow pathogen and prepare inoculum at the desired density
- Infect animal model and cell lines
- Analyze mRNAs using species-specific cDNA microarrays
- Look for similar mRNA expressions between the animal model and tissue cell line.

Assuming similar gene expression patterns are found between the tissue culture and animal models, move to Stage 2 testing.

Stage 2:

- Establish and infect human intestinal cell line
- Analyze mRNAs from human tissue cultures, look for similar patterns as seen in the animal model using human cDNA microarray.

The literature suggests that pathogens elicit characteristic sets of mRNAs in host tissues. These mRNAs could be used to define the pathogen present. Alternatively, a common set of mRNAs may be produced in response to many different pathogens. In either case, the identification of specific mRNAs in host tissue will indicate significant risk. By establishing a relationship between animal models and tissue culture, the case will be made that human tissue culture can serve as a model for human infection in terms of mRNA expression. This addresses effects of exposure and identifies disease mechanisms from exposure. An important secondary goal of the project is to eliminate using animal testing for pathogen exposure research.

Effectiveness of UV Irradiation for Pathogen Inactivation in Surface Waters

Karl Linden[1], Mark Sobsey[2], and Gwy-Am Shin[2]
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Poster Abstract

Ultraviolet (UV) irradiation is now recognized as an effective and cost-competitive measure to achieve significant level of inactivation of *Cryptosporidium* while not producing appreciable level of harmful disinfection by-products (DBPs) at practical doses. However, the effectiveness of UV technology against new and emerging pathogens and uncertainties in application of UV disinfection for unfiltered surface waters still needs to be assessed before widespread use of this technology as a primary disinfectant in drinking water treatment processes. The primary objectives of this research project are to evaluate the susceptibility (or resistance) and repair potential of select Contaminant Candidate List (CCL) pathogens and indicator microorganisms to or after UV disinfection from low- and medium-pressure (LP and MP) UV sources, and to investigate the extent of microbial association with particles in unfiltered systems and the effects of this particle association and other water quality parameters on UV disinfection potential.

Preliminary results indicate that both LP and MP UV irradiation are very effective against most of the indicator and emerging microorganisms tested. However, some of the indicator microorganisms like coliphage MS2, bacteriophage PRD-1, and *Bacillus subtilis* endospores as well as CCL and emerging pathogens like *Mycobacterium terriae* (a substitute for *Mycobacterium avium* complex), adenovirus type 2, and *Toxoplasma gondii* oocysts showed relatively high resistance against both UV irradiation. Although the effectiveness of LP and MP UV appeared to be similar against most of the microorganisms tested, there was some remarkable difference between these two UV technologies in terms of their effectiveness against adenovirus 2. To determine the level of particle association and its effect on UV disinfection, raw surface water samples have been collected from various utilities across the United States, and the waters have been examined for particle associated coliform and aerobic endospores using physical particle disruption techniques such as homogenization and blending. However, the levels of the indigenous microbes in the raw waters were typically low (< 1,000/100 mL), so that it was not feasible to assess the degree of particle association in these waters based on those physical methods.

Currently, the use of microscopic techniques (nucleic acid staining/probes along with confocal microscopy) are being investigated to determine the level of particle association in those raw waters. Regarding the development of new assay systems for some of the CCL microorganisms, we have been successful in developing a new assay system (Long-template [LT] RT-PCR) for Norwalk virus and a new molecular biology assay (RT-PCR) for adenovirus 40 or 41, which are being and will be used in the current and future inactivation study on these viruses by LP and MP UV. In addition, a method has been established to perform wavelength specific studies using a polychromatic UV light source (MP UV lamps) with a set of UV bandpass filters, and this setup will be utilized to develop wavelength effectiveness information for select CCL microorganisms like adenovirus 2, *M. terriae*, and *T. gondii* oocysts. Finally, protocols have been established to examine repair phenomenon following UV disinfection in various conditions in real water treatment situations, and these protocols will be implemented to evaluate the presence and extent of repair after UV irradiation in the select CCL and emerging microorganisms in the later phases of this research.

Survey of U.S. Public Health Laboratories: Microbial Pathogens on the Candidate Contaminant List

***Elizabeth D. Hilborn*[1]*, Michael O. Royster*[2]*, and Doug J. Drabkowski*[3]**
[1]*Office of Research and Development, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC;* [2]*Crater Health District, Petersburg, VA;* [3]*Association of Public Health Laboratories, Washington, DC*

Poster Abstract

During March 1998, the U.S. EPA published the Candidate Contaminant List (CCL) of drinking water contaminants; these chemical contaminants and microbial pathogens will be evaluated by the U.S. EPA for potential regulation. Microbial pathogens contained in the list include: *Aeromonas hydrophila*, adenovirus, calicivirus, coxsackie virus, echo virus, *Helicobacter pylori*, *Microsporidia* spp., and *Mycobacterium avium*-intracellulare complex (MAIC). Because few of these pathogens are reportable and detection methods among laboratories may vary, an estimate of the public health burden of illness is needed to prioritize pathogens for regulatory action. State public health laboratories (SPHL) serve as reference laboratories in many states. If SPHL are likely to receive requests to detect CCL pathogens in human clinical specimens, they may serve as future active surveillance sites to help establish population-based estimates of illness with CCL pathogens.

During early 2000, a survey of SPHLs was performed by the Association of Public Health Laboratories. The survey goal was to ascertain the number of clinical specimens submitted, the number of specimens in which evidence of infection with a CCL pathogen was verified, and analytic methods used to detect evidence of infection. Each state laboratory director was asked to report fiscal year 1999 (FY 99) data via a self-completed questionnaire.

Forty-seven of 50 (94 percent) SPHL representatives completed and returned questionnaires. During FY 99, the number of clinical specimen submissions, percent positivity, and analytic methods varied by CCL pathogen. Number of submissions ranged from 1,009 for analysis of calicivirus, to 199,641 for analysis of MAIC. Percent positivity ranged from less than 1 percent of specimens examined for evidence of *A. hydrophila*, coxsackie virus, and *Microsporidia* infection, to 40 percent of specimens examined for evidence of calicivirus infection. Analytic methods used by SPHLs included: culture, immunologic and molecular assays, and direct visualization of pathogens. SPHLs solely reported using polymerase chain reaction (PCR) to detect calicivirus in clinical specimens; this technique resulted in the highest percent detection (40 percent as compared to < 5 percent for all other pathogens).

This survey provided information about which CCL pathogens are currently detected at SPHL and analytic methods used during 1999. SPHL may be useful in active surveillance systems for nontuberculous *Mycobacterium* spp., adenovirus, and enteroviral (coxsackie virus, echo virus) infections. SPHL are least likely to be good locations for surveillance of *H. pylori*, *Microsporidia* spp., and calicivirus. The use of PCR to detect evidence of calicivirus in clinical specimens resulted in the highest percent detection of calicivirus among all CCL pathogens. However, PCR may be underutilized in SPHLs. Increased use of molecular techniques may increase the diagnostic efficiency of CCL pathogens within SPHLs.

Comparative Diversity of Fecal Bacteria in Agriculturally Significant Animals To Identify Alternative Targets for Microbial Source Tracking

Joyce M. Simpson[1], Samuel P. Myoda[2], Donald J. Reasoner[1], and Jorge W. Santo Domingo[1]
[1]Water Supply and Water Resources Division, National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH; [2]Division of Water Resources, Delaware State Department of Natural Resources and Environmental Control, Dover, DE

Poster Abstract

Animals of agricultural significance contribute a large percentage of fecal pollution to waterways via run-off contamination. The premise of microbial source tracking is to utilize fecal bacteria to identify target populations that are directly correlated to specific animal feces, thus permitting identification of contamination sources and implementation of remediation practices.

To identify alternative targets for source tracking studies, comparison of fecal bacterial populations was performed using Denaturing Gradient Gel Electrophoresis (DGGE) targeting the V3 region of the 16S rDNA gene. Fecal populations from individual horses, cattle, swine, sheep, and goats were compared (see Figure 1).

The greatest diversity was found in the ruminant animal species. Within the ruminants, between 40 and 51 percent of the bands within the fecal patterns were dominant populations (i.e., occurred in greater than 50 percent of animals tested) and 7 percent were highly dominant (occurred in greater than 80 percent of animals tested). Within the non-ruminants, only 14 to 18 percent of the bands were dominant, and 4 percent were highly dominant. Eleven bands were common to all fecal samples, and eight bands were present in ruminants only. Another eight bands were predominantly found in ruminants, and three bands were predominant in non-ruminants. No bands specific to non-ruminants were found in any of the animals tested.

A comparison using Dice's similarity coefficient and Ward's dendrogram algorithm indicated that fecal patterns tended to cluster according to digestive physiology (i.e., ruminants clustered with ruminants) rather than by species. Non-ruminant species tended to cluster more closely within species than to each other and were not as intermixed as ruminant results.

Phylogenetic examination of the common and divergent banding populations should provide information to determine if there are suitable alternative organisms that may be used to track fecal pollution. Elucidation of novel organisms related to fecal contamination would potentially increase the ability to identify sources more accurately, thereby allowing the appropriate remediation response to be expeditiously selected.

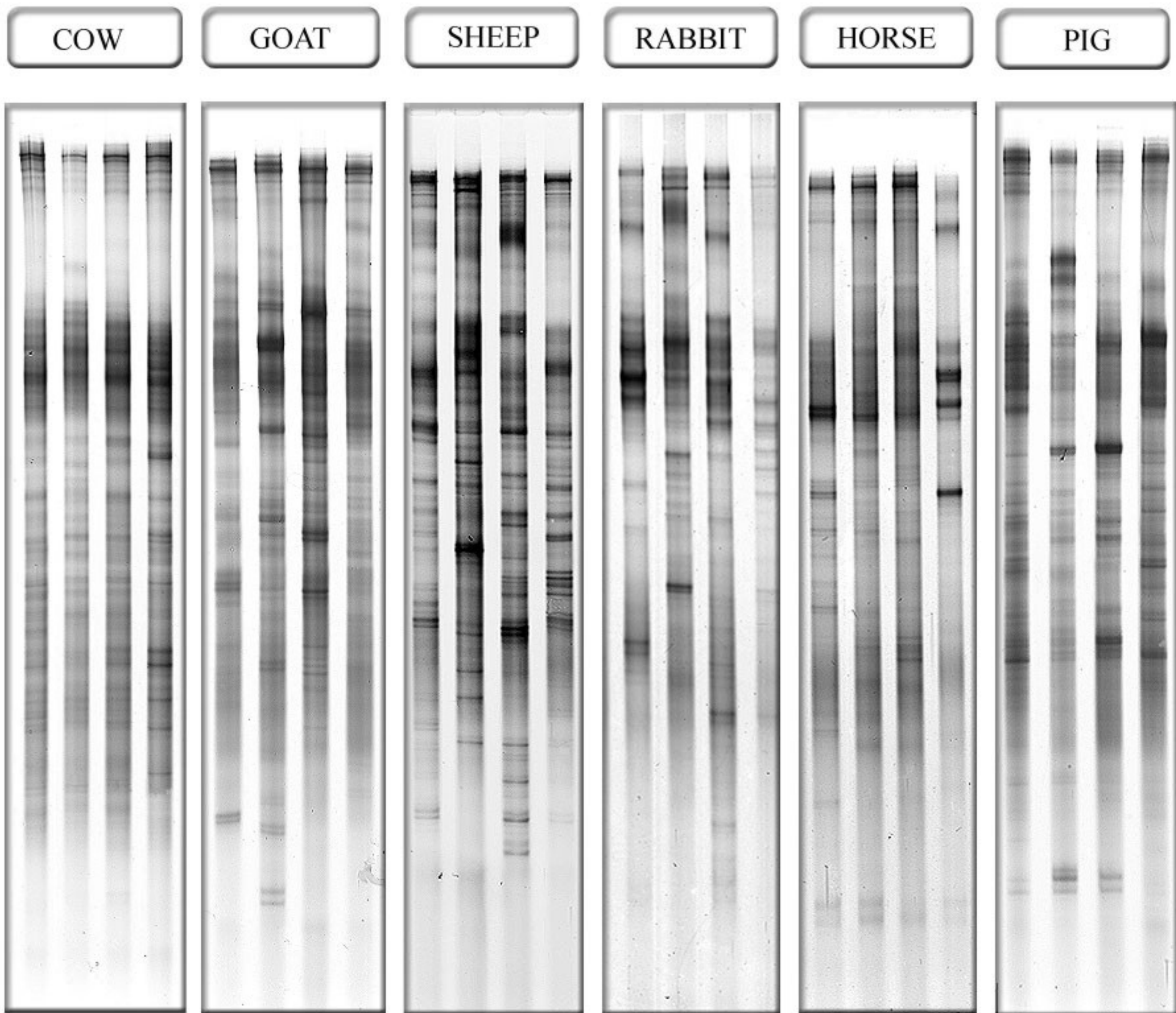

Figure 1. DGGE gel representing typical fecal banding patterns observed for different animal species.

