MICROBIAL RISK ASSESSMENT FOR RECLAIMED WATER

FINAL REPORT

May 10, 1995

EOA, Inc.

Eisenberg, Olivieri, & Associates
Environmental and Public Health Engineering
1410 Jackson Street
Oakland, CA 94612

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Eisenberg, Olivieri, & Associates
Environmental and Public Health Engineering

May 10, 1995

Mr. Kenneth A. Thompson
Manager of Water Quality
Irvine Ranch Water District
P.O. Box 57000
Irvine, California 92619-7000

Subject: Microbial Risk Assessment for Reclaimed Water

Dear Ken:

Attached is the final report for the microbial risk assessment for reclaimed water project. We have enjoyed working with you on this project. If you have any questions, please feel free to call.

Very truly yours,
EOA, Inc.

Jonathan I. Konnan, M.S.
Senior Engineer

Adam W. Olivieri, Dr.P.H., P.E.
Principal Engineer

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1410 Jackson Street • Oakland, CA 94612 • (510) 832-2852 • Fax 832-2856

MICROBIAL RISK ASSESSMENT FOR RECLAIMED WATER

Prepared for:
Irvine Ranch Water District
Irvine, California

Prepared by:
EOA, Inc.,
in Association with
the University of California
School of Public Health

May 10, 1995

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- Irvine Ranch Water District
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PROJECT TEAM

Adam W. Olivieri, Dr.P.H., P.E.
Joseph Eisenberg, Ph.D.
Jonathan I. Konnan, M.S.
Edmund Seto, M.S.

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Microbiologists	Robert C. Cooper, Ph.D. (Principal Investigator)	Emeritus Professor, University of California at Berkeley, School of Public Health
	Rick E. Danielson, Ph.D.	California Department of Health Services
Environmental Engineers	James Crook, Ph.D., P.E.	Black and Veatch
	Robert Spear, Ph.D. (Project Technical Reviewer)	Professor, University of California at Berkeley, School of Public Health
Epidemiologist	Herschel E. Griffin, M.D.	San Diego State University, Graduate School of Public Health
Virologists	Edward Lennette, Ph.D., M.D.	California Public Health Foundation
	John Riggs, Ph.D.	BioVir Laboratories
Infectious Disease Physician	Jack Colford, M.D., M.P.H.	University of California at San Francisco, Veterans' Affairs Medical Center

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EXECUTIVE SUMMARY

The Irvine Ranch Water District retained EOA, Inc., in association with the University of California School of Public Health, to explore new approaches to microbial risk assessment applicable to assessing the population risks of ingestion of reclaimed water. The general technical approach proposed for development in this project was based on earlier work by project investigators relating to the risk of infectious waterborne disease for the U.S. Army. To supplement the experience and expertise of the project team, a Technical Advisory Committee was established to provide input on all phases of the project.

The primary objectives of the project were to: (1) develop an epidemiologically based risk assessment model that allows for the assessment of the public health risk of exposure via ingestion to microbial agents associated with water recycling projects; (2) conduct a formal analysis of uncertainty of key model parameters; and (3) estimate the level of microbial risk associated with several combinations of recycled water treatment methods and uses.

A principal failing of much of the earlier work in this area was its neglect of population-related aspects of risk, e.g., immune status and community-specific exposure factors. To address these issues from the outset, a risk assessment model was developed that was based closely on models used in infectious disease epidemiology. The great advantage of this form of model is that it can be used to integrate and organize the diverse data bearing on disease risk, account for immunity to disease, model aspects of the transmission dynamics of the agent in the environment, and explicitly acknowledge the uncertainty and variability in the many parameter values characteristic of comprehensive models.

The uncertain and variable nature of environmental systems such as those modeled in this study makes an absolute assessment of risk questionable. Therefore, a comparative risk approach was developed in which a background simulation scenario was analyzed for each pathogen under study. The risk associated with each of several alternatives for reclaimed water use was then compared with a background prevalence level. All subsequent uncertainty analysis was performed by assessing whether or not a simulation produced an output above or below a mean background level plus one standard deviation.

Unfortunately, in many biological models there is sufficient uncertainty in parameter values to make the selection of any particular parameter set about which to conduct a typical sensitivity analysis a questionable procedure. The technique know as regional sensitivity analysis (RSA) was utilized to overcome the short comings of the typical sensitivity analysis approach. RSA involves describing the uncertainty or variability in each model parameter by a statistical distribution function and then, within the structure of the risk model, use the set of parameter distributions to define the system under study. The strength of this type of analysis is that it overtly acknowledges both uncertainty and variability in the parameter values and avoids the pitfalls of worst-case analysis.

Giardia lamblia was selected as the agent to consider in detail because the literature was more extensive than for any of the other agents reviewed. In particular, there were reports which provided data on the non-outbreak background incidence of giardiasis in a

community setting which allowed a "calibration" of some of the biological parameters of the model. RSA was used to establish a set of "valid" parameters that reproduced non-outbreak conditions from routine surveillance data.

This information was then applied to investigate the risk associated with ingestion of reclaimed water containing *Giardia* while swimming in an impoundment. As in all of the studies carried out under this project, computer simulation was used to calculate the disease risk for defined ranges of parameter uncertainty and variability. In the *Giardia* case study, the simulations forecast significantly higher prevalence of giardiasis in a community with a public swimming impoundment than in a community without such an impoundment. Filling the impoundment with tertiary treated wastewater did not increase mean prevalence levels above those obtained when the impoundment was filled with water from a pathogen-free source. However, the predicted disease prevalence is highly variable under both scenarios due to the uncertainty and variability of the model's parameter values and this may have masked differences in predicted public health impact. The results of this simulation study of a hypothetical closed community point towards two findings: 1) Shedding by swimmers may be an important source of pathogens in swimming areas, and 2) The levels of uncertainty and variability inherent in the system preclude a conclusive assessment of the importance of shedding by swimmers relative to treated wastewater in producing a given level of pathogen exposure. Results from RSA suggest, in ranking order, which parameters contribute to this uncertainty. The uncertainty in the risk was driven principally by uncertainties and variabilities in the biological parameters rather than the exposure-related parameters. For example, uncertainties and/or variability in the rate of shedding of pathogen by infected swimmers highly influenced the risk of disease as did the rate at which diseased individuals return to the non-infectious state.

Including calibrating the model for background prevalence, a total of nine exposure scenarios (including seven water reclamation alternatives) were studied as summarized in Figure ES-1. Even in the presence of substantial uncertainty, it can be seen that, in the case of *Giardia*, exposures in restricted recreational impoundments and from industrial cooling towers result in public health risks indistinguishable from background levels. It should be noted that the prevalences calculated are case-specific and, while the information found in the literature relating to the values of the biological parameters can be used in more general analyses, the exposure-related parameters must be selected for specific sites, populations and water reuse applications.

A significant advantage of the type of model used in this study is that it can track exposure events, like treatment plant failures, that produce outbreaks of infections and disease that vary over time. While the *Giardia* study did not overtly utilize this feature, the model was also applied in this dynamic mode to an outbreak of enteric disease, now attributed to *Cryptosporidium,* which occurred in Milwaukee in 1993. In contrast to the risk assessment mode of the *Giardia* analysis, the *Cryptosporidium* study focused on understanding the risk-related factors central to the Milwaukee outbreak in mechanistic terms that might inform future control or water treatment strategies.

The same approach to the analysis was applied to *Cryptosporidium* as for *Giardia* in which only parameter sets consistent with the background prevalence of the disease were used

in attempting to simulate the outbreak conditions. Simulations of the outbreak scenario were then performed. Only 124 cases were found out of 500,000 simulations that met the outbreak definition. The fact that so few cases were found suggests that conditions governing which parameter set combinations are consistent with the outbreak scenario, as defined by our initial parameter ranges and output criteria for the outbreak, are highly constraining. Therefore much information is contained in these 124 parameter sets. Based on univariate statistical analyses, three parameters were highly constrained within their sampled range, two associated with the concentration of oocysts in the treated water and a third which relates to the baseline transmission rate. Six additional biological parameters were important to reconstruct the outbreak pattern. These results are very informative in that they contain considerable information about both the exposure intensity and the population distribution of various biological parameters that underlie the Milwaukee outbreak. The results should be subjected to a more sophisticated multivariate statistical analysis which has the potential to identify interactions between the biological and exposure-related parameters and thereby add significantly to the current understanding of the circumstances contributing to this epidemic of waterborne disease.

A literature review was performed for seven microorganisms, in addition to *Giardia* and *Cryptosporidium*, with the objective of determining if the modeling approach could be easily extended to other organisms and exposure situations. Six of the microorganisms appeared suitable and were selected for the risk assessment which included the nine exposure scenarios used in the *Giardia* analysis. This literature review provided the data to assign parameter ranges for both the biological and the exposure alternatives. A general observation was that the rate at which diseased individuals return to the non-infectious state, σ, followed by T_E, the fraction of pathogen remaining after water treatment, were most often identified as important determinants of disease risk for all the microorganism and water reclamation alternative combinations.