Developing Dynamic Infection Transmission Models for Microbial Risk Assessment (MRA) Applications

Patricia Murphy, Brenda Boutin, Jeff Swartout, Glenn Rice, Jon Reid, and Michael Broder
Office of Research and Development, National Center for Environmental Assessment,
U.S. Environmental Protection Agency, Edison, NJ

Poster Abstract

Drinking water MRAs generally are conducted with methods and assumptions that are analogous to existing chemical risk assessment methodologies. These assessments usually consider only a primary environmental transmission route, such as drinking water, where a human host is exposed and infected solely through this route. There are, however, several issues unique to infectious diseases that the traditional chemical framework does not address, including: (1) the potential for secondary transmission (ST), where the infectious agent is passed directly or indirectly from an infectious human to other susceptible humans; (2) acquired immunity to the infectious agent, where previous exposures render the human host either completely or partially resistant, and the duration of that immunity; and (3) the environmental population dynamics of pathogens, which are living organisms.

To explore the influence of these additional, interdependent factors on traditional MRA approaches, the National Center for Environmental Assessment initiated collaborative research with investigators at the University of California-Berkeley (UCB) and the University of Michigan (UM) to develop and apply infection transmission models for waterborne pathogen exposures. The academic research teams extended existing approaches for compartmental modeling of dynamic infection transmission systems to include infection routes appropriate for enteric waterborne pathogens, that is, exogenous sources, human-human contact, and human-water-human pathogen circulation. Using data from the scientific literature, computer simulation approaches were used to study how model output changed in relation to alternative assumptions for the studied exposure scenarios, alternative values for the input variables, and alternative analytical forms of the model, that is, deterministic or stochastic.

This project has demonstrated that existing infection transmission models can be extended and modified to integrate diverse and complex information on host, agent, and environmental characteristics that affect pathogen exposure and risk. Results show that ignoring or mis-specifying ST effects in the context of MRAs leads to mis-characterization of individual- and population-level risks and mis-estimation of the health benefits attributable to different drinking water treatment interventions.

Existing MRA methodologies for estimating health risks from waterborne exposure to pathogens with a significant potential for human-human transmission require conceptual and analytical modifications to accurately capture host-agent-environment interdependencies that determine human exposure and risk. The final report from this research is under development. It will provide the focus for an upcoming expert workshop that will develop specific recommendations on when and how ST effects should be modeled and incorporated into assessments and will form part of the basis for a 2008 EPA guidance document on MRA tools.

Virulence Factors of *Aeromonas:* A Molecular Genetic Characterization

Keya Sen and Mark Rodgers
Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency, Cincinnati, OH

Poster Abstract

Surveys of finished drinking water from eight small systems (2001) and eight large systems (2000) conducted by the U.S. EPA, revealed that 7 out of 16 water utilities encompassing several states (NY, KY, IA, OH) were contaminated with *Aeromonas* species. Altogether 205 organisms were isolated by EPA Method 1605. The goal of this project was to determine whether the *Aeromonas* species isolated from these drinking water utilities had the potential to be pathogenic.

A molecular genetic approach was chosen. Published literature was searched to identify the genes that played a role in the pathogenesis of the organism. Only those genes were selected that definitively played a role in the virulence of the species when tested in animal models or cell cultures. The isolates were tested for the virulence factors elastase, lipase, flagella A and B genes, the cytotoxic enterotoxin, Act (achytoen), and the cytotonic enterotoxins, Alt and Ast. Oligonucleotide primers were developed against these genes, and when used in the polymerase chain reaction (PCR), a portion of the gene was amplified in the positive control. A positive control was an *Aeromonas* species known to have the virulence gene being tested. PCR was performed in three duplex assays with the 205 isolates using the following primer sets together: elastase (ahyB) and lipase (pla); fla and alt; act and ast.

Preliminary findings showed that pla was present in 86 percent, act in 69 percent, fla in 55 percent, ahyB in 40 percent, and alt and ast in 45 percent and 35 percent of the isolates, respectively. Only one isolate had all six virulence genes. Multiple species were isolated from most of the utilities. Different combinations of virulence factors also were observed, sometimes even in different strains of the same species. However, a dominant strain having the same set of virulence factors was usually isolated from different rounds of sampling from a single tap.

These results suggest the importance of examining as many *Aeromonas* isolates as possible from a water sample, as within the same species the occurrence of certain virulence factors may vary. The results also suggest that the *Aeromonas* strains isolated from water utilities have the potential to be pathogenic. However, additional virulence factors, which have not yet been identified or characterized, may be needed to cause actual disease.

Isolates having different combinations of virulence factors will be tested in animal models to determine whether there are one or more combinations of virulence factors that are necessary for establishing diarrhea in the models. *Aeromonas* isolates from the UCMR survey of finished drinking water, which is being conducted in 2003, also will be tested for the above virulence factor genes.

Effects of pH and Temperature on the Kinetics of *Aeromonas hydrophila* Inactivation With Combined Chlorine

Kwanrawee Sirikanchana and Benito J. Mariñas
Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, Urbana, IL

Poster Abstract

Aeromonas hydrophila is a waterborne opportunistic pathogen commonly detected in natural water sources (e.g., surface water and groundwater) and also in drinking water, even in the presence of detectable levels of chlorine residual. There is a need to elucidate the mechanisms responsible for the survival of *A. hydrophila* during and/or after the disinfection process. Previous studies have shown that the inactivation kinetics of *A. hydrophila* with monochloramine is independent of monochloramine concentration in the range of 0.01 – 10 mg/L as Cl_2 at constant pH and constant temperature. However, water quality parameters including pH and temperature could affect the inactivation kinetics of *A. hydrophila*. The main objective of this study is to characterize the effects of pH and temperature on the inactivation kinetics of *A. hydrophila* (ATCC 7966) with combined chlorine. Experiments are performed in batch reactors with the temperature controlled at target values in the range of 1-30 ^{o}C with a recirculating water bath. The solution pH is maintained constant at target values in the range of 6-10 with phosphate and borate buffers. Disinfectant concentrations range from 0.01 to 10 mg/L.

The kinetics of *A. hydrophila* inactivation with monochloramine under all conditions investigated was characterized by an initial lag phase followed by pseudo-first order inactivation. The temperature effect was found to be consistent with Arrhenius law at each pH. Experiments performed at pH 6, 8, and 10 for each temperature revealed strong pH dependence. As the pH decreased from pH 10 to 6, the inactivation kinetics was faster due to both a shorter lag-phase and a faster rate of post-lag phase inactivation. In general, the concentration of monochloramine used did not affect the kinetics of *A. hydrophila* inactivation with this disinfectant, thereby confirming the validity of the CT concept (a given value of the product of disinfectant concentration and contact time resulted in a given degree of inactivation independently of the disinfectant concentration used) at each pH and temperature tested for the concentration range investigated.

The U.S. Environmental Protection Agency's Research on Microorganisms in Drinking Water Workshop

Marriott Kingsgate Conference Hotel
151 Goodman Drive
Cincinnati, OH 45219

August 5-7, 2003

AGENDA

Tuesday, August 5, 2003

10:00 – 10:10 a.m. Welcome
Hugh McKinnon, Director, National Risk Management Research Laboratory

10:10 – 10:25 a.m. Introductory Remarks
J. Paul Gilman, Assistant Administrator and Agency Science Advisor
Office of Research and Development

10:25 – 10:50 a.m. Overview of the U.S. EPA's Drinking Water Research Program
Fred Hauchman, Drinking Water National Program Manager, NHEERL

10:50 – 11:15 a.m. Overview Presentation From the U.S. EPA's Office of Ground Water and Drinking Water
Gregory Carroll, Chief, Technical Support Center, OW/OGWDW

11:15 – 11:35 a.m. Overview of Water Security Research and Technical Support Activities
Jonathan Herrmann, Water Security Team Leader, and **Hiba Shukairy**, Technical Support Center, OW/OGWDW

11:35 – 12:00 noon Overview of Regional Concerns for Microorganisms and Drinking Water
Bruce Macler, EPA Region 9 (San Francisco)

12:00 – 1:00 p.m. **Lunch**

1:00 – 4:15 p.m. **Topic Area 1: Research Supporting Office of Water's Ground Water/Source Water Regulatory Activities**
Moderators: **Stig Regli**, OW/OGWDW, and **Pat Murphy**, NCEA

1:00 – 1:30 p.m. Safe Drinking Water Act (SDWA) Requirements and Microbial Research Needs (Surface Water, Ground Water, and Distribution Systems)
Presented by **Stig Regli**, OW/OGWDW

1:30 – 1:50 p.m. Microbial Dose-Response Modeling: A Predictive Bayesian Approach (EIMS #54468)
Presented by **Jeff Swartout**, NCEA

1:50 – 2:10 p.m. The Use of Randomized Trials of In-Home Drinking Water Treatment To Study Endemic Water Borne Disease
Presented by **Timothy J. Wade**, NHEERL

2:10 – 2:30 p.m. **Break**

2:30 – 2:50 p.m. Screening Models To Predict Probability of Contamination by Pathogenic Viruses to Drinking Water Aquifers
Presented by **Bart Faulkner**, NRMRL

2:50 – 3:10 p.m. Integrated Approach for the Control of *Cryptosporidium parvum* Oocysts and Disinfection By-Products in Drinking Water Treated With Ozone and Chloramines
Presented by **Benito Marinas**, University of Illinois at Urbana–Champaign

3:10 – 3:30 p.m. Prevalence and Distribution of Genotypes of *Cryptosporidium parvum* in United States Feedlot Cattle
Presented by **Robert Atwill**, University of California–Davis

3:30 – 4:15 p.m. **Panel Discussion**

4:15 – 5:45 p.m. **Poster Session I**
All posters will be set up during both Poster Sessions with one-half of the posters staffed at each session.

5:45 p.m. **Adjournment**

<u>Wednesday, August 6, 2003</u>

8:15 – 5:40 p.m. **Topic Area 2: Research Supporting Office of Water's Contaminant Candidate List (CCL)**
Moderator: **Cynthia Nolt-Helms**, NCER

8:15 – 8:50 a.m. CCL and National Drinking Water Advisory Council (NDWAC) Process
Presented by **Tom Carpenter**, OW/OGWDW

8:50 – 9:15 a.m. The Roles of Pathogen Risk Assessment in the Contaminant Candidate List Process (EIMS #22389)
Presented by **Glenn Rice**, NCEA

9:15 – 9:40 a.m. Overview: CCL Pathogens Research at NRMRL
Presented by **Don Reasoner**, NRMRL

9:40 – 10:00 a.m. **Break**

10:00–11:50 a.m. **<u>Topic Area 2.1: CCL Protozoa</u>**
Moderators: **Carrie Moulton**, OW/OGWDW, and **Alan Lindquist**, NERL

10:00 – 10:20 a.m. Detection of *Cyclospora cayetanensis* and Microsporidial Species Using Quantitative Fluorogenic 5' Nuclease PCR Assays (EIMS #56083)
Presented by **Frank Schaefer**, NERL

10:20 – 10:40 a.m. Development of Detection and Viability Methods for Wateborne *Microsporidia* Species Known to Infect Humans
Presented by **Rebecca Hoffman**, University of Wisconsin at Madison

10:40 – 11:00 a.m. Development and Evaluation of Procedures for Detection of Infectious *Microsporidia* in Source Waters
Presented by **Paul Rochelle**, Metropolitan Water District of Southern California

11:00 – 11:20 a.m. Development and Evaluation of Methods for the Concentration, Separation, Detection, and Viability/Infectivity of Three Protozoa From Large Volumes of Water
Presented by **Saul Tzipori**, Tufts University

11:20 – 11:50 a.m. **Panel Discussion**

11:50 – 12:50 p.m. **Lunch**

12:50 – 2:20 p.m. **Poster Session II**
All posters will be set up during both Poster Sessions with one-half of the posters staffed at each session.

2:20 – 3:50 p.m. **Topic Area 2.2: CCL Viruses**
Moderators: **Robin Oshiro**, OW/OST, and **Betsy Hilborn**, NHEERL

2:20 – 2:40 p.m. Use of PCR-Based Methods for Virus Occurrence Studies (EIMS #56084)
Presented by **Shay Fout**, NERL

2:40 – 3:00 p.m. Norwalk Virus Dose Response and Host Susceptibility
Presented by **Peter Teunis**, National Institute of Public Health and Environment, The Netherlands

3:00 – 3:20 p.m. Development of a Rapid, Quantitative Method for the Detection of Infective Coxsackie and Echo Viruses in Drinking Water
Presented by **Marylynn Yates**, University of California–Riverside

3:20 – 3:50 p.m. **Panel Discussion**

3:50 – 4:10 p.m. **Break**

4:10 – 5:40 p.m. **Topic Area 2.3: CCL Bacteria**
Moderators: **Jim Sinclair**, OW/TSC, and **Don Reasoner**, NRMRL

4:10 – 4:30 p.m. Disinfection of *Helicobacter pylori* and *Aeromonas* Species
Presented by **Don Reasoner**, NRMRL

4:30 – 4:50 p.m. Genomic and Physiological Diversity of *Mycobacterium avium* Complex
Presented by **Gerard Cangelosi**, Seattle Biomedical Research Institute

4:50 – 5:10 p.m. *Mycobacterium avium* Complex (MAC) in Drinking Water: Detection, Distribution, and Routes of Exposure
Presented by **Phanida Prommasith**, Harvard School of Public Health

5:10 – 5:40 p.m. **Panel Discussion**

5:40 p.m. **Adjournment**

Thursday, August 7, 2003

8:00 – 9:00 a.m. **Topic Area 3: Distribution Systems and Biofilms**
Moderators: **Lisa Almodovar**, OW/OST, and **Mark Meckes**, NRMRL

8:00 – 8:20 a.m. The Effect of Chlorine, Chloramine, and Mixed Oxidants on Biofilms in a Simulated Water Distribution System
Presented by **Mark Meckes**, NRMRL

8:20 – 8:40 a.m. Molecular Characterization of Drinking Water Microbial Communities
Presented by **Jorge Santo Domingo**, NRMRL

8:40 – 9:00 a.m. **Panel Discussion**

9:00 – 9:20 a.m. **Break**

9:20 – 12:30 p.m. **Topic Area 4: Cross-Cutting Research and Emerging Topics**
Moderators: **Keya Sen**, OGWDW/TSC, and **Rebecca Calderon**, NHEERL

9:20 – 9:40 a.m. The Application of Mass Spectrometry to the Study of Microorganisms (EIMS #18338)
Presented by **Jody Shoemaker**, NERL

9:40 – 10:00 a.m. Cyanobacteria and Their Toxins (EIMS #54617)
Presented by **Elizabeth Hilborn**, NHEERL

10:00 – 10:20 a.m. Transport of Chemical and Microbial Contaminants From Known Wastewater Discharges: Potential Chemical Indicators of Human Fecal Contamination (EIMS #18337)
Presented by **Susan Glassmeyer**, NERL

10:20 – 10:40 a.m. High Throughput DNA-Based Tools To Study Water Microbial Communities
Presented by **Jorge Santo Domingo**, NRMRL

10:40 – 11:00 a.m. **Break**

11:00 – 11:20 a.m. Detection of Emerging Microbial Contaminants in Source and Finished Drinking Water Using DNA Microarrays
Presented by **Timothy Straub**, Pacific Northwest National Laboratory

	11:20 – 11:40 a.m.	Mammalian Cell Response to Pathogens Presented by **Sam Hayes**, NRMRL
	11:40 – 12:30 p.m.	**Panel Discussion**
12:30 p.m.	**Adjournment of Public Workshop**	
12:20 – 1:30 p.m.	**Lunch**	
1:30 – 4:00 p.m.	**EPA-Only Discussion Session**	
4:00 p.m.	**Adjournment of EPA-Only Discussion Session**	

U.S. Environmental Protection Agency
Organization Abbreviations

ORD, Office of Research and Development

ORD Laboratories and Centers:

NHEERL – National Health and Environmental Effects Laboratory
NERL – National Exposure Research Laboratory
NCEA – National Center for Environmental Assessment
NRMRL – National Risk Management Research Laboratory
NCER – National Center for Environmental Research

OW, Office of Water

OW Offices:

OGWDW – Office of Ground Water and Drinking Water
OST – Office of Science and Technology
TSC – Technical Support Center

The U.S. Environmental Protection Agency's Research on Microorganisms in Drinking Water Workshop

Marriott Kingsgate Conference Hotel
151 Goodman Drive
Cincinnati, OH 45219

August 5-7, 2003

POSTER TITLES AND SESSIONS

Poster Session I: Tuesday, August 5, 4:15 – 5:45 p.m.
Poster Session II: Wednesday, August 6, 12:50 – 2:20 p.m.
All posters will be set up during both poster sessions.

Poster Session	Topic Area 1: Research Supporting Office of Water's Ground Water/Source Water Regulatory Activities
I	Microbial Drinking Water Contaminants: Endemic and Epidemic Waterborne Gastrointestinal Disease Risks in the United States Presented by **Rebecca L. Calderon**, NHEERL
II	Evaluating Microbial Indicators and Health Risks Associated With Bank Filtration Presented by **Twila Kunde**, Lovelace Clinic Foundation
I	A Prospective Epidemiological Study of Gastrointestinal Health Effects Associated With Consumption of Conventionally Treated Groundwater Presented by **Stuart Hooper**, Emory University
II	Using Neural Networks To Create New Indices and Classification Schemes Presented by **Gail Brion**, University of Kentucky
	Topic Area 2: Research Supporting Office of Water's Contaminant Candidate List (CCL)
	Topic Area 2.2: CCL Viruses
II	Dose-Response Assessments for NLV and Coxsackievirus in Drinking Water (EIMS #22389) Presented by **Brenda Boutin**, NCEA
I	Methods Used To Analyze a Norovirus Outbreak (EIMS #56084) Presented by **Jennifer Cashdollar**, NERL
II	Development of a Molecular Method To Identify Astrovirus in Water (EIMS #56080) Presented by **Ann C. Grimm**, NERL
I	Detection and Occurrence of Human Caliciviruses in Drinking Water Presented by **Gwy-Am Shin**, University of North Carolina at Chapel Hill

Topic Area 2.3: CCL Bacteria

I Sensitivity of Three *Encephalitozoon* Species to Chlorine and Chloramine Treatment as Detected by an *In Vitro* Microwell Plate Assay
Presented by **Cliff Johnson**, NRMRL

II Inactivation of *Aeromonas* by Chlorine and Monochloramine
Presented by **L.A. DeMaria** (**Laura Boczek**), NRMRL

I *Mycobacterium paratuberculosis* and Nontuberculous Mycobacteria in Potable Water (EIMS #18289 & #18341)
Presented by **Terry Covert**, NERL, and **Stacy Pfaller**, NERL

II Detection of *Helicobacter pylori* Using a Highly Variable Locus Upstream of the 16S Ribosomal RNA Gene
Presented by **Manoucher Shahamat**, University of Maryland

I Using Real-Time PCR To Detect Toxigenic Strains of *Microcystis aeruginosa*
Presented by **Carrie Moulton**, Technical Support Center, OW/OGWDW

II Role of Adaptive Response in the Kinetics of *Mycobacterium avium* Inactivation With Monochloramine
Presented by **Benito Mariñas**, University of Illinois at Urbana–Champaign

Topic Area 3: Distribution Systems and Biofilms

II Phylogenetic Analysis of Prokaryotic and Eukaryotic Microorganisms in a Drinking Water Distribution System Simulator
Presented by **Margaret M. Williams**, NRMRL

I Identification and Characterization of *Aeromonas* Isolates From Drinking Water Distribution Systems
Presented by **Jennifer Birkenhauer**, NERL

II Pathogenicity of Biofilm Bacteria (EIMS #18286)
Presented by **Dennis Lye**, NERL

Topic Area 4: Cross-Cutting Research and Emerging Topics

II Effectiveness of UV Irradiation for Pathogen Inactivation in Surface Waters
Presented by **Gwy-Am Shin**, University of North Carolina at Chapel Hill

I Survey of U.S. Public Health Laboratories: Microbial Pathogens on the Candidate Contaminant List (EIMS #54616)
Presented by **Elizabeth D. Hilborn**, NHEERL

II Comparative Diversity of Fecal Bacteria in Agriculturally Significant Animals To Identify Alternative Targets for Microbial Source Tracking
Presented by **Joyce M. Simpson**, NRMRL

I Developing Dynamic Infection Transmission Models for Microbial Risk Assessment (MRA) Applications (EIMS #18473)
Presented by **Pat Murphy**, NCEA

II Virulence Factors of *Aeromonas:* A Molecular Genetic Characterization
Presented by **Keya Sen**, Technical Support Center, Office of Ground Water and Drinking Water

I Effects of pH and Temperature on the Kinetics of *Aeromonas hydrophila* Inactivation With Combined Chlorine
Presented by **Benito Mariñas**, University of Illinois at Urbana–Champaign

The U.S. Environmental Protection Agency's Research on Microorganisms in Drinking Water Workshop

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August 5-7, 2003

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Appendix 1: Presentations of Regional Research Needs and Office of Water Regulatory Activities and Research Needs

Regional Concerns for Microoganisms in Drinking Water

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Regions Mostly Need Help With Applications

- The here and now of regional operations offices day-to-day activities require
  - Information to prioritize work
  - Tools to make decisions
  - Tools for regulatory monitoring and compliance
- Some of these are being worked on, some are not

Information to Prioritize Work

- Regions and State counterparts are swamped
  - Have to aggressively prioritize
  - Ask: What is the magnitude of the problem to be solved? (that is, beyond the political...)
- For microbial pathogens, mostly epidemiology
  - What is the extent of microbial disease from drinking water?
  - Do we have a microbial problem on beaches?