The original motivation for this project arose from a need to improve methods for carrying out risk assessments for waterborne pathogens by providing a modeling approach that integrates public health information and data in a way that acknowledges the specifics of the situation in particular communities. The analysis requires an explicit statement of what is known about the infectious disease processes involved, the size of the population at risk, and the site-specific details of the exposure scenarios. It became clear only during the course of the project that our use of models whose origins lie in epidemiological analysis did not support the development of simple regulatory procedures which neglect community-specific determinants of risk. This conclusion suggests that the differences in particular community and exposure scenarios are likely to be significant and produce widely varying levels of risk. The costs of risk mitigation are generally community or situation-specific which suggests that the approach to risk assessment applied in this study leads naturally to exploring site-specific strategies for risk management.

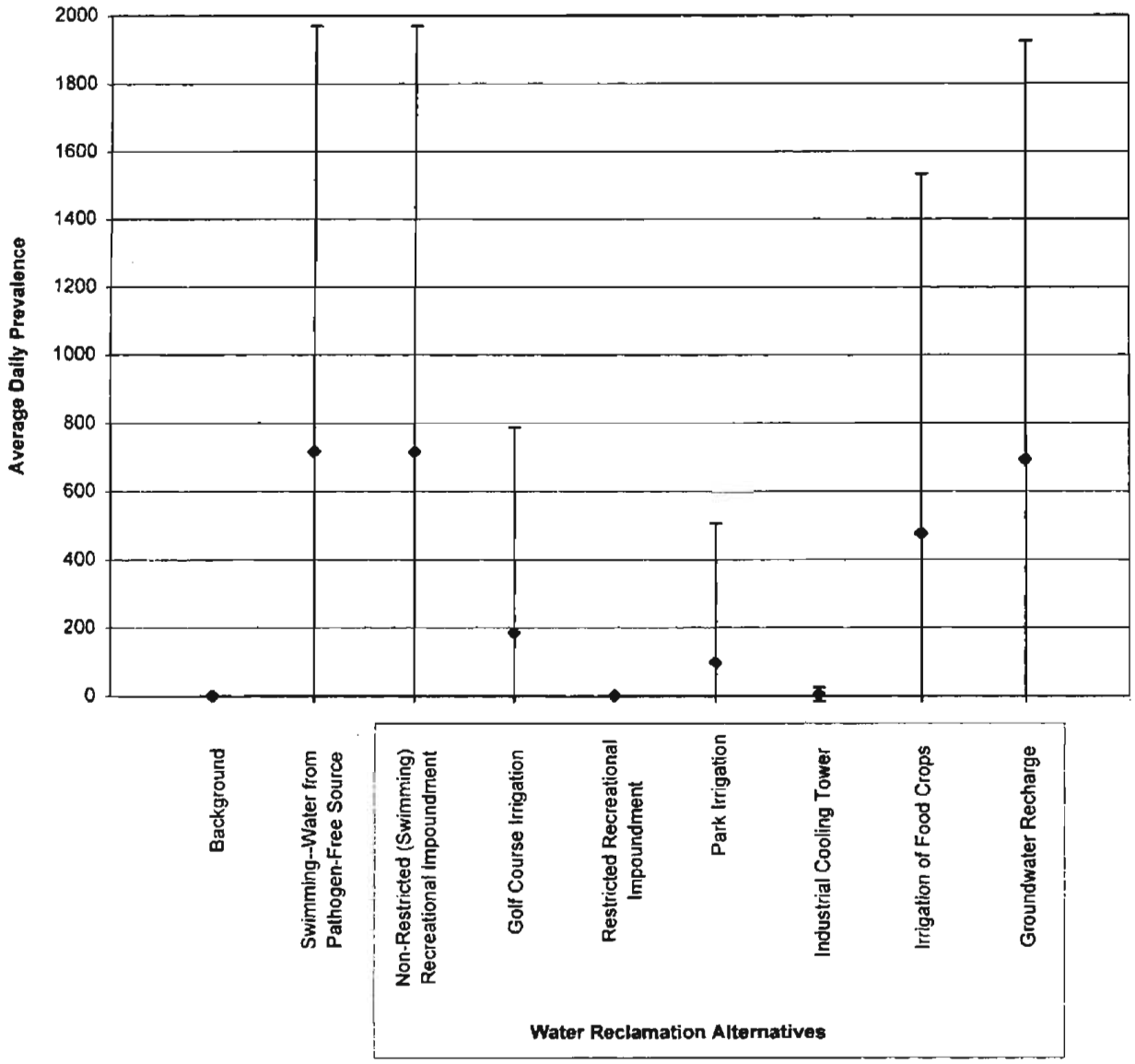
However, a number of generic water reclamation alternatives were evaluated in this study. The results provide a foundation for identifying those parameters that tend to influence the risk assessment results as well as a general sense of the relative relationship and significance, in terms of public health risk, among various alternative uses (i.e., exposure scenarios) and background levels of risk. This information, while not community or

situation-specific, can be useful in generally evaluating the relative degree of public health risk posed by different water reclamation alternatives. From the risk management perspective, we believe that although risk assessment modeling using generic or site-specific input data can be valuable in providing general information pertaining to risk, the results should not be considered definitive. Risk assessment modeling should instead be considered one of many tools available to policy makers in the decision making process.

While this project has been successful in showing the feasibility of a new and effective approach to assessing the public health risks of waterborne pathogens, the risk assessment methodology remains in an early stage of development. More work is particularly needed in studying the dynamic properties of these models in the risk assessment application. It is clear that expanding the scope of the model to incorporate seasonal patterns of exposure and the susceptibility status of different groups within the population will be fruitful directions to pursue. In addition, the uncertainty analysis needs to be refined to include nonlinear multivariate techniques, first in the case of *Cryptosporidium*.

Figure ES-1

***Giardia* Average Daily Prevalence**



The figure above shows the results for *Giardia*. 6,000 simulations were performed for each water reclamation alternative. *Giardia* is described in Appendix A, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

1.0 INTRODUCTION

This report documents the work performed and the results of the risk assessment. It is divided into six chapters. Chapter 1.0 is an introduction which includes a brief overview of the risk assessment process and a discussion of past modeling efforts. Chapter 2.0 presents a conceptual description of the new risk assessment model and applies the model to examine the risk associated with ingestion of the protozoan parasite *Giardia lamblia* while swimming in an impoundment filled with reclaimed water. The uncertainties in the process are examined. Chapter 3.0 describes the identification and selection of the microorganisms included in the risk assessment, the literature review performed for these microorganisms, and the water reclamation alternatives selected for modeling and their corresponding exposure assumptions. In Chapter 4.0, the model is applied to an outbreak of cryptosporidiosis associated with inadequate drinking water treatment which occurred in Milwaukee during the spring of 1993. Chapter 5.0 summarizes the results of modeling a number of microorganism and water reclamation alternative scenarios. Chapter 6.0 discusses the overall results and identifies areas where further research is needed.

1.1 Motivation for Study

There is continual interest in the use of reclaimed water in California and other regions of the United States and the world. The extended California drought dramatized the need for reuse in urban areas and it is becoming clear that, even without a drought, population pressures are such that reclaimed water will be an important water supply adjunct. With this interest in the use of reclaimed water, the effectiveness of the water treatment process, including disinfection of pathogenic agents, has become a central public health issue. Regulators in charge of monitoring the prevalence of infectious diseases within the human population are in constant need of a way to estimate the risk of waterborne disease transmission. This need has become more acute with the increased use of reclaimed water.

As current exposures to environmental pathogens are often quite low, field epidemiology can no longer produce sufficiently sensitive information for assessing these risks. An alternative to the epidemiological approach is the quantitative estimation of the intensity of human exposure and the probability of human response from this exposure. This approach is highly developed in assessing cancer risks arising from environmental exposures to chemical agents, and has resulted in a field of study called quantitative risk assessment.

Because environmental risk assessment is subject to a variety of uncertainties, the process is often cast in probabilistic terms. Moreover, field data are frequently unavailable to quantify some elements of the process, and mathematical modeling is used to bridge these data gaps. The principal advantage of mathematical modeling in risk assessment applications is that it makes assumptions explicit, including structural mechanisms relating human exposure to pathogens and the public health outcome, and quantitative assumptions such as water treatment efficacy and the dose-response relationship. A mathematical model organizes data and assumptions in a framework leading to quantitative predictions and can be an indispensable tool for decision making. However, the model itself brings no new data or information to the process. Thus the biological significance of a model's output is completely dependent on the appropriateness and accuracy of the assumptions used to build the model.

1.2 Previous Related Work

Attempts to provide a quantitative framework for the assessment of human health risks associated with the ingestion of waterborne pathogens have generally focussed on the

probability of individual infection or disease as a result of a single exposure event. Most models described in the literature are of the same generic form.[1-4] They give a point estimate of the probability of a particular exposure leading to infection or disease in a single individual and, except for Dudley's work[1], carry little or no information about the uncertainty or variability in this estimate. Much of quantitative risk assessment, in particular, focuses on a point estimate of the response probability, often using worst-case assumptions for exposure and other parameters. From a public health perspective, the probable number of people infected in an exposed population is more meaningful than the probability of individual infection. In the past, the probability of individual infection (using worst-case assumptions) has sometimes been multiplied by the population number in an attempt to predict the disease incidence in the population. This may lead to unrealistically high risk forecasts.