Microbial Epidemiology Questions for Drinking Water

- Do we have a national public health problem from undisinfected wells?
  - >50% of public wells not disinfected
  - But, is there a problem?
- Is there remaining microbial disease from treated water?
- Answers useful for reg implementation

Microbial Epidemiology Questions for Beaches

- What is the extent of waterborne illness from bathing exposures?
  - Is freshwater exposure different from marine?
  - Can we confirm the significance of bather-to-bather contamination?
  - Are some exposure situations more problematic?
- Are the current beach criteria accurate?

Microbial Tools to Make Decisions

- Drinking water decisions primarily involve determining "fecal contamination"
- Beach decisions need information on sources of fecal contamination
  - Is it pathogenic to humans?
  - Or, is it "false positive" and non-pathogenic?

Drinking Water Tools

- Need confidence in vigor of current fecal indicators
  - Do they adequately represent range of pathogens? (apparently, no)
  - Need an approach that is more definitive
- Need suitable surrogates to determine adequacy of disinfection treatment
  - Chlorine, UV, ozone, etc

Beach Microbial Needs

- Biggest problem is to determine when a bathing beach may be contaminated with microbials pathogenic to humans
- Current fecal indicators can be positive for apparently non-pathogenic situations
  - Birds versus people
- Indicators may not match risk

Monitoring and Compliance Needs

- Cheaper
- Easier
- More definitive
- For drinking water, *Cryptosporidium parvum*
- For bathing beaches, approach for human pathogens.

SDWA Requirements & Microbial Research Needs (Surface Water, Ground Water, & Distribution Systems)

Stig Regli
OGWDW/USEPA
8/5/2003

EPA Regulation Setting Requirements Under SDWA

- Must publish MCLGs for contaminants that
  - may have adverse health effects
  - occur in public water systems at frequencies & levels of public health concern
  - provide meaningful opportunity for health risk reduction for persons served by PWS
- MCLGs shall be set at levels at which no known health effects occur and which allows an adequate margin of safety

EPA Requirements Under SDWA (cont'd)

- Must promulgate MCLs as close to the MCLG as is "feasible"
  - "feasible" means with use of "best available technology" (taking costs into consideration)
- **Must promulgate treatment technique requirement if not economically or technically feasible to monitor**
- Must perform regulatory impact analysis (RIA) for each regulation

SDWA - Risk Assessment

- For each regulation specify to extent practicable:
  - Estimates of public health effects for each population
  - Expected, upper, and lower bound risk estimates for each population
  - Each significant uncertainty identified in risk assessment
  - Peer reviewed studies that support estimates above & methodologies used to reconcile data inconsistencies

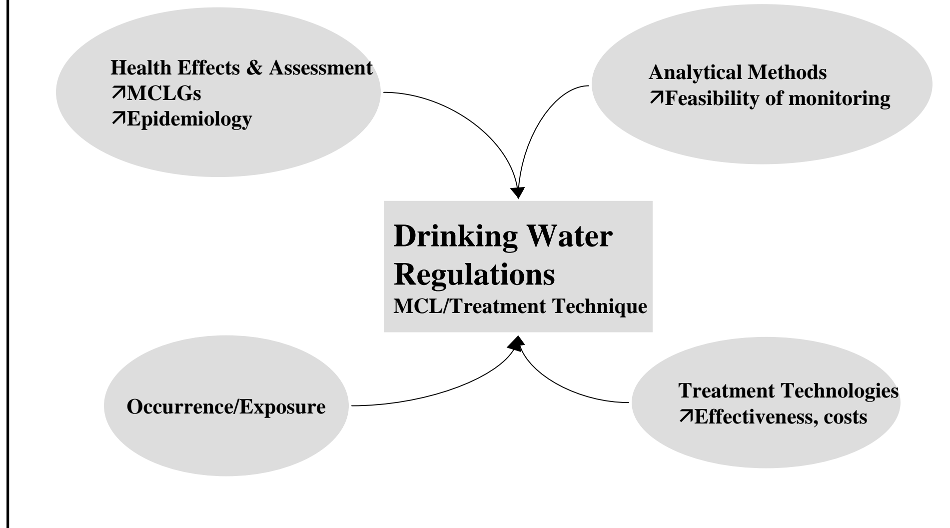
SDWA – Health Risk Reduction & Cost Analysis (HERCA)

- Quantifiable & nonquanitifiable reduction of health risk
  - Above also for reductions in co-occurring contaminants
- Quantifiable and nonquantifiable costs
- Incremental costs & benefits associated with each alternative standard
- Effects of contaminant on general population and groups within general population
  - Children, pregnant women, elderly, individuals with serious illness, or subpopulations at greater risk
- Any increase in health risk that may occur

SDWA – Feasible technologies

- List feasible technologies, treatment techniques, and other means for achieving compliance
- List any technology, treatment technique, or other means affordable for small systems:
  - >3300 to 10,000
  - >500 to 3300 people
  - >25 to 500 people

Research Inputs into DW Regulations



Long Term 2 Enhanced Surface Water Treatment Rule (LT2)

- SDWA requires EPA to promulgate LT2 with Stage 2 Disinfection Byproducts Rule
- Goal: provide equivalent level of protection for all systems using surface water
- Covers: 5500 systems, 174 million people
- Status: propose 2003, promulgate 2004

LT2 Components

- Systems monitor Cryptosporidium in source water to determine if more treatment is needed
  - Small systems monitor *E.coli;* if levels are low they can avoid monitoring Cryptosporidium
- Tool box of treatment options to achieve different Cryptosporidium removal credits
- Unfiltered systems must provide at least 2 log inactivation of Cryptosporidium
- Finished water reservoirs must be covered or disinfected (4 log virus inactivation)
- Source water treatment level reevaluated six years after first round of monitoring

LT2 Research Issues

- What treatment strategies are available for small systems & how can they be evaluated?
- What are appropriate indicators to determine source water pathogen risk?
- What are appropriate indicators for assessing effectiveness of surface water treatment?
- What proportion of the total waterborne pathogen risk is linked to source/treatment issues?
- Does control for Giardia & Cryptospridium adequately control for other pathogens?

Ground Water Rule (GWR)

- SDWA requires EPA to promulgate GWR before Stage 2 DBPR promulgation
- GWR Goal: identify GW systems vulnerable to fecal contamination & require remedial action for such systems
- Covers: 154,000 systems serving 118 million people
- Status: proposed FRN 5/10/00, promulgate 03/04

GWR Components

- Periodic sanitary surveys
- Hydrogeologic sensitivity assessments
- Source water monitoring (E.coli, enterococci, or coliphage) for systems if:
  - Sensitive hydrogeologic setting
  - Total coliform hit in distribution system
- Corrective action if significant deficiency or fecal indicator is positive
- Compliance monitoring for disinfected systems (show $\geq$ 4 log virus inactivation)

GWR Issues

- What are the most appropriate indicator(s) for vulnerability to fecal contamination?
- What tools are available to make indicator monitoring more cost effective (e.g, micro arrays, molecular techniques)?
- How does the sensitivity of naturally occurring vs. lab-adapted viruses to different disinfectants compare?
- What proportion of the total waterborne pathogen risk is linked to source/treatment related GW issues?

Revised TCR & Distribution System (DS) Requirements

- SDWA requires 6 year review of all rules including TCR
- TCR revision to be coupled with development of DS requirements
- DS Goal: protect public health from distribution system contamination
- Status: now in problem definition phase; proposed FRN anticipated 2006

DS Issues

- How do we assess public health risk associated with: cross connections, intrusion, contamination following repair or replacement, biofilms, nitrification, uncovered storage & water age?
- Which DS deficiencies pose greatest risk to public health?
- What are appropriate indicators of DS deficiencies?
- How effective is current technology for reducing the most important potential DS health risks?
- What proportion of the total waterborne pathogen risk is linked to distribution system issues?

TCR Issues

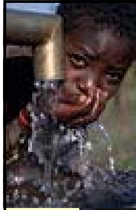
- What are the most appropriate monitoring strategies for routine monitoring? After a TC-positive?
  - Location, frequency, sample volume
- What control and prevention strategies are effective?
- What are the most appropriate approaches for indicating distribution system risk?
  - Microbes: TC, E.coli, etc.
  - Non-microbial: hydraulics, disinfectant residual, etc.?

National Estimate

- SDWA requires EPA & CDC to develop national estimate of waterborne disease
- Status: Approaches for generating estimate are in development (OGWDW, ORD, CDC)
  - FRN indicating estimates & uncertainties using different approaches to be published 2004
  - Much more data will be needed to address uncertainty of estimates (beyond 2004)

National Estimate – Issues

- What percent of national incidence of GI illness is associated with drinking water?
  - Which estimation methodologies are most reliable?
- What percent of waterborne illness is associated with source/treatment issues versus DS issues?
- Can ongoing estimates of national drinking waterborne disease be used as a benchmark for evaluating benefits of drinking water regulations?



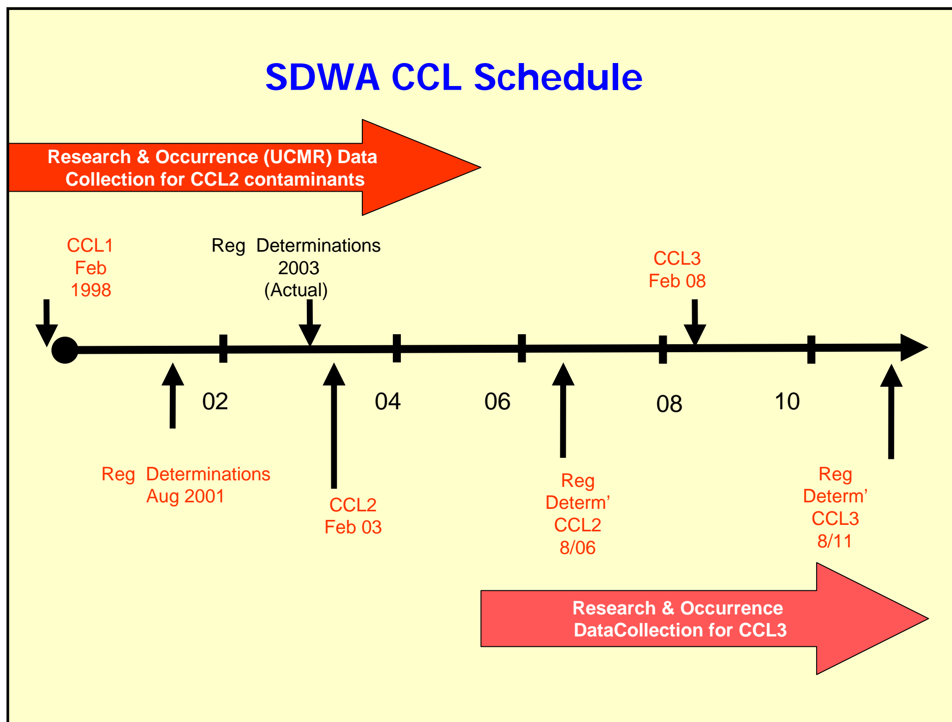
The Contaminant Candidate List: Determining the Need for Future Drinking Water Standards

US Environmental Protection Agency Research on Microorganisms in Drinking Water Workshop
August 5-7, 2003