The project team took a somewhat different point of view in a risk analysis of waterborne disease carried out for the U.S. Army.[5-7] In this work a population perspective was taken and the analysis was carried beyond the risk of infection to an individual by estimating the probability distribution of the number of infected/diseased people in the exposed population. One feature of the Army model was its probabilistic treatment of dose-response data (i.e., data which provide a quantitative linkage between the number of organisms ingested and the probability of infection or overt disease). From this model's population perspective, each member of the population received a different dose and also had a different probability of responding to this dose. The combination of these two factors resulted in each member of the population carrying a different probability of becoming infected or diseased.

1.3 New Risk Assessment Model

In general, the above models assume that the populations are homogeneous and the disease transmission processes static. In designing the new risk assessment model for this project, we took advantage of a large literature describing the use of deterministic and stochastic dynamic population models in the study of epidemics.[8] These epidemiological models emphasize the importance of the changing immune status of a population over time and are therefore dynamic, requiring a subdivision of the population by susceptibility status. This work motivated the development of an epidemiological risk assessment model that accounts for immunity and the transmission dynamics of the system.

One central issue in biological risk assessment is how to extract information from biological data, which tends to be highly uncertain and variable. In particular, the uncertainty and variability of factors affecting infectious disease transmission limit the usefulness of traditional curve-fitting techniques. An alternative goodness-of-fit procedure that explicitly acknowledged these uncertainties and variabilities was therefore used. The approach consisted of assigning probability distributions to each parameter, sampling these distributions during Monte Carlo simulations, and using a binary classification to assess the output of each simulation.

As a case study, we chose *Giardia lamblia* as the pathogen, and swimming in an impoundment filled with reclaimed water as the exposure scenario. A simulation study in which the model was used as a comparative analysis tool was designed. Three transmission scenarios were compared to analyze the relative risk of contracting giardiasis while swimming. The first scenario, based on a Vermont study,[9] described a situation in which reclaimed water was not the exposure vehicle. The results were used to establish a baseline prevalence with which to compare the effects of the next two scenarios. The second scenario was swimming in an impoundment supplied with reclaimed water from a pathogen-free source but includes shedding of pathogens by the swimmers, and the third

explored the additional effect of filling the impoundment with water reclaimed from the wastewater of the community.

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2.0 CASE STUDY - THE RISK TO HUMANS FROM *GIARDIA LAMBLIA* IN A RECREATIONAL SWIMMING IMPOUNDMENT

To evaluate the utility of the risk assessment methodology, we chose a specific application of the risk assessment model for in-depth exploration. For this application, *Giardia lamblia* was chosen as the pathogen because of its public health importance and because it is one of the more researched waterborne pathogens. The water reclamation alternative chosen, a swimming impoundment, was also selected because of its public health importance and to allow exploration of the effect of swimmers shedding pathogen at the exposure site.

2.1 Conceptual Description of Model

The structure of the model is illustrated in Figure 2.1. The model is composed of five state variables and 14 parameters, as summarized in Table 2.1. Four of the state variables represent the human population, which is divided into four epidemiological groups:

X - susceptible individuals
Y - infectious/asymptomatic individuals
Z - non-infectious/asymptomatic individuals
D - infectious/symptomatic individuals

Individuals in state X are susceptible to infection. For the remaining groups, the terms infectious or non-infectious define whether or not individuals sheds pathogen in their stool, and the terms symptomatic and asymptomatic define whether or not an individual exhibits symptoms of disease. The state variables X, Y, Z and D keep track of the population levels in each group. The remaining state variable, W, keeps track of the concentration of pathogen in the water to which the population is exposed. The movement of individuals from one state to another and the concentration of pathogen are governed by the set of five differential equations shown in Table 2.1.

The rate at which members of the population move from state X to state Y is governed by two factors. One is the background rate of infection, which accounts for non-outbreak transmission due to exposure routes other than ingestion of reclaimed water. The second is a dose-response term specific to the scenario under evaluation, which is dependent on the pathogen concentration in reclaimed water and the amount of water ingested.

Once in state Y, an individual can move in any given time step to either state D or state Z. The rates of these two transitions, represented respectively by the parameters ρ and α, are dependent on each other, i.e., at any given time step an individual in state Y will, with probability of 1, either stay in this state, move to state D, or move to state Z.

Individuals in state D, who show symptoms of disease and shed pathogen, move to state Z at a rate of σ. Individuals in state Z are asymptomatic and do not shed pathogen. The parameter σ is defined as the rate at which symptoms of disease disappear as an individual recovers, i.e., the reciprocal of the duration of symptoms. This definition was chosen because state D is used to calculate average daily prevalence in the population, which is the model output used to assess risk. To minimize the number of state variables, it was assumed that an infectious/symptomatic individual will transition directly to the non-infectious/asymptomatic state.

Individuals in state Z revert back to state X at a rate of γ. By definition, γ is the reciprocal of the period of time for an immune individual to become susceptible, i.e., the rate of immunity loss. Thus it is assumed that non-infectious/asymptomatic individuals in state Z are immune.

In addition to movement of individuals among the epidemiological states, the model also describes the concentration of the waterborne pathogen at the exposure site. The pathogen may arrive at the exposure site in three ways. First, individuals in state Y directly shed pathogen into water in the swimming area of the impoundment during swimming at a rate of λ. Second, individuals in both states Y and D shed into raw wastewater, which is then treated before discharge into the impoundment. And third, the parameter I represents the pathogen loading from raw wastewater that originates from external sources and is also treated prior to discharge into the impoundment.

Assumptions made in designing the model include the following:

- The period of time that an individual is asymptomatic and infectious is short relative to the duration of the symptomatic and infectious period.
- Background disease transmission occurs independently of the water reclamation scenario under study.
- All wastes from all members of the population are sent to the treatment plant.
- Exposure to pathogen occurs via ingestion of reclaimed water containing pathogen.
- The discharge rate of reclaimed water into the impoundment from the treatment plant is equal to the rate of volume loss due to evaporation. (This assumption results in the swimming area having a constant volume, and is valid if the impoundment is used only for recreation and not for other uses, such as drinking water storage).

To describe the 14 model parameters, 26 pieces of data were required. Therefore 26 sampling parameters were established, each with a lower and upper bound selected to account for the variability of the available data found during the literature search performed for this project (Table 2.2). Chapter 3.0 describes the literature search. Appendix A contains a write-up for *Giardia* based on the literature search results and Appendices B through I contain write-ups for other microorganisms selected for the risk assessment (see Chapter 3.0).

The 26 parameters were sampled from uniform distributions, except for values that spanned three or more orders of magnitude, in which case log uniform sampling was used. Table 2.2 lists the 26 sampling parameters and classifies the parameters as biological, community or water treatment-based parameters. Seven of the 14 model parameters are dependent on other sampling parameters. Table 2.3 shows the relationship between the seven dependent parameters and the appropriate sampling parameters.

2.2 Analysis and Simulation Approach

In general, biological systems have large variability due to both genetic differences among individuals and environmental factors that are not explicitly modeled. Standard analytical tools, such as curve-fitting techniques and sensitivity analysis, become less useful when data such as that produced from surveillance of infectious diseases are so variable. Traditionally, a sensitivity analysis procedure involves selecting a point in the parameter space and perturbing the parameter values about this point. Unfortunately, in many biological models there is sufficient uncertainty in parameter values to make the selection of any particular parameter set about which to conduct the sensitivity analysis a questionable procedure. This is particularly true with infectious disease data, which are often hard to quantify. For example, with respect to studies of giardiasis, Veazie et al.[1]

report an average disease duration of 14.8 days with a range of 1-120, Shaw et al.[2] report that in half of the cases the disease lasted seven days and a fourth more than 30 days, and Dykes et al.[3] report a mean of 22 days with a range of 10-40 days. Likewise, prevalence rates range from 2-7% in Western European countries, to 10-13% in Oregon, to 8-40% in South America, the Middle East, and South East Asia. These rates depend not only on the geographical location but also on the methods used to collect the data. To address this problem, a technique termed Regional Sensitivity Analysis (RSA) was used for this project.

RSA involves describing, *a priori*, the uncertainty and variability in each model parameter by a probability distribution function. Multiple simulations called Monte Carlo simulations are run and for each simulation a different set of parameter values is used. The parameter values are chosen by randomly sampling each parameter from its distribution. Assigning a bounded uniform distribution to each parameter allows us to take into account data from various literature sources without bias toward one value or another.

A binary classification algorithm is then applied to each simulation output, in which the simulation output either passes or fails a set of criteria. The multivariate parameter distribution associated with a pass classification can be analyzed through a variety of statistical procedures to assess parameter sensitivity. The binary classification is basically a goodness-of-fit criterion based on whether or not the output is representative of the data. The strength of this approach is that it acknowledges both the uncertainty and variability in parameter values in a structured fashion. The RSA procedure has now been applied to a variety of problems.[4-7]

Due to the nature of this study, the approach used was slightly different from previous applications of RSA. The simulation approach consisted of a three-scenario comparative study, in which the first scenario used the same binary classification scheme as RSA. The remaining scenarios then used the "valid" parameter sets obtained from the first scenario to generate a distribution of prevalence levels.

Specifically, a classification scheme was used to identify the ten parameter values that describe the background scenario of the model, in which exposure to reclaimed water is not the vehicle of disease transmission. Each simulation was classified as acceptable if its output was consistent with available disease incidence data for non-outbreak conditions. For other scenarios, the remaining parameters that describe human exposure to reclaimed water were sampled, combined with the valid parameter sets from the background scenario, and used as a complete model representative of a community using reclaimed water. The outputs generated by running the model with this complete parameter set were statistically analyzed to identify parameters whose values strongly influence the magnitude of risk.

For this study of *Giardia* transmission, surveillance data from non-outbreak conditions in Vermont[8] were used to obtain baseline values for nine of the parameters not associated with reclaimed water transmission. The Vermont study found that between 1983 and 1986 the annual incidence rate was 45 cases per 100,000 per year. Selecting a range around this value, the incidence rate criterion was set at 20 - 60 cases per 100,000 per year. To calculate the incidence rate from the simulation runs, the following equation was used:

$$I = \rho \cdot Y_{365} \cdot 365 / N$$

where I is the annual incidence rate, ρ is the fraction of individuals in state Y who move to state D per day, Y_{365} is the number of individuals in state Y at day 365, and N is the total

population. This equation assumes that the system is at steady state, a good approximation for these non-outbreak simulations.

Using the above criterion, Scenario 1 simulations were performed until 6,000 sets of parameter values were produced consistent with non-outbreak conditions in Vermont. The number 6,000 was selected to produce a body of data sufficiently large for meaningful statistical analysis without making the simulation process unreasonably time-consuming. Since none of the parameters related to exposure to reclaimed water was required for this scenario, these simulations used only ten of the 26 sampling parameters (X_0, ρ_T, ρ_P, α_{Rand}, a, σ, γ, μ, β_0, δ). Five of the remaining 16 sampling parameters (I_C, R_F, T_E, P_S and λ_F) were set to zero, which removes their effect on the output of the model and results in the rest of these 16 sampling parameters being mathematically canceled.

Once established, the parameter sets for which a Scenario 1 simulation resulted in an annual incidence of 20-60 were used as a basis to run Scenarios 2 and 3. Therefore, in the last two scenarios the ten parameter values were predetermined while the remaining 16 parameters were obtained by randomly sampling the parameter distributions. For Scenario 2, the treatment parameter, T, was set to zero reflecting the use of water from a pathogen-free source, whereas for Scenario 3 it was randomly sampled.

One-year periods were simulated on a Sun Sparc Station using the MCSim simulation software package.[9] The output variable used in the analysis was average daily prevalence, which was defined as the proportion of population that was symptomatic (in state D) calculated for each day of the simulation averaged over the one-year simulation period. Average prevalence incorporates both the number of cases and the duration of the disease, resulting in a measure of disease intensity, whereas incidence accounts for the number of cases but not the duration of disease. Average prevalence can be compared with incidence by the following approximation:

$$P \approx I \cdot d$$

where I is the incidence and d is the duration of the disease.

2.3 Parameterization

This section describes the use of sampling parameters in the model. The parameters are divided into three groups: biological, treatment, and community parameters. Biological parameters are generally based on properties of the microorganism under study, including those associated with host-parasite interaction. Community parameters are based on properties of the community and the exposure scenario under study. The two remaining parameters that relate to water treatment comprise the final group. The following is a detailed description of the parameters. The model parameters and differential equations are summarized in Table 2.1. The sampling parameters are described and the ranges from which these parameters were sampled are given in Table 2.2. Dependent parameters and their relation to sampling parameters are shown in Table 2.3.

Biological Parameters

1. ρ_P, ρ_T The dependent parameter ρ, the fraction of individuals in state Y who move to state D per day, is a ratio of sampling parameters ρ_p, the fraction in state Y that move to state D and ρ_T, the incubation period:

$$\rho = \rho_p / \rho_T$$

2. α_{Rand} The dependent parameter α, the fraction of individuals in state Y who move to state D per day, depends on the value of ρ. For each unit of time, a fraction of the population in state Y will move to state D, at the rate ρ. The remaining population in state Y either remains in state Y or moves to state Z. The sampling parameter α_{Rand} is sampled from a uniform distribution of 0 to 1 and determines the fraction of the remaining population in state Y that moves to state Z. Therefore α is defined as follows:

$$\alpha = \alpha_{Rand} \cdot (1 - \rho)$$

3. β_0 The dependent parameter β_0, the background transmission rate, is based on the expected non-outbreak incidence of disease in the community. The sampled range of β_0 is established by running a series of calibration simulations. First, simulations of the model are run with the value of β_0 sampled from an arbitrarily large range. The incidence rates generated from these calibration simulations are compared with the expected non-outbreak incidence rate range, and if within this range, are classified as passes. The sampling range of β_0 is adjusted to reflect the distribution of β_0 for the passed simulations. Hence, the sampling range of β_0 is narrowed by these calibration simulations to provide a high likelihood of matching the expected non-outbreak incidence of disease in the community.

4. β_{Exp} For *Giardia*, the standard single-hit exponential model to describe the probability of infection when an individual is exposed to a certain dose of pathogen was used. This model assumes that infection is a two-step process: 1) the host is exposed to a certain number of microorganisms, and 2) a fraction of the microorganisms ingested survive and cause disease. From these assumptions the probability of an infection resulting from the ingestion of d organisms is:[10]

$$P_{ij} = 1 - \exp (-\beta_{Exp} \cdot d)$$

where d is the dose that an individual is exposed to and β_{Exp} is the fraction of ingested organisms that survive. This function has been used to calculate the risk of disease due to exposure to various waterborne pathogens, including viral diseases[11] and *Giardia lamblia*.[12] The range of the sampled parameter β_{Exp} is determined using a maximum likelihood estimator (MLE) approach, derived in a previous study on risk assessment of pathogens in drinking water.[12] The likelihood equation for the exponential function was maximized:

$$L = 2\sum_j \left(p_j \ln \left[\frac{P_{ij} (p_j + n_j)}{p_j}\right] + n_j \ln \left[\frac{(1 - P_{ij}) (p_j + n_j)}{n_j}\right] \right)$$

where p_j is the number of diseased at each dosage, n_j is the number of non-diseased at each dosage (see Appendix A for a description of the dose-response data), and P_{ij} is the probability of infection, as described above.

For this study, the dose d is described by the following function:

$$d = W \cdot \beta_I \cdot \beta_{TsHour} \cdot \beta_{TsDay} / 365$$

where W is the state variable representing the concentration of pathogen in water in the swimming area, β_I is the volume of water ingested per hour swimming, and β_{TsHour} and β_{TsDay} together represent the amount of time in hours spent swimming per year. Dividing by 365 accounts for the fact that d represents pathogen ingested per day. These four sampling parameters will be described later in the community parameter section.

5. λ_F The dependent parameter λ is the number of pathogen shed per liter of water in the swimming area per day per infectious/asymptomatic individual. This parameter is a function of three community parameters (β_{TsHour}, the number of hours spent swimming per day; β_{TsDay}, the number of days spent swimming per year; and P_S, the proportion of the population that swims) and one biological parameter (λ_F, the number of pathogen shed per swimmer hour):

$$\lambda = (\lambda_F \cdot P_S \cdot \beta_{TsHour} \cdot (\beta_{TsDay} / 365))/\textit{volume of swimming area}$$

The calculation of the volume of the swimming area will be discussed later.

Two methods were used to calculate λ_F, as described below.

Method 1

In the first method it was assumed that the ratio of concentration of pathogen to concentration of indicator organisms in an infected person's stool is equal to the ratio of the rate of shedding of pathogen by an infected person during swimming to the rate of shedding of indicators during swimming. This relationship is described by the following equation:

$$\frac{\text{[pathogen in stool]}}{\text{[indicators in stool]}} = \frac{\text{rate of pathogen shedding}}{\text{rate of indicators shedding}}$$

where the rate of shedding of pathogen can be used to represent the sampled parameter λ_F.

Data are available in the literature on three of the four terms in the above equation: the number of indicators (total coliforms) per gram of stool; the number of pathogen per gram of stool for an infected person; and the number of indicators (total coliforms) shed per hour swimming. Therefore, this relationship can be used to provide an approximation of the remaining term, λ_F, the number of pathogen shed per hour swimming by an infected person.

The following ranges were estimated using data obtained from the literature:

- For *Giardia lamblia*, [pathogen in stool] = 10^6 to 10^8 per gram of feces (see Chapter 3.0, Table 3.3).

- For total coliforms, [indicators in stool] = 10^7 to 10^9 per gram of feces.[13]

- For total coliforms, from a study by Hanes and Fossa,[14] the rate of shedding by swimmers = 10^7 to 5 X 10^8 indicators per hour swimming.*

Solving the above equation for the rate of pathogen shedding yields an approximation of the number of pathogen shed per infected person per hour swimming that ranges from 10^6 to 5 X 10^{11}. The five order of magnitude range reflects the uncertainty in the data. However, the distribution between this range is not uniform; rather, it is a function of three uniform distributions. The resulting distribution contains long tails on either extreme.