Tom Carpenter
CCL Team Lead
Office of Ground Water and Drinking Water

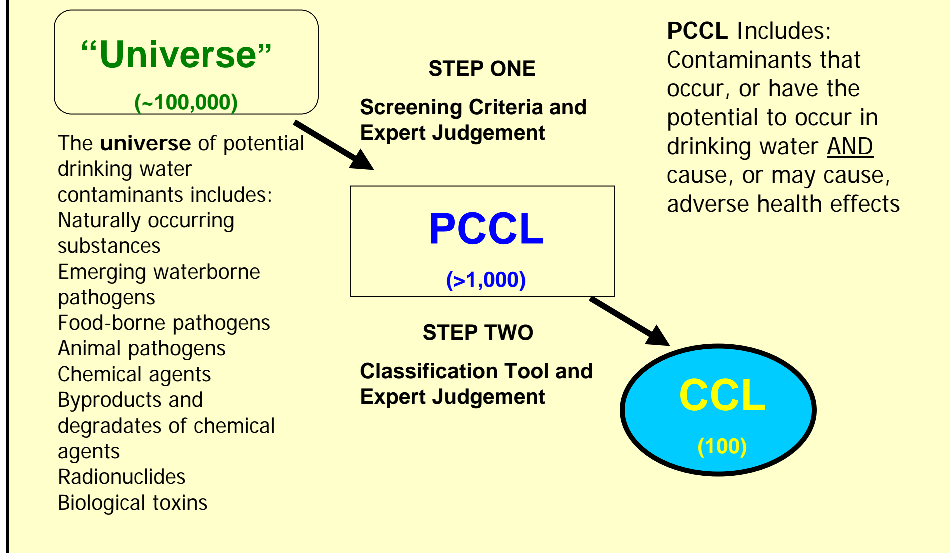
Overview of Presentation

- Statutory Requirements for the CCL
- NRC recommendations for future CCLs
- NDWAC Schedule
- Overview of the Methods and Issues
- Efforts Underway



Future CCL Development

- **Same SDWA requirements as CCL1 and similar components needed to develop future CCLs**
  - Develop list
  - Initiate the regulatory determination process
- **National Academy of Sciences Panel Reports**
  - Last report of trilogy recommends strategies for future CCLs
- **Evaluate NRC Report "Classifying Drinking Water Contaminants for Regulatory Consideration" Provides Recommendations**
  - Extensive process for identifying and narrowing contaminant universe
  - Recommendations for data quantity and quality
  - Significant quantitative aspect
  - Validation of recommended approach using case example



NRC Recommendations (cont'd)

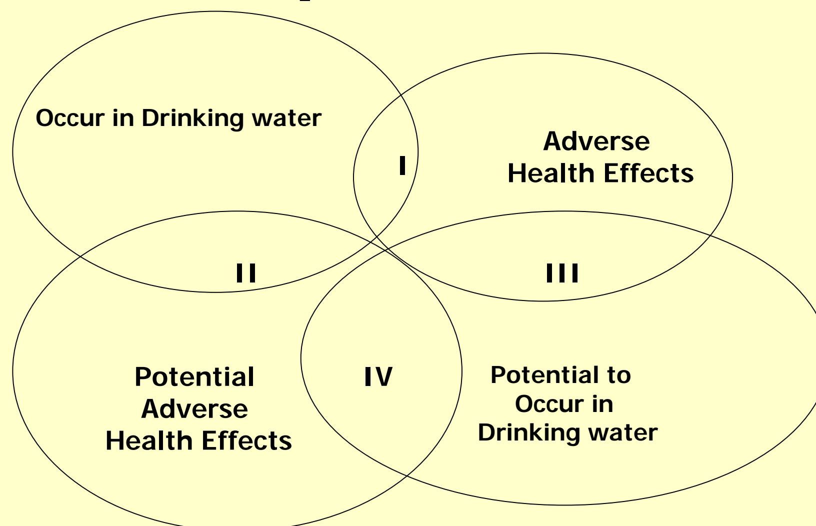
- **Strongly recommends a classification approach that should not sacrifice complexity for transparency**
  - allows for complex decision process that scores and weights classification attributes of contaminants based on pattern recognition
  - calibrate and validate using existing contaminants as training sets
- **Evaluate new molecular/genetic methods to identify new/emerging microbiological contaminants as part of new approach**
  - base evaluation of microbes on similarities of virulence, physical, and/or genetic attributes (Virulence Factor Activity Relationships)
  - relies on new genomic and molecular analytical methods and indicators
  - VFAR is long-term goal -- need to identify interim products as proof of concept

Methodology Issues NDWAC is discussing

- Prototype Classification Approach
  - How did NRC arrive at this approach
    - evaluation of several prioritization approaches
    - common characteristics were selection of contaminant pool, determination of exposure and toxicity, what was the prioritization method
    - most examples used for chemicals not/pathogen
  - NRC Panel started at the beginning
    - had limited resources
    - wrestled with how the attributes of exposure and toxicity inter-relate to one another
    - NRC recommendations are not a complete road map, there is more work to do

Methodology issues discussions (cont'd)

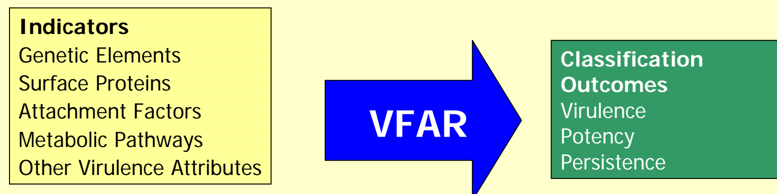
The universe of potential contaminants



Methodology issues discussions (cont'd)

- The universe to the PCCL
  - Intersection of the major characteristics identify contaminants to carry to the PCCL
  - Seek expert judgement on the screening process
  - Screening criteria need to be connected to the data sources
  - Identify data elements to capture and compile the universe from these evaluations
  - Develop screening criteria
    - NDWAC discussing guidelines and concept
    - generate from the data sources to identify potential and known criteria
    - apply "automated" screening

Virulence Factor Activity Relationships



- Classifies pathogens
- Will not be fully developed for CCL2
- Relies on molecular technologies and gene sequencing
- Research and analytical capabilities are improving
- EPA research efforts are underway for microbes (i.e., *Aeromonas*)
- NRC strongly recommends interagency participation (e.g. participation in National Science and Technology Council's Biotechnology Research Group)
- Next steps are to identify and coordinate range of research needs

Methodology issues discussions (cont'd)

- VFARs: A new process for pathogens
  - Genomics and proteomics are rapidly emerging technologies and should provide new indicators
  - Identify pilot and prototype projects
    - Literature reviews, State of the science, available data sources
    - Develop model systems to test "virulence" of the potential pathogens
    - Develop interagency partnerships
    - Identify/develop/modify analytical methods for VFAR indicators

NDWAC Charge

- Discuss, evaluate, and provide advice on methodologies, activities, and analysis needed to implement the NRCs recommendations on an expanded approach for the CCL listing process. This may include advice on:
  - an overall implementation strategy
  - classification attributes and criteria
  - pilot projects to validate new classification approaches
  - proof of concept activities to support VFAR analysis
  - communication issues
  - additional issues not addressed in the NRC report

CCL NDWAC Work Group

- **Request NDWAC advice to assist EPA in developing methodologies that can be used for future CCLs**
- **NDWAC Work Group with 3 Activity Groups**
  - CCL may need parallel paths for pathogens and chemical contaminants
  - One microbiological/pathogen and one chemical subworkgroup
  - Both technical activity group should include classification/information technology expertise
- **Convened Work Group September 2002**
- **6 plenary meetings to date**
- **2 remaining meetings through the Fall of 2003**

Novel approach: comparative genetics

Identify virulence genes from genomes by location

- Many virulence-associated genes cluster together (pathogenicity islands)
- Genes flanking virulence genes may be co-expressed and have related functions www.tigr.org select genome browser

Identify virulence genes from genomes by expression

- Transcriptosomes (grouping of genes according to their transcription regulation patterns) may identify virulence-associated genes

Identify virulence genes from genomes by primary sequence

- GC-content, flanking IS sequences or repeats, can identify recently acquired genes. Surface-exposed genes are generally more AT-rich which makes them prone to mutation www.cbs.dtu.dk/services/GenomeAtlas

Identify virulence genes by multiple sequences

- substitution rates (Ka/Ks) identify genes under strong selection
- polymorphisms within (surface expressed) genes indicate avoidance of immune response

Microarrays: promises, outcome

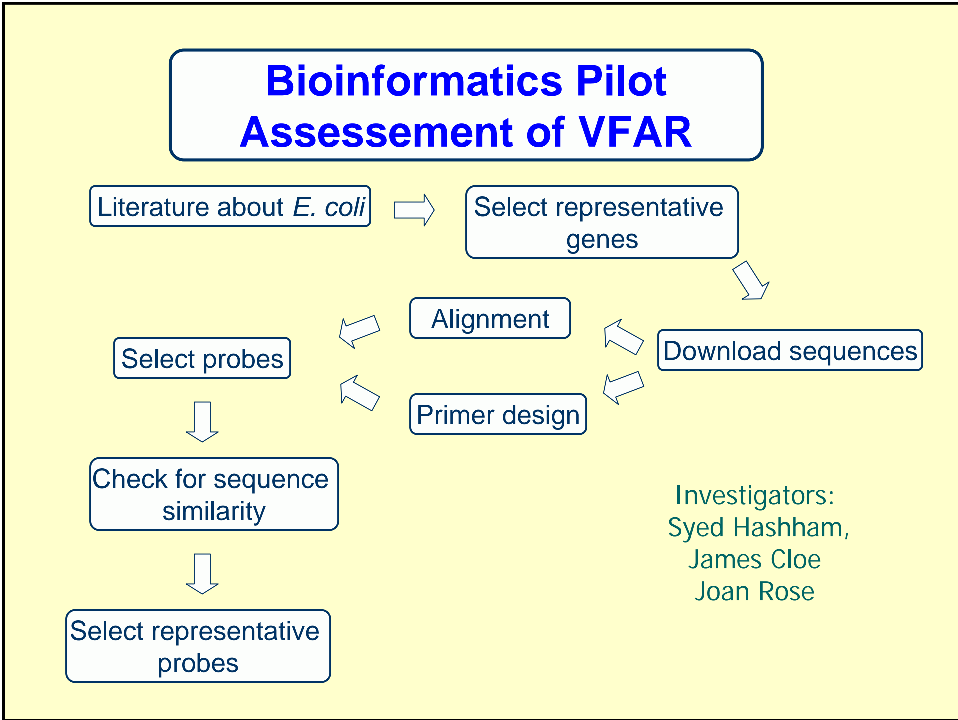
"Applications of functional genomics of food microorganisms: novel risk assessment procedures" (Curr. Opin.Biotech. 1999, 10:511)

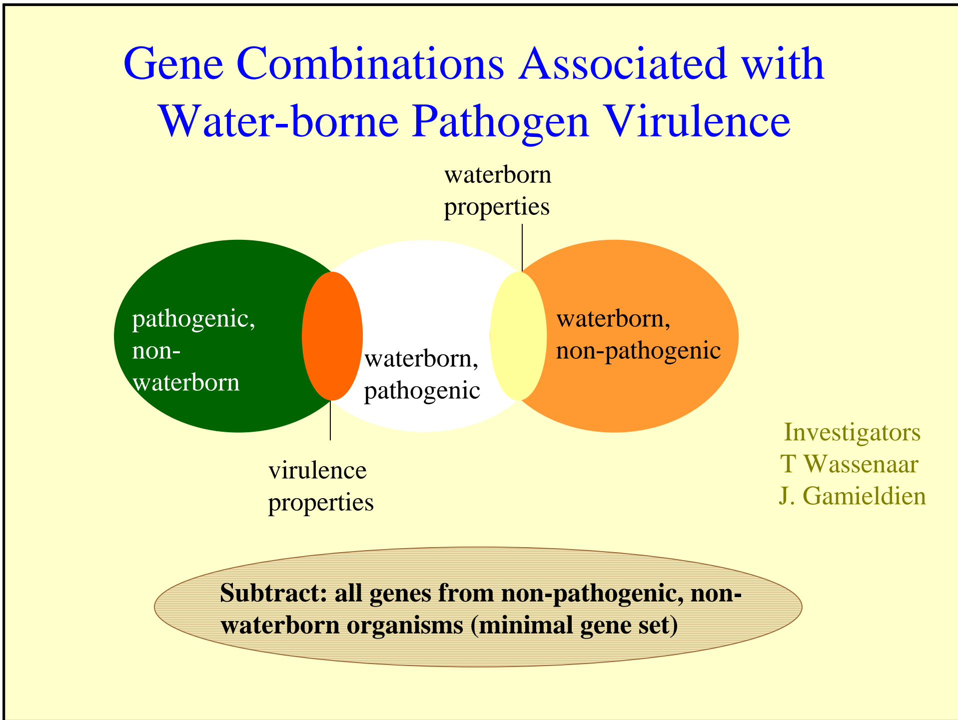
Applications of MA to food pathogens (published as of May 2002)