To narrow the five order of magnitude range, only the most probable values within this range were sampled when running the model. The new ranges were determined by sampling independently and uniformly within the above respective ranges for *[pathogen in stool]*, *[indicators in stool]* and *rate of indicator shedding* 1,000 times and solving the above equation for each sampled set. Thus 1,000 values were generated for the number of pathogen shed into water during one hour of swimming. The distribution of these values was determined and the approximate lowest and highest 2.5% of the distribution was removed. The resulting distribution for λ_F ranged from 10^8 to 4 X 10^{10}.

For documentation supporting part of this methodology, see Appendix J.

Method 2

In the second method for calculating λ_F, it was assumed using professional judgement that an individual swimmer releases between 10^{-3} and 10^{-2} grams of feces per hour into the swimming water. The number of pathogen shed per swimmer hour, λ_F, was calculated using the following relationship:

$$\lambda_F = \textit{grams of feces released per swimmer hour} \cdot [\text{pathogen in stool}]$$

Using a range of 10^6 to 10^8 for [pathogen in stool] (as in the Method 1) results in a range for λ_F of 10^3 to 10^6 pathogen per swimmer hour.

This second method resulted in a range that was about three orders of magnitude below that calculated using the first method. This discrepancy suggests that assignment of uncertainty bounds for these factors is itself uncertain, e.g., the data supplied by Hanes and Fossa[14] was not sufficient to assign an accurate lower bound. Furthermore, the discrepancy in results between the two methods for calculating λ_F illustrates the need to study the shedding dynamics in more detail. A separate series of simulation runs were performed to consider the output generated at the lower and higher ranges of shedding. The details and results of this study will be discussed later.

*This range was selected around the mean of 2.3 X 10^8 total coliforms shed per person per hour obtained from Hanes and Fossa, 1970. The same study reported a median of 5.13 X 10^7, which implies a highly skewed distribution. It is therefore possible that the range of 10^7 to 5 X 10^8 does not adequately cover the lower end of the data. Unfortunately, without intraquartile information this cannot be assessed.

6. P_F P_F is the number of pathogen per milligram of feces. It is used in calculating the dependent parameter R, as will be discussed later.

7. a The dependent parameter a, the number of new susceptible individuals who migrate into the population per day, is set equal to the birth rate of the community, which was assumed to be equal to the global birth rate provided by Raven and Johnson.[15]

The remaining sampled biological parameters δ, σ, γ, μ, and ζ were described previously (see Table 2.1) and are identical to their respective dependent parameters. The parameter μ, the fraction of individuals who die from natural causes per day, is set equal to the death rate of the community, which was assumed to be equal to the global death rate provided by Raven and Johnson.[15]

Treatment Parameters

The dependent parameter T, the fraction of pathogen remaining per day after water treatment and dilution, is a function of two sampled parameters (see Table 2.3):

1. T_E The sampled parameter T_E represents the treatment efficiency of the water treatment facility, and is expressed as the fraction of pathogen that remains after treatment. Hence, a "log 5" removal corresponds to a T_E value of 10^{-5}.

2. T_L The sampled parameter T_L is the fraction of the swimming impoundment's volume that is lost to evaporation per day. An evaporation rate of 5 - 7.5 feet per year was assumed based on review of data provided by the State of California Department of Water Resources.[16] By using an assumed impoundment surface area of 10.4 acres and volume of 1.7×10^8 liters[b] and the above evaporation rate, the fraction of the swimming impoundment's volume that evaporates per day was computed.

Community Parameters

1. X_0 X_0 is both the initial susceptible population and the total population, since it is assumed that all members of the population are susceptible at the beginning of each simulation. It is assumed to be 100,000 individuals in order to produce output values "per 100,000."

2. P_S The sampled parameter P_S represents the fraction of the population that swims. The transmission parameter β is calculated by multiplying the dose response equation describing the probability of a susceptible swimmer becoming infected by P_S:

$$\beta = (1\text{-}\exp(-\beta_{Exp} \cdot .d) * P_S$$

to produce a susceptible individual's probability of becoming infected due to the ingestion of reclaimed water. As described earlier, P_S is also used to calculate λ, reflecting that only the fraction of the population in state Y that swims sheds pathogen directly into the swimming water.

[b]The surface area and volume are based on averages for Lake Temescal, an artificial lake in Oakland, California used by the public for swimming.

3. β_{TsHour}, β_{TsDay}

The dependent parameter β, the infection rate due to ingestion of pathogen in reclaimed water, is a function of the number of hours of exposure per day (β_{TsHour}) and the number of days of exposure per year (β_{TsDay}). As described earlier, these parameters are used in the β transmission function, where dose is dependent on the fraction of time spent swimming, and to calculate λ, reflecting that the infected population contributes pathogen directly to the swimming water only when swimming.

4. β_I The sampled parameter β_I is the amount of water ingested per swimmer per hour. As shown earlier, it is used in the calculation of dose for the β transmission function.

5. I_C, I_L The sampled parameters I_C and I_L are used to calculate I, the number of pathogen from external sources per liter wastewater entering the treatment plant. The parameter I_L is the number of liters of external wastewater input per day, and the parameter I_C is the concentration of pathogen in the external wastewater. I is calculated as follows:

$$I = (I_C \cdot I_L) / (I_L + R_L \cdot (X+Y+Z+D))$$

where $(I_L + R_L \cdot (X+Y+Z+D))$ is the volume of wastewater entering the treatment plant per day and $(I_C \cdot I_L)$ is the number of pathogen per day input from the external wastewater.

6. R_F, R_L The dependent parameter R, the number of pathogen per liter of wastewater entering the treatment plant per infectious individual, is a function of three community parameters (R_F, the number of milligrams of feces released into wastewater per individual per day; R_L, the number of liters of wastewater produced per individual per day; and I_L, the number of liters of external wastewater input per day) and one biological parameter (P_F, the number of pathogen per milligram of feces).

$$R = (R_F \cdot P_F) / (I_L + R_L \cdot (X+Y+Z+D))$$

where $(I_L + R_L * (X+Y+Z+D))$ is the volume of wastewater entering the treatment plant per day and $(R_F * P_F)$ is the number of pathogen shed per infectious individual per day.

7. W_V, P_{VS}

The sampled parameter W_V is the total volume of the swimming impoundment and the sampled parameter P_{VS} is the fraction of W_V used for swimming. These two parameters are used in calculating the concentration of pathogen in the swimming area, as described below.

R is the number of pathogen per liter of wastewater, or pathogen concentration, entering the treatment plant per infectious individual. Multiplying R by the state variables Y and D, which together represent all the infectious individuals in the population, gives the pathogen concentration in the influent to treatment plant contributed by the population:

$$[\text{pathogen in influent}]_{\text{POPULATION}} = R \cdot (Y+D)$$

I is the number of pathogen per liter wastewater, or pathogen concentration, entering the treatment plant from external sources. The total concentration of pathogen in the influent to the treatment plant is the sum of the influent pathogen concentration from the population and the influent concentration from wastewater from external sources:

$$[\text{pathogen in influent}]_{\text{TOTAL}} = (R \cdot (Y + D)) + I$$

The concentration of pathogen in the treatment plant effluent is equal to the total pathogen concentration in the influent multiplied by T_E, the fraction of pathogen remaining after treatment:

$$[\text{pathogen in effluent}] = [\text{pathogen in influent}]_{\text{TOTAL}} \cdot T_E$$

A fraction of this effluent is discharged into the swimming impoundment per day:

$$\textit{Volume Discharged Per Day} = W_V \cdot T_L$$

where W_V is the total volume of the swimming impoundment and T_L is the fraction of the swimming impoundment's volume that is lost to evaporation per day. It is assumed that the amount of water discharged by the treatment plant into the swimming impoundment per day is equivalent to the volume of water evaporated per day.

The number of pathogen discharged per day into the impoundment is determined by multiplying the volume of water discharged into the impoundment per day by the concentration of pathogen in the effluent:

$$\textit{Number of Pathogen Discharged Per Day} = \textit{Volume Discharged Per Day} \cdot [\text{pathogen in effluent}]$$

The number of pathogen discharged per day divided by volume of the impoundment is the average daily concentration of pathogen in the impoundment water from the tertiary treated water route (complete mixing is assumed):

$$[\text{pathogen in impoundment}]_{\text{TREATED WATER}} = \textit{Number of Pathogen Discharged Per Day}/W_V$$

The number of pathogen released per day by swimmers directly into the swimming area is determined by multiplying λ, the number of pathogen shed per day per infectious/asymptomatic individual and Y, the number of infectious/asymptomatic individuals. The sampled parameter P_{VS} represents the fraction of W_V that is used for swimming, and it is assumed that pathogen shed by swimmers completely mix in the swimming area but do not enter the remainder of the impoundment. Hence the average daily concentration of pathogen in the swimming area from the shedding route is:

$$[\text{pathogen in swimming area}]_{\text{SHEDDING}} = (\lambda \cdot Y) / (W_V \cdot P_{VS})$$

The daily change in W, the average daily concentration of pathogen in the water in the swimming area, is calculated from the concentrations of pathogen from the tertiary treated water and shedding routes and the pathogen die-off rate as follows:

$$dW/dT = [\text{pathogen in impoundment}]_{\text{TREATED WATER}} + [\text{pathogen in swimming area}]_{\text{SHEDDING}} - \zeta W$$

2.4 Results

Scenario 1 - Background Transmission Rate

Scenario 1 modeled the infectious process of giardiasis due to factors other than those associated with reclaimed water. β, the infection rate due to the ingestion of pathogen in reclaimed water, was therefore set to zero. Simulations were run for Scenario 1 until 6,000 simulations were produced with an incidence rate between 20 and 60 cases per 100,000 per year, consistent with the Vermont background rate. The mean average daily prevalence was approximately 2 per 100,000, with a standard deviation of 1.53 (range: 0.27 - 11.62). Figure 2.2 is a histogram that shows the distribution of background prevalence values in the population.

A multiple linear regression analysis was performed using the ten parameters sampled in the background transmission scenario ($R^2 = 0.8386$). The most important determinants of the level of disease prevalence were the parameters σ, the fraction of individuals in state D who move to state Z per day ($P<0.0005$), and to a lesser extent, β_0, the baseline transmission rate ($P<0.0005$). A regression was performed using only the two parameters ($R^2 = 0.7636$).

Scenario 2 - Swimming Impoundment Filled with Water from a Pathogen-Free Source

In Scenario 2 the swimming impoundment was filled with water from a pathogen-free source. T_E, the fraction of pathogen remaining after water treatment, was therefore set to zero. Shedding of pathogen into the water occurred when infectious individuals went swimming and susceptible individuals were exposed to this pathogen during swimming. Shedding was represented by the parameter λ, the number of pathogen shed per liter of water in the swimming area per day per asymptomatic/infectious individual. For these simulations, λ_F, the rate of pathogen shedding per infectious swimmer, was assigned a range using Method 1, described previously (10^8 to 4×10^{10} pathogen/hour). The parameter sets from the 6,000 simulations produced for Scenario 1 were reused one at a time during a sampling of the remaining 16 parameter distributions. The resulting 26 parameters were used to perform 6,000 new simulations. The mean average daily prevalence of these 6,000 new simulations increased to approximately 717 cases per 100,000. In addition, the standard deviation increased to 1,252.

Using a multiple linear regression analysis, the parameters from the background scenario were found to be the most significant determinants of prevalence value when all the parameters are sampled within the ranges given in Table 2.2 (with the exception that T_E is set to zero). The parameters σ, γ, ρ_T, α_{Rand}, ρ_P, and β_0 were all significant to $P<0.0005$. Of the 16 parameters not used in the background scenario, only the parameters β_{TsHour} and β_{TsDay} were significant to $P<0.05$. The remaining 14 non-background parameters were statistically insignificant.

Scenario 3 - Swimming Impoundment Filled with Tertiary Treated Water

For Scenario 3, the 6,000 simulations were rerun analogously to Scenario 2, with the addition that the parameter T_E was sampled. T_E was sampled between 5×10^{-7} and 0.02, corresponding to 1.8 to 6.3 log removal treatment. This range was selected based on the approximate removal of protozoa by tertiary wastewater treatment (See Chapter 5.0, Section 5.4). The mean average daily prevalence value for this reclaimed water scenario was approximately 717 cases per 100,000, which was the same result for Scenario 2. To further observe the effect of treatment on average daily prevalence, the results of simulations from Scenarios 2 and 3 were paired and the percent differences between each pair were binned into four levels of treatment efficiency (Figure 2.3). The results suggest that even with the lowest treatment efficiency, less than a 1% difference exists in average daily prevalence between Scenarios 2 and 3.

To assess which parameters are most significant in controlling the output for Scenario 3, a multiple linear regression analysis was performed on the model parameters. Similarly to Scenario 2, biological parameters strongly determined the prevalence output when all the parameters are sampled within the ranges given in Table 2.2. Specifically, when a linear model was used with all 26 parameters included, the resulting R^2 was 0.917. A best-subsets regression was performed using C_P as the evaluation criteria (see Appendix K). This procedure identified ρ_P (the fraction of state Y that moves to state D per day), ρ_T (the incubation period), α_{Rand} (the fraction of state Y that does not move to state D that moves to state Z per day), σ (the fraction of state D that moves to state Z per day), and γ (the fraction of state Z that moves to state X per day) as a particularly good subset of the total parameter set. A linear model with these five variables produced a resulting R^2 of 0.9158, indicating that for the sampling ranges used, the model using only these variables describes the output nearly as well as the model using all the parameters. After considering the residuals from the linear model, the squared values of the five important parameters were added to the model and the best-subsets regression was rerun. A linear model with ρ_P, ρ_T, α_{Rand}, σ, γ, ρ_P^2, ρ_T^2, α_{Rand}^2, σ^2, and γ^2 produced an R^2 of 0.9824. A linear model with two of the squared terms removed (ρ_P^2 and γ^2) produced an R^2 of 0.9755. A t-statistic calculation suggested that the following five parameters (in order of decreasing importance) highly influence the output of the model when all the parameters are sampled within the ranges given in Table 2.2 (t-statistics and P values shown in parentheses):

- σ — fraction of state D that moves to state Z per day ($t = -179.787$, $P<0.0005$)
- α_{Rand} — fraction of state Y that does not move to state D that moves to state Z per day ($t = -115.837$, $P<0.0005$)
- ρ_T — the incubation period ($t = -115.346$, $P<0.0005$)
- γ — fraction of state Z that move to state X per day ($t = 73.665$, $P<0.0005$)
- ρ_P — fraction of state Y that moves to state D per day ($t = 57.275$, $P<0.0005$)

Although the regression analysis showed that these five parameters control the output for Scenario 3 when all the parameters are sampled within the ranges given in Table 2.2, it is still unclear why there is a large difference in average daily prevalence between Scenario 1 and Scenarios 2 and 3. One might initially conclude that biological parameters account for this difference, since these parameters were most important in predicting the prevalences for Scenarios 2 and 3 and parameters that influence shedding, treatment efficiency, and exposure were of lesser importance. This conclusion would be incorrect, however, since the ranges for all the sampling parameters were set such that all Scenario 2 and 3 simulations produced high prevalences relative to Scenario 1. To assess the impact of the

parameters in determining the change from background to elevated ranges of prevalence, the sampled ranges of one or more of the parameters would need to be expanded to the point where the prevalences generated spanned the range from background to elevated levels.

Analysis of Shedding Range

The fact that swimming in an impoundment filled with water from a pathogen-free source increases the average daily prevalence output of the model compared to background suggests that shedding of pathogen by swimmers is a potential cause of the increase in prevalence. Furthermore, as described previously, the rate of shedding is highly uncertain. For these reasons, λ_F was chosen for further exploration.

In the previous scenarios, λ_F was sampled from 10^8 - 4 X 10^{10} pathogen per swimmer hour, the range calculated using the most probable region of a shedding distribution (Method 1). For this analysis, additional simulations were performed expanding the range of λ_F to 1 - 10^{11}. This very wide range encompasses the ranges calculated using both Methods 1 and 2.

The results of these simulations are shown in Figure 2.4. Figure 2.4 suggests a strong relationship between the rate of shedding and the probability of a simulation producing a high prevalence. Above a rate of 10^9 pathogen per swimmer hour, all simulations produced relatively high prevalences. Shedding therefore clearly drives the output of the model above this rate when all other parameters are sampled from the ranges given in Table 2.2. It appears that relatively high shedding rates result in relatively high concentrations of pathogen in the swimming water, which because of the dose-response relationship results in increased prevalence.

At rates of shedding between 10^5 and 10^9 pathogen per swimmer hour, Figure 2.4 shows that many simulations produced much lower prevalences. At approximately 10^6 pathogen per swimming hour, the number of simulations producing high and low prevalences were approximately equal. Within the 10^5 to 10^9 range, the rate of shedding did not completely control whether high or low prevalence resulted and the prevalence produced by a given simulation depended on the sampled values of other parameters in the model. However, the rate of shedding did influence the probability that simulations resulted in high or low prevalences.

At rates of shedding from 1 to 10^6 pathogen per swimmer hour, Figure 2.4 shows that the majority of simulations resulted in low prevalences. However, there was no shedding rate below which all simulations resulted in low prevalences. Even with shedding set at a rate of one pathogen per swimmer hour there were still simulations which resulted in high prevalences. Therefore, as with the 10^5 to 10^9 range of shedding, other parameters were able to push the results of the simulations to high prevalences regardless of the rate of shedding within the 1 to 10^5 range. To determine which parameter played the most important role in producing high prevalences within this range of shedding, a linear multiple linear regression was performed (R^2 = 0.5789). The fraction of pathogen remaining after treatment, T_E, predominantly drove the results to high prevalences for this range of shedding ($P<0.0005$). Figure 2.5 is a three-dimensional plot illustrating how prevalence increases with both increasing λ_F and decreasing treatment efficiency (increasing T_E). It illustrates that if the rate of shedding was within the 1 to 10^5 range, then relatively high values of T_E (corresponding to relatively low treatment efficiency) were capable of driving the output to a high prevalence.