Detection/ differentiation	•detection of bacterial virulence genes by microarray performs better than PCR •direct detection of *E. coli* on chicken carcasses •differentiate *Staph.* spp. by low-density microarray of PCR products
Diversity/ conservation	•identification of diversity in gene content of *C. jejuni* •identification of homologs conserved within pathogenic *Salmonellas*
Gene regulation	•gene regulation under Fe-limitation (in *Pasteurella*) proves complex and pleiotrophic. How to differentiate primary from secondary, down-stream effects? •gene expression during acid-adaptation of *E. coli* was studied •*sarA* and *agr* of *S. aureus* regulate known virulence genes, also many others

Genomic Data Searches VFAR Discovery Phases

- Phase I September-October, 2002
  - Limited virulence factor keyword search of GenBank
  - Basic Local Alignment Search Tool (BLAST) alignments
- Phase II October-November, 2002
  - Comprehensive keyword search of GenBank
  - Comparison of other available genomic databases
- Phase III November-December 2002
  - Keyword search of whole genomes
  - Selective virulence factor sequence alignments against other whole genomes
  - BLAST alignment of complete virus genomes


Bioinformatics Pilot
Assessement of VFAR
Literature about E. coli
Select representative genes
Download sequences
Alignment
Primer design
Select probes
Check for sequence similarity
Select representative probes
Investigators:
Syed Hashham,
James Cloe
Joan Rose


Gene Combinations Associated with Water-borne Pathogen Virulence
waterborn properties
pathogenic, non-waterborn
waterborn, pathogenic
waterborn, non-pathogenic
virulence properties
Investigators
T Wassenaar
J. Gamieldien
Subtract: all genes from non-pathogenic, non-waterborn organisms (minimal gene set)

For Additional Information or Questions

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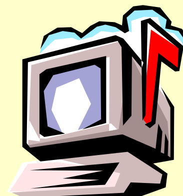

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Appendix 2: Additional NCER STAR Drinking Water Grant Microbial Research

Experimental Infection of Healthy Adults with a *Cryptosporidium* Genotype 1 Isolate (TU502)

Cynthia Chappell[1], P. Okhuysen[2], R. Langer[1], D. Akiyoshi[3], and S. Tzipori[3]
[1]Center for Infectious Diseases, The University of Texas Health Science Center at Houston, School of Public Health, Houston, TX; [2]Department of Internal Medicine, The University of Texas Health Science Center at Houston Medical School, Houston, TX; [3]Division of Infectious Diseases, School of Veterinary Medicine, Tufts University, North Grofton, MA

Cryptosporidium parvum causes diarrheal illness worldwide. Molecular studies have identified two distinct genotypes with different transmission cycles. Genotype 1 (G1) strains are primarily a human-to-human transmission, and genotype 2 (G2) strains are zoonotic. Previous dose-response studies in healthy adults employed five genotype 2 isolates, which varied widely in infectivity, yielding ID_{50}'s between 9 and 1042 oocysts. This study is the first report of experimental G1 infections in healthy adults. The G1 isolate (TU502) used in this study originated from a human case and was amplified in gnotobiotic piglets. A single dose (10, 30, 100, or 500 oocysts) of TU502 was administered to 16 volunteers, which were monitored for 6 weeks. Results showed that the TU502 ID_{50} was similar to the most infectious of the G2 isolates. The onset of diarrhea and oocyst shedding following TU502 challenge were similar to the G2 isolates; however, the duration of diarrhea and oocyst shedding showed important differences. The typical 4-7 days of diarrhea seen with G2 isolates was prolonged in TU502 volunteers, lasting up to 22 days. Further, 83 percent of volunteers challenged with the G2 isolates cleared their oocysts by $\leq$14 days as compared to 60 percent of volunteers receiving TU502. Two subjects shed for 24 and 35 days, respectively. Total oocysts shed per person ranged from 5×10^6 to 1×10^{10}, the latter occurring in the volunteer with the longest episode of diarrhea and oocyst shedding. These data suggest that the G1 isolate, TU502, was highly infectious in healthy adults and was associated with a longer diarrheal illness followed by an extended period of oocyst shedding. These characteristics suggest a high risk of infection from environmental sources and a risk of secondary transmission from contact with symptomatic and asymptomatic oocyst shedders. These findings are consistent with the high proportion of G1 isolates associated with outbreaks of human cryptosporidiosis. This work was supported, in part, by EPA STAR Grant #R-82918001 and NIH GCRC Grant #RR-02558.

Experimental Challenge of Healthy Adult Volunteers With *Cryptosporidium muris* Oocysts

Cynthia Chappell[1], P. Okhuysen[2], R. Langer[1], and S. Tzipori[3]
[1]Center for Infectious Diseases, The University of Texas Health Science Center at Houston, School of Public Health, Houston, TX; [2]Department of Internal Medicine, The University of Texas Health Science Center at Houston Medical School, Houston, TX; [3]Division of Infectious Diseases, School of Veterinary Medicine, Tufts University, North Grofton, MA

Cryptosporidium muris has long thought to be a pathogen of animals, but not humans. However, a recent study indicated that infections with *C. muris* might occasionally occur in immunocompromised persons. In addition, probable *C. muris* cases were reported in two children but were not confirmed. Detection of cases is complicated by the fact that monoclonal antibodies commonly used in *Cryptosporidium* assays do not recognize *C. muris* and might yield false negative results. The purpose of this study was to determine if *C. muris* oocysts are infectious for healthy adults. Six serologically negative, healthy volunteers were challenged with 10^5 *C. muris* oocysts and monitored for infection and illness for a minimum of 6 weeks. All six volunteers became infected, but only one developed a diarrheal illness, which lasted for 4 days. In contrast, previous studies have shown that *C. parvum* isolates were associated with illness attack rates of 52-86 percent. Interestingly, the duration of *C. muris* oocyst shedding was longer (40-45 days or more) than with *C. parvum* (3-12 days, depending on the isolate tested). In some volunteers, the study period was extended due to continued oocyst shedding. These data indicate that healthy adults are susceptible to infection with *C. muris* oocysts and in some cases (17 percent) might experience a diarrheal illness. Further, the longer period of oocyst shedding might be important for secondary transmission. These findings support the need for dose-response studies to more fully describe the risk of *C. muris* infectivity and illness in the community setting.

Appendix 3: STAR Grant Presentation Abstracts and Agenda From the USEPA/USGS Meeting on *Cryptosporidium* Removal by Bank Filtration, September 9-10, 2003

William Blanford

(Abstract Not Provided)

Study of Particle and Pathogen Removal During Bank Filtration of River Waters

Edward J. Bouwer[1], Charles R. O'Melia[1], W. Joshua Weiss[1], Kellogg J. Schwab[2], Binh T. Le[2], and Ramon Aboytes[3]
[1]Johns Hopkins University, Baltimore, MD; [2]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD; [3]American Water Belleville Laboratory, Belleville, IL

Project Goals and Objectives

The overall objective of this research project is to evaluate the merits of riverbank filtration (RBF) for removing/controlling pathogens in drinking water and to investigate the potential for using removal of particles and other water quality indicators as surrogates for pathogen removal in riverbank systems.

Approach

This research consists of: (1) field studies to document actual changes in pathogen and particle concentrations from rivers of similar source quality in the context of variations in subsurface travel distances, pumping rates, porous medium properties, and residence times; and (2) parallel laboratory column studies with aquifer media to provide insights into process mechanisms and the relationship between pathogens and potential surrogate parameters upon transport through riverbank media under a variety of physical and chemical conditions.

Preliminary Findings

Field monitoring results from the three study sites indicate that RBF serves as a consistently significant barrier to the transport of microorganisms from the river water sources (see Table 1). *Cryptosporidium* oocysts were detected in 11 out of 16 sampling rounds in the Ohio River, 12 out of 16 sampling rounds in the Wabash River, and 8 out of 16 sampling rounds in the Missouri River, but never in any of the corresponding well waters. Similarly, *Giardia* cysts were detected in 4 of the sampling rounds in the Ohio River, 2 sampling rounds in the Wabash River, and 3 sampling rounds in the Missouri River, but never in any of the corresponding well waters. With the exception of *Bacillus* and bacteriophage ϕX174, all of the monitored organisms were always below the detection limit in the well waters, with corresponding reductions of average concentrations ranging from 0.8 logs to more than 6 logs.

Preliminary design of column experiments has been accomplished. Riverbed sediment was collected from the Potomac River in western Maryland to serve as representative riverbank media for the column studies. The sediment was dried and sieved to remove fine and coarse material but otherwise was not manipulated or cleaned prior to packing into glass columns. Preliminary experiments used 2.5 cm diameter, 30 cm long columns. Breakthrough curves for bacteriophage MS2, bacteriophage PRD1, and Polio virus indicate that under several ionic strength conditions, polio virus is removed by a substantially greater extent than MS2, suggesting that the phage might be useful as a conservative indicator for the transport of the human virus through riverbank media.

Significance of Findings

The field monitoring data support the use of RBF at controlling the transport of potentially harmful microorganisms from river water sources. Reductions in average bacteria concentrations are often well in excess of 2 logs. This is significant because bacteria have been proposed as potential surrogate parameters for the protozoans (which are more difficult to measure accurately in the field due to their low and variable concentrations in natural systems).

Next Steps

Future column studies are intended to examine the relationship between the protozoans and the other microbes in aquifer media, as well as other potential surrogate/indicator parameters (including latex microspheres, natural river water particles, and turbidity measurements).

Table 1. Field monitoring results: January 2002 through July 2003 (log removals given in brackets). Averages were calculated as the sum of counts divided by the sum of volumes sampled over all sampling rounds; detection limit was calculated as 1 divided by the sum of the volumes.

Ohio River	8.7×10^{4}	7.6×10^{2}	1.3×10^{6}	1.5×10^{4}	4.6×10^{1}	1.7×10^{3}	2.7×10^{-2}	7.3×10^{-2}
Well #9	1.7×10^{2} [2.7]	<1.1 [>2.8]	<5.0 [>5.4]	$<1.0 \times 10^{1}$ [>3.2]	<1.1 [>1.6]	1.1 [3.2]	$<1.2 \times 10^{-3}$ [>1.3]	$<1.2 \times 10^{-3}$ [>1.8]
Well #2	8.0×10^{2} [2.0]	<2.0 [>2.6]	<5.0 [>5.4]	$<1.0 \times 10^{1}$ [>3.2]	<2.0 [>1.4]	<2.0 [>2.9]	$<2.4 \times 10^{-3}$ [>1.0]	$<2.4 \times 10^{-3}$ [>1.5]
Wabash River	2.8×10^{5}	2.2×10^{3}	6.1×10^{6}	5.7×10^{5}	3.6×10^{1}	2.4×10^{3}	1.7×10^{-2}	1.0×10^{-1}
Collector Well	3.5×10^{3} [1.9]	<1.1 [>3.3]	<5.0 [>6.1]	<5.0 [>5.1]	<1.1 [>1.5]	<1.1 [>3.3]	$<1.3 \times 10^{-3}$ [>1.1]	$<1.3 \times 10^{-3}$ [>1.9]
Well #3	$<2.0 \times 10^{2}$ [>3.2]	<2.0 [>3.0]	<5.0 [>6.1]	<5.0 [>5.1]	<2.0 [>1.3]	<2.0 [>3.1]	$<2.5 \times 10^{-3}$ [>0.8]	$<2.5 \times 10^{-3}$ [>1.6]
Missouri River	4.2×10^{5}	8.9×10^{2}	6.1×10^{5}	3.1×10^{4}	3.4×10^{1}	2.6×10^{3}	2.4×10^{-2}	6.3×10^{-2}
Well #4	6.3×10^{4} [0.8]	<1.1 [2.9]	$<1.0 \times 10^{1}$ [>4.8]	$<1.0 \times 10^{1}$ [>3.5]	<1.1 [>1.5]	<1.1 [>3.4]	$<1.1 \times 10^{-3}$ [>1.3]	$<1.1 \times 10^{-3}$ [>1.8]
Well #5	1.1×10^{3} [2.6]	<2.5 [>2.6]	$<1.0 \times 10^{1}$ [>4.8]	$<1.0 \times 10^{1}$ [>3.5]	<2.5 [>1.1]	<2.5 [>3.0]	$<2.5 \times 10^{-3}$ [>1.0]	$<2.5 \times 10^{-3}$ [>1.4]

Evaluating Microbial Indicators and Health Risks Associated With Bank Filtration

Floyd J. Frost
Lovelace Clinic Foundation, Albuquerque, NM

The purpose of the proposed project is to compare serological responses to *Cryptosporidium* antigens in users of bank-filtered water (one community with only bank filtration and disinfection and one community with bank filtration, conventional filtration, and disinfection) with the responses of similar people residing in an area that uses disinfected but unfiltered high-quality groundwater. The hypothesis is that, if bank filtration completely removes *Cryptosporidium* oocysts, the serological responses of the three populations should be similar. The specific goals of the study are to: (1) identify approaches to collecting sera from similar populations in different geographic locations so that rates of serological responses can be compared; (2) pilot test the approach in three different geographical locations by collecting sera from cities that use bank filtration and nearby cities that use high-quality groundwater for a drinking water source; (3) analyze the sera for serological responses to *Cryptosporidium* and *Giardia* antigens and compare the frequency and intensity of responses between the bank filtration cities and the groundwater cities; and (4) compare serological responses in the same cities at times when bank filtration efficacy is likely to be optimal and when it is likely to be least effective.

Sera from 50 people from each of three communities (users of bank filtered and chlorinated, bank filtered plus direct filtration plus ozonation, and chlorinated groundwater) will be collected at baseline and at five followup blood draws. A questionnaire on risk factors will be collected at each blood draw. Sera will be tested for the presence of antibody responses to two *Cryptosporidium* antigens (15/17-kDa and 27-kDa) and for serological changes (seroconversion). The baseline level of serological responses as well as the rates of seroconversion will be compared for each population (50 baseline and 250 periods for estimating rates of seroconversion). Comparisons will adjust for collected risk factor data from each individual. For purposes of extrapolating these results to other locations, a series of source and finished water quality indicators will be measured for each water source.

No results are available at this time. Analysis of sera will take place once all sera are collected. Then, all sera from a subject will be run on the same Western blot to reduce variations between blots. Blood draws will continue every 4 months. Data entry protocols will be developed and implemented for data entry of the questionnaires. Sample analysis will commence once all of the samples are collected, because the analysis of each subject's samples will be on the same Western blot. The distribution systems analysis also will commence.

This abstract also was presented as a poster at the Research on Microorganisms in Drinking Water Workshop in Cincinnati, OH, August 5-7, 2003.

Application of a Multipath Microsphere Tracer Test To Understanding Transport of Bacteria and Protozoa at a Bank Filtration Site

Rick Langford[1], Dirk Schulze-Makuch[1], and Suresh Pillai[2]
[1]University of Texas, El Paso, TX; [2]Texas A&M University, Kingsville, TX

The objective of this research study is to determine whether bank filtration is effective in removing microbial pathogens in an arid environment. The study site uses the Rio Grande that experiences significant annual fluctuations in both water quantity and quality. A well-characterized site with numerous monitoring wells has been established. The pumping well is 17 m from the stream bank. The water table during the experiment was 2 m below the land and the stream surface. The aquifer is composed of medium and fine-grained sand comprising two flow units. Observation wells are screened over 1 or 1.5 m intervals. The average hydraulic conductivity was about 2×10^{-3} m/s based on a test analysis. However, the responses indicated that sediment heterogeneities affected the hydraulic behavior at the field site.

A 427-hour tracer test using bromide and fluorescent microspheres provides initial results that are relevant to the transport of pathogens through the subsurface under riverbank filtration conditions. Bromide was injected into an observation well at the channel margin. Differently colored fluorescent microspheres (0.25 μm, 1 μm, 6 μm, and 10 μm) were injected into the stream bottom and into two observation wells. Conclusions from the tracer test include the following:

- Both bromide and microspheres continued to be observed throughout the 18 days of the experiment.
- The bromide recovery in the pumping well and in the deeper observation wells showed early and late peaks with long tails indicating that the geological medium at the field site behaves like a double-porosity medium, allowing the tracer to move relatively quickly through the higher conductivity units while being significantly retarded in the low hydraulic conductivity units.
- Some wells showed consistently higher concentrations of bromide.
- The 1 μm micospheres were abundant in the observation wells and allowed tracing of flowpaths. These showed multiple peaks similar to the bromide results. This indicates highly preferential transport paths in the sediment.
- Microspheres from the three injection sites had distinctly different transport paths and rates.
- Both bromide and microspheres appeared in the stream soon after injection, moving apparently against a 2 m head difference.
- The 6 μm and 10 μm microspheres were observed in low concentrations and were episodically detected in the stream and in two widely spaced observation wells.

The significance of these results is that:

- Inorganic microspheres might mimic the episodic occurrence of microorganisms in wells.
- Even in this relatively homogeneous aquifer, preferential transport within the aquifer results in highly divergent transport paths and rates. Microspheres from one of the injection sites traveled essentially perpendicular to the expected transport direction.
- Even small variations in the sand grain size can effectively compartmentalize the aquifer.

The next steps of this project will include field studies to observe the migration and persistence of selected organisms (*Escherichia coli*, enterococci, coliphages, cysts, oocysts, and enteroviruses) in the pumping well and observation wells under different pumping rates. Continued combined chemical sampling, along with the microbial sampling, will document whether the changes in water chemistry alter the behavior of the organisms.

Pathogenic Microbe Removal During Riverbank Filtration

Joseph N. Ryan[1], Yumiko Abe[1], Rula Abu-Dalo[1], Menachem Elimelech[2], Garrett Miller[2], Zachary Kuznar[2], Ronald W. Harvey[3] and David W. Metge[3]
[1]University of Colorado at Boulder, Boulder, CO; [2]Yale University, New Haven, CT;
[3]U.S. Geological Survey, Reston, VA

Project Goals and Objectives

Our incomplete understanding of processes and properties affecting the transport of pathogenic microbe transport during riverbank filtration is currently limiting our ability to predict the effectiveness of this water treatment option. We are conducting a series of fundamental experiments designed to better understand the effects of microbe size, physical and geochemical heterogeneity of the porous media, and high pumping rates on the transport of *Cryptosporidium parvum* oocysts in alluvial valley aquifers used for riverbank filtration.

Our major objective for this research is to develop a model of oocyst transport in porous media that can accommodate the physical and geochemical heterogeneity present in alluvial valley aquifer used for riverbank filtration. Our goal is that this model can be used to predict the oocyst removal during riverbank filtration. To do this, we are in the process of providing: (1) improved characterization of the properties of *C. parvum* oocysts related to transport in porous media; (2) improved understanding of the mechanisms of oocyst removal in porous media; (3) including special features of riverbank filtration in alluvial valley aquifers; and (4) incorporating the improved characterization and mechanistic understanding into a two-dimensional model of microbe transport during riverbank filtration.

Approach

The experiments are being conducted in a stagnation point flow apparatus, flow-through columns, an intermediate-scale two-dimensional aquifer tank (5 m length, 0.5 m height, 10 cm width). The stagnation point flow experiments are being used to examine the effects of the surface charge heterogeneity and the DLVO secondary minimum on oocyst attachment and release. The flow-through column experiments are being used to explore the effects of grain-scale heterogeneities on oocyst transport and to provide data for modeling of the intermediate-scale tank experiments. The tank experiments are being used to examine random physical and geochemical heterogeneities above the grain scale. The porous media being used in the column and tank experiments are designed to simulate the complex and variable stratigraphies and geochemical gradients encountered in alluvial valley aquifers.

The following tasks are being conducted to achieve the project objectives and goals: (1) stagnation point flow and column experiments to test the effect of microbe size on attachment to porous media, velocity enhancement, and straining; (2) column and tank experiments to test the effect of grain size on attachment to porous media, velocity enhancement, and straining; (3) stagnation point flow, column, and tank experiments to test the effect of geochemical heterogeneity on attachment to porous media; and (4) stagnation point flow and column experiments to test the effect of flow rate on oocyst deposition in the DLVO secondary minimum and release.

Preliminary Findings

We have conducted column experiments and a tank experiment examining the effects of physical and geochemical heterogeneity on oocyst transport. The tank was filled with a heterogeneous porous medium consisting of sands of 5 grain sizes and 12 ferric oxyhydroxide surface coverages. Oocyst transport in each of the porous media was tested in column experiments. Oocysts (formalin-inactivated) and polystyrene latex microspheres (4.6 μm diameter) were injected into the tank and monitored over 5 days. Physical heterogeneity (the difference in hydraulic conductivity as a result of grain size) was much more important than geochemical het-

erogeneity in controlling oocyst transport. Microspheres broke through at the same time as the oocysts, but the microspheres were removed much more rapidly than the oocysts.

In column experiments, we showed that straining is contributing to the removal of oocysts in fine-grained sands. Straining was demonstrated by comparing the transport of polystyrene latex microspheres (0.32 to 4.1 μm) to oocyst transport. Removal was consistent for microspheres from 0.32 to 1.9 μm and increased significantly for 4.1 μm microspheres (see Figure 1).

Stagnation point flow experiments have been conducted to explore the effect of ionic strength on oocyst deposition. The dynamics of oocyst deposition in these experiments—the oocysts come into contact with the glass deposition surface, but do not remain in the position of first contact—clearly indicate that the oocysts are depositing in the secondary minimum of the DLVO potential energy profile.

Significance of Findings

The physical and geochemical heterogeneity experiments show that oocyst transport modeling can focus on variations in the hydraulic conductivity of porous media. Geochemical heterogeneity is far less important because oocysts deposit equally well on unfavorable and favorable surfaces. The straining and secondary minimum experiments show that: (1) straining must be considered in oocyst transport modeling; (2) oocyst deposition depends on solution ionic strength; and (3) oocyst deposition is reversible.

Next Steps

The next steps in this research project will focus on column experiments to explore the effects of microbe size, grain size, and flow rate on oocyst deposition, release, and velocity enhancement.