2.5 Discussion

The use of a comparative simulation design allowed us to contrast the risk from swimming in an impoundment filled with water from a pathogen-free source with the risk from swimming in an impoundment filled with tertiary treated water. The results of both of these scenarios were compared with non-outbreak prevalence to observe the relative impact of adding pathogen to swimming water either directly by shedding during swimming or indirectly through the use of tertiary treated water. Any simulation for which the prevalence was in the range of 0.27 to 11.62 per 100,000 was considered within the background prevalence range. Given the model structure and the ranges of values selected for model parameters used in this study, the simulations forecast significantly higher prevalence of giardiasis in a community with a public swimming impoundment than in a community without such an impoundment. Filling the impoundment with tertiary treated wastewater (at least a 1.8 log removal of pathogen) did not increase mean prevalence levels above those obtained when the impoundment was filled with water from a pathogen-free source. However, the uncertainty and variability of many of the parameter values are quite high. The standard deviation of output prevalence in each of the two swimming scenarios (Scenarios 2 and 3) was more than 1.5 times the mean value. It is possible that these uncertainties masked any significant difference between the two scenarios in terms of mean prevalence and therefore predicted public health impact. For the swimming impoundment filled with tertiary treated water (Scenario 3), preliminary analysis showed that the uncertainty of the shedding dynamics highly influenced the output of the model.

The analysis of shedding uncertainty demonstrated the power of using the model as a tool for analyzing parameters whose values are uncertain and/or variable. The rate of pathogen shedding by a swimmer is not a parameter that is easily found in the literature. Furthermore, any value found would be highly variable. By performing simulations over a wide range of values for shedding which probably more than encompass the uncertainty and variability of this parameter, a picture of how prevalence changed with rate of shedding was obtained. Hypothetically, the results might have shown that throughout this large range the level of prevalence did not change. If this were the case, then it could have been concluded that λ_F was not a significant parameter, and even though its value was highly uncertain and variable it was not critical to the results. However, Figure 2.4 shows that the rate of shedding clearly played a role in determining whether prevalences were high or low when all other parameters were sampled within the ranges given in Table 2.2. By further dividing the wide shedding rate range into subranges and analyzing high (10^9 - 10^{11} pathogen per swimmer hour), medium (10^5 - 10^9 pathogen per swimmer hour), and low (1 - 10^5 pathogen per swimmer hour) rates of shedding, the role of λ_F in the model was clarified. For high rates of shedding it controlled the results, driving outputs to high prevalences. For medium rates of shedding, it produced simulations that resulted in both high and low prevalences. For low rates of shedding, the majority of simulations resulted in low prevalences and T_E, the fraction of pathogen remaining after treatment, became a critical parameter in the model.

It can be concluded that although the shedding uncertainty is not resolved, it is already well understood within the context of the model. If future research suggests that shedding is most likely to be in the high range, then the model will always produce high prevalences if the ranges for the other parameters do not change. If, however, research suggests that shedding is most likely to be in the low range, then researchers should begin to focus on the level of wastewater treatment. If future research suggests medium levels of shedding, then the model could once again become a critical tool in identifying other parameters that determine whether prevalence is high or low.

The rate of shedding is just one parameter whose uncertainty and variability is a factor in the model output. More work is needed to understand how other parameters affect the output of the model. For example, a similar wide-range analysis could be performed for other model parameters. The results of these studies would help identify other parameters that need better definition and inform the process of selecting parameter distribution ranges that reduce the overall uncertainty in the model output.

Chapter 2.0 - References

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Table 2.1

Differential Equations, State Variables, Parameters and Input Variables

Equations:

$$\frac{dX}{dt} = a + \gamma Z - \mu X - \beta_0 X - \beta(W)X$$

$$\frac{dY}{dt} = \beta_0 X + \beta(W)X - \mu Y - \rho Y - \alpha(\rho)Y$$

$$\frac{dD}{dt} = \rho Y - \delta D - \mu D - \sigma D$$

$$\frac{dZ}{dt} = \alpha(\rho)Y + \sigma D - \gamma Z - \mu Z$$

$$\frac{dW}{dt} = (I + R(Y+D)) \cdot T + \lambda Y - \zeta W$$

State Variables:

X	Number of susceptible individuals
Y	Number of infectious/asymptomatic individuals
Z	Number of non-infectious/asymptomatic individuals
D	Number of infectious/symptomatic individuals
W	Concentration of pathogens in reclaimed water

Parameters:

ρ	Fraction of individuals in state Y who move to state D per day (day^{-1})
α	Fraction of individuals in state Y who move to state Z per day (day^{-1})
σ	Fraction of individuals in state D who move to state Z per day (day^{-1})
γ	Fraction of individuals in state Z who move to state X per day (day^{-1})
δ	Fraction of individuals in state D who die due to modeled disease per day (day^{-1})
μ	Fraction of individuals who die from natural causes per day (day^{-1})
λ	Number of pathogen shed per liter of water in swimming area per day per infectious/asymptomatic individual ($day^{-1} \cdot liter^{-1}$)
β_0	Baseline transmission rate (day^{-1})
β	Infection rate due to ingestion of pathogen in reclaimed water (day^{-1})
R	Number of pathogen per liter of wastewater entering treatment plant per infectious individual ($liter^{-1}$)
T	Fraction of pathogen remaining per day after water treatment and dilution (day^{-1})
ζ	Fraction of pathogen in water which become non-viable per day (day^{-1})
a	Number of new susceptible individuals who migrate into population per day (day^{-1})
I	Number of pathogen from external sources per liter wastewater entering treatment plant ($liter^{-1}$)

Table 2.2

Parameterization for *Giardia lamblia* and Swimming Impoundment

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
Biological Parameters:					
ρ_T	Incubation period	3 - 60	days	3-60 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.2 -0.7		20 - 70% infected develop symptoms	ρ
α_{Rand}	Fraction of state Y that does not move to state D that moves to state Z per day	0 - 1		Randomly generated number from 0 to 1	α
γ	Rate of movement from state Z to state X	5.6e-3 - 0.033	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-6 months)	γ
σ	Rate of movement from state D to state Z	0.01 -0.2	day^{-1}	Reciprocal of duration of symptoms (5-100 days)	σ
δ	Fraction in state D who die due to disease per day	0	day^{-1}	0% case-fatality due to disease	δ
a	Rate of migration of new susceptible individuals into population	6.85e-5 - 9.59e-5	day^{-1}	Birth rate	a
μ	Rate of death due to natural causes	1.37e-5 - 4.11e-5	day^{-1}	Death rate	μ
P_F	Concentration of pathogen in feces	1e3 - 1e5	pathogen/ mg	Concentration of 1e6 - 1e8 pathogen per gram of feces	R, λ
λ_F	Rate of pathogen shedding per infectious swimmer	1e8 - 4e10	pathogen/ hour	Most probable range of shedding distribution (see text)	λ
β_o	Background transmission rate	0 - 0.00021	day^{-1}	Calibration simulations using yearly incidence of disease of 20-60 per 100,000 (see text)	β_o
β_{Exp}	Disease transmission function parameter	0.008 - 0.04		Result of fitting transmission function to dose response data (see text)	β
ζ	Rate of pathogen die-off	4.2e-3 - 0.01	day^{-1}	Reciprocal of survival time of pathogen in fresh water (100-240 days)	ζ
Community Scenario Parameters:					
X_o	Initial number of individuals in state X	1e5		All output "per 100,000"	X
P_S	Fraction of population that swims	1		Assumption that 100% of population swims	β, λ
β_I	Rate of water ingestion during swimming	0.03 - 0.05	liters/hour	Assumption that 30-50 ml/hour of water is ingested during swimming	β
β_{TsHour}	Number of hours per day exposure occurs	0.5 - 3	hours/day	Assumed average of 2.6 hours for children and 0.5 hours for adults swimming per day	β
β_{TsDay}	Number of days per year exposure occurs	1 - 40	days/year	Assumption that individuals swim 1-40 days per year	β
I_L	Flow rate in of external wastewater	0	liters/day	No external wastewater modeled	I
I_C	Concentration of pathogen in external wastewater	0	pathogen/ liter	No external wastewater modeled	I

Table 2.2

Parameterization for *Giardia lamblia* and Swimming Impoundment

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
Community Scenario Parameters: (continued)					
R_L	Volume of wastewater produced per individual per day	400 - 600	liters/day	Assumption that average volume of wastewater produced by an individual per day is 500 liters	R
R_F	Weight of feces released into wastewater per day per individual	2.5e4 - 2e5	mg/day	Assumption that an average person produces 25 to 200 grams of feces per day	R
W_V	Volume of impoundment	5e7 - 5e8	liters	Hypothetical impoundment volume	W
P_{VS}	Fraction of impoundment used for swimming	0.05 - 0.15		Assumption that 5-15% of impoundment volume is used by swimmers	W
Water Treatment Scenario Parameters:					
T_E	Fraction of pathogen remaining after water treatment	5e7 - 0.02 (Log Uniform)		Assumed log 1.8 to 6.3 removal of pathogen	T
T_L	Fraction of impoundment volume which evaporates per day	1e-3 - 1.6e-3	day^{-1}	Assumed 5 - 7.5 ft/year evaporation rate for hypothetical impoundment with 10.4 acre surface area and volume of 1.7e8 liters	T