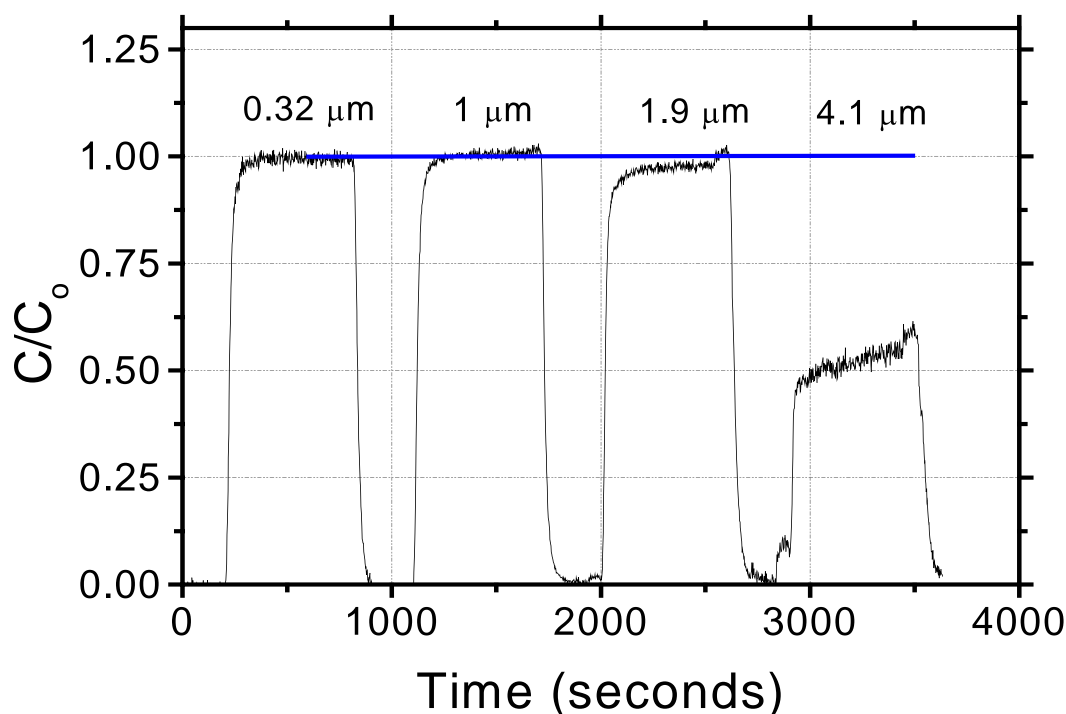

Figure 1. Breakthrough of carboxyl-modified polystyrene latex microspheres through a column filled with quartz sand (d_{50} of 0.210 mm) as a function of time. Microsphere concentration normalized to the influent microsphere concentration (C/C_0). Diameters of the microspheres shown above the breakthrough curves. The experiments were conducted at a pH of 5.6-5.8, an ionic strength of 1 mM, and a flow rate of 2 mL min^{-1}.

NCER Calendar of Events

September 2003

Title: The U.S. Environmental Protection Agency / U.S. Geological Survey Meeting on *Cryptosporidium* Removal by Bank Filtration

Date: Tuesday, September 9 - Wednesday, September 10, 2003

Location: U.S. Geological Survey National Center
Main Auditorium
12210 Sunrise Valley Drive
Reston, VA 20192

See **Logistics** below for information regarding **hotel reservations** at the Sheraton Reston Hotel, Reston, VA.
Please make your reservations by **Wednesday, August 27, 2003.**

Contact: Angela Page, (202) 564-5172 or page.angelad@epa.gov
Philip Berger (202) 564-5255 or berger.philip@epa.gov and
Tina Conley (202) 564-3209 or conley.tina@epa.gov

Purpose: The U.S. Environmental Protection Agency (EPA) Office of Research and Development, National Center for Environmental Research (NCER) and the EPA's Office of Water together with the U.S. Geological Survey (USGS) are sponsoring a meeting to discuss the research being conducted on *cryptosporidium* removal by bank filtration. The **USEPA/USGS Meeting on *Cryptosporidium* Removal by Bank Filtration** will be held on September 9-10, 2003 at the USGS facility in Reston, VA. The meeting will consist of a series of plenary sessions where EPA, NCER's Science To Achieve Results (STAR) grantees, USGS, U.S. Department of Agriculture, university and state researchers will present their research. This public meeting is open to all who are interested in hearing about the research in this exciting area.

Please mark your calendars now and make your hotel reservations by August 27.

Registration: Fill out the Registration Form at:
http://www.scgcorp.com/epachildhealth2003/registration.asphttp://www.scgcorp.com/USEPA_USGS/registration.asp

The U.S. Environmental Protection Agency/U.S. Geological Survey Meeting on *Cryptosporidium* Removal by Bank Filtration

U.S. Geological Survey National Center
12201 Sunrise Valley Drive
Reston, VA 20192

September 9-10, 2003

AGENDA

Tuesday, September 9, 2003

10:00 – 10:15 a.m. Welcome
James LaBaugh, USGS Office of Ground Water

10:15 – 10:30 a.m. Overview of the U.S. EPA's Office of Research and Development and The Science To Achieve Results (STAR) Program
Cynthia Nolt-Helms, EPA, Office of Research and Development

10:30 – 10:45 a.m. Overview Presentation From the U.S. EPA's Office of Ground Water and Drinking Water
Dan Schmelling, EPA, Office of Ground Water and Drinking Water

10:45 – 11:00 a.m. Overview of the U.S. EPA's Water Security Program
Regan Murray, EPA, Homeland Security Research Center

11:00 – 2:00 p.m. **Field Studies of *Cryptosporidium*, Surrogate and Indicator Transport in Saturated Porous Media**
Moderator: **Glenn Patterson**, USGS, Reston, VA

11:00 – 11:25 a.m. Initial Results From the Rio Grande Bank Filtration Site
Presented by **Rick Langford**, University of Texas, El Paso, TX, STAR Grant

11:25 – 11:50 a.m. Using Riverbank Filtration To Improve Water Quality
Presented by **Ed Bouwer**, The Johns Hopkins University, Baltimore, MD, STAR Grant

11:50 – 12:50 p.m. **Lunch**

12:50 – 1:15 p.m. Bank Filtration Studies at the City of Lincoln, Nebraska Wellfield
Research conducted by **Jason Vogel**, USGS; Presented by **Philip Berger,** EPA, Washington, DC

1:15 – 1:40 p.m. Assessment of the Microbial Removal Capabilities of Riverbank Filtration
Presented by **Robin Collins**, New England Water Treatment Technology Assistance Center at the University of New Hampshire, Durham, NH, Funded by EPA

1:40 – 2:00 p.m. **General Discussion**

2:00 – 2:15 p.m. **Break**

2:15 – 3:45 p.m. **Laboratory and Simulation Studies of *Cryptosporidium*, Surrogate and Indicator Transport in Saturated Porous Media**
Moderator: **Ingrid Verstraeten**, USGS, Baltimore, MD

2:15 – 2:40 p.m. Effect of Heterogeneity on Transport of *Cryptosporidium parvum* in Saturated Porous Media
Presented by **Joe Ryan**, University of Colorado, Boulder, CO, STAR Grant

2:40 – 3:05 p.m. *Cryptosporidium* Transport in Porous Media
Presented by **Scott Bradford**, USDA Salinity Laboratory, Riverside, CA

3:05 – 3:30 p.m. Streamline-Based Simulation of *Cryptosporidium* Transport in Riverbank Filtration
Presented by **Reed Maxwell**, Lawrence Livermore National Laboratory, Livermore, CA

3:30 – 3:45 p.m. **General Discussion**

3:45 – 4:00 p.m. **Break**

4:00 – 5:00 p.m. **Estimating *Cryptosporidium* Removal and Health Effects**
Moderator: **John Grace**, Maryland Department of the Environment, Baltimore, MD

4:00 – 4:25 p.m. *Cryptosporidium* Removal at the Louisville, Kentucky Wellfield
Presented by **Steve Hubbs**, Louisville Water Company, Louisville, KY

4:25 – 4:50 p.m. Serological Monitoring of Pathogen Occurrence
Presented by **Floyd Frost**, Lovelace Respiratory Research Institute, Albuquerque, NM, STAR Grant

4:50 – 5:00 p.m. **General Discussion**

5:00 p.m. **Adjournment**

Wednesday, September 10, 2003

9:00 – 11:45 a.m. **Ground Water Flow, Heat Flow, and Environmental Tracer Studies at Bank Filtration Sites**
Moderator: **Tom Grubbs**, EPA, Washington, DC

9:00 – 9:25 a.m. Infiltration Rate Variability and Ground Water Flow at the Cincinnati Wellfield
Presented by **Bill Gollnitz**, Greater Cincinnati Water Works, Cincinnati, OH

9:25 – 9:50 a.m. Application of Different Tracers To Evaluate the Flow Regime at Riverbank Filtration Sites in Berlin Germany
Presented by **Gudrun Massmann**, Free University of Berlin, Berlin, Germany

9:50 – 10:15 a.m. Diatom Proteins as a Surface Water Indicator in Ground Water
Presented by **Tim Reilly**, USGS, Trenton, NJ

10:15 – 10:30 a.m. **Break**

10:30 – 10:55 a.m. Inclined Well Studies at the Cincinnati Wellfield
Presented by **Bruce Whitteberry**, Greater Cincinnati Water Works, Cincinnati, OH

10:55 – 11:20 a.m. Heat as a Tracer at Sonoma County Bank Filtration Site
Presented by **Jim Constantz**, USGS, Menlo Park, CA (via PlaceWare)

11:20 – 11:45 a.m. **General Discussion**

11:45 – 12:45 p.m. **Lunch**

12:45 – 3:55 p.m.

Moderator: **Mike Finn**, EPA, Washington, DC

12:45 – 1:10 p.m. *Cryptosporidium* Transport in Soil-Aquifer Treatment
Presented by **William Blanford**, Louisiana State University, Baton Rouge, LA, STAR Grant (to University of Arizona)

1:10 – 1:35 p.m. *Cryptosporidium* Transport in Unsaturated Flow
Presented by **Christophe Darnault**, Environmental Engineering &
Technology, Inc., Newport News, VA

1:35 – 2:00 p.m. Comparison of Batch and Flow Experimental Data on Retention of Manure-Borne *Cryptosporidium parvum* Oocysts in Soils
Presented by **Yakov Pachepsky**, USDA, Beltsville, MD

2:00– 2:15 p.m. **Break**

2:15 – 2:40 p.m. Release of *Cryptosporidium* and *Giardia* Dairy Manure Due to Flowing Water
Presented by **Scott Bradford**, USDA Salinity Laboratory, Riverside, CA

2:40 – 3:05 p.m. Unsaturated Zone Processes in the Sonoma County Recharge Basins
Presented by **Jim Constantz**, USGS, Menlo Park, CA (via PlaceWare)

3:05 – 3:30 p.m. One-Dimensional Variably Saturated Microbial Transport Simulations
Presented by **Bart Faulkner**, EPA, Ada, OK

	3:30 – 3:55 p.m.	**General Discussion**
3:55 – 4:10 p.m.	**Break**	
4:10 – 4:50 p.m.	***Cryptosporidium* Removal by Bank Filtration Summary, Regulations and Caveats** Moderator: **Ronald Fayer**, U.S. Department of Agriculture, Beltsville, MD	
	4:10 – 4:35 p.m.	*Cryptosporidium* Removal by Bank Filtration Summary, Regulations, and Caveats Presented by **Philip Berger**, EPA, Washington, DC
	4:35 – 4:50 p.m.	**General Discussion and Closing Remarks**
4:50 p.m.	**Adjournment**	