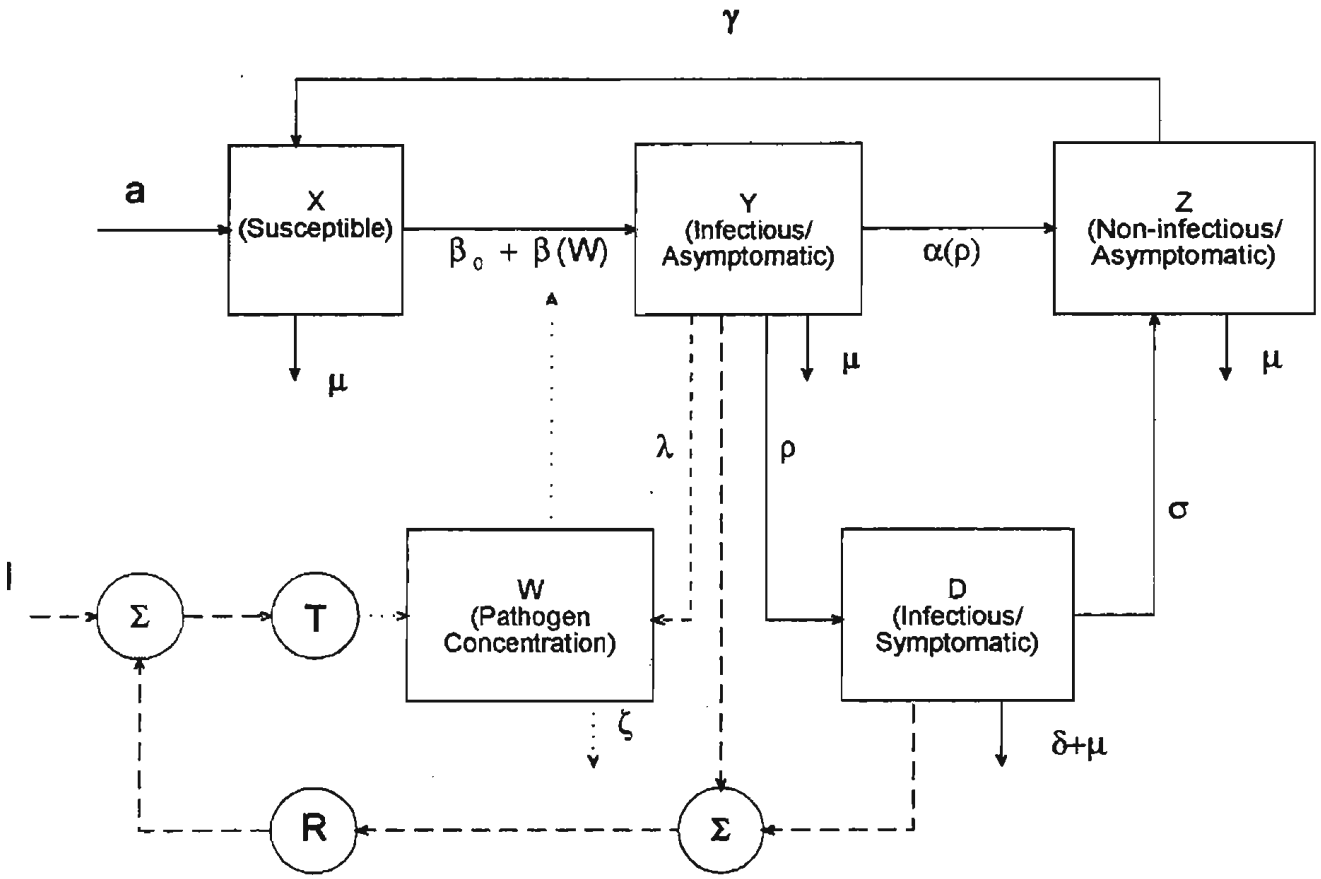
Table 2.3

List of Dependent Parameters and the Functional Dependence on the Sampled Parameters

Dependent Parameter	Description	Relation to Sampled Parameters
ρ	Rate of movement from state Y to state D	$\rho = \rho_P / \rho_T$
α	Rate of movement from state Y to state Z	$\alpha = \alpha_{Rand} \cdot (1 - \rho)$
β	Infection rate due to ingestion of pathogen in reclaimed water	$\beta = F(W \cdot \beta_I \cdot \beta_{Tshour} \cdot (\beta_{Tsday} / 365) \cdot P_S)$ where $F(d) = 1 - \exp (d \cdot -\beta_{Exp})$
λ	Rate of pathogen shedding by infectious swimmers	$\lambda = \lambda_F \cdot PS \cdot \beta_{Tshour} \cdot (\beta_{Tsday} / 365) / (P_{VS} \cdot W_v)$
I	Concentration of pathogen entering treatment plant from external sources	$I = I_C \cdot I_L / (I_L + R_L \cdot (X+Y+Z+D)$
R	Concentration of pathogen entering treatment plant per infectious individual	$R = R_F \cdot P_F / (I_L + R_L \cdot (X+Y+Z+D)$
T	Fraction of pathogens remaining per day after water treatment and dilution	$T = T_E \cdot T_L$

Figure 2.1

Model Structure



Movement of individuals.

Pathogen entering treatment plant.

Pathogen released directly into swimming water by infected swimmers.

Pathogen remaining in treated water entering swimming impoundment.

Indicates that the infection rate, β, is a function of the concentration of the pathogen, W.

F:\R02\REPORT\FIG2-1.CDR

Figure 2.2

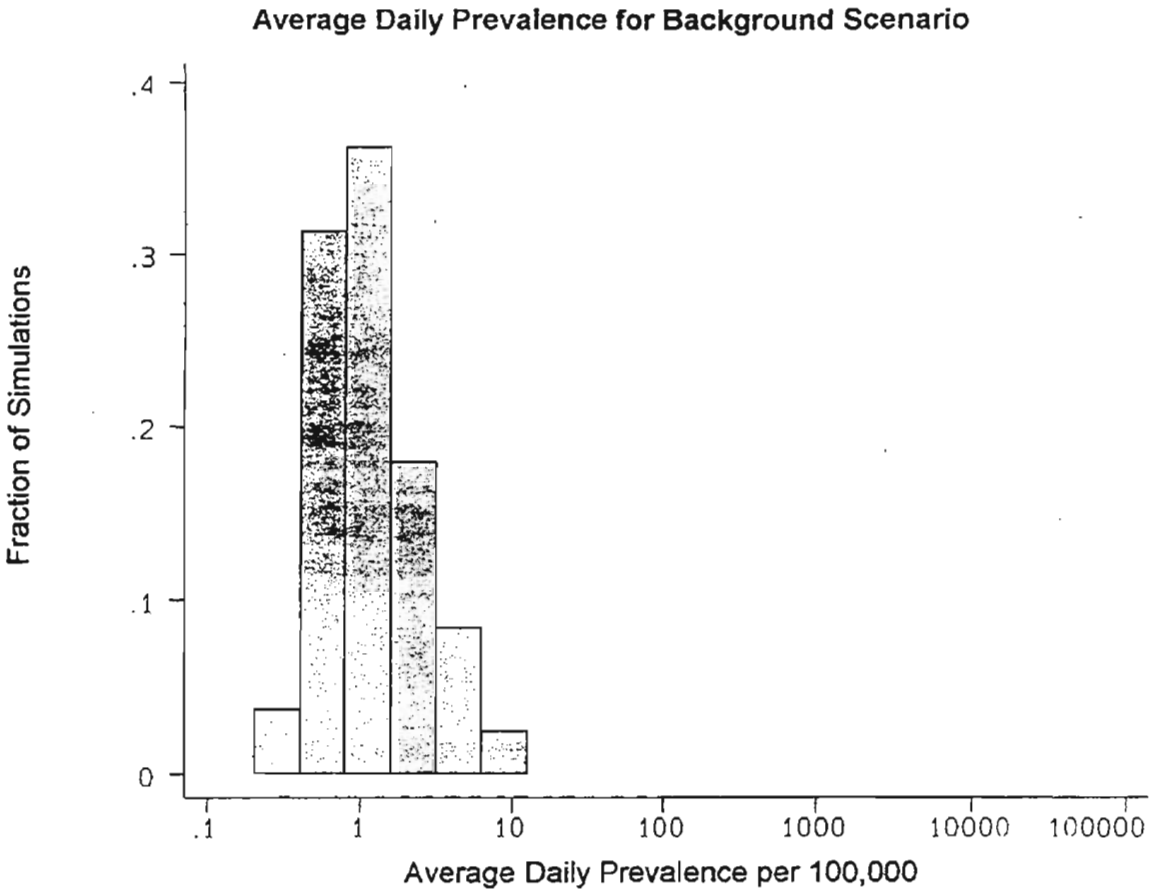

Average Daily Prevalence for Background Scenario
Fraction of Simulations
.4
.3
.2
.1
0
.1
1
10
100
1000
10000
100000
Average Daily Prevalence per 100,000

Figure 2.3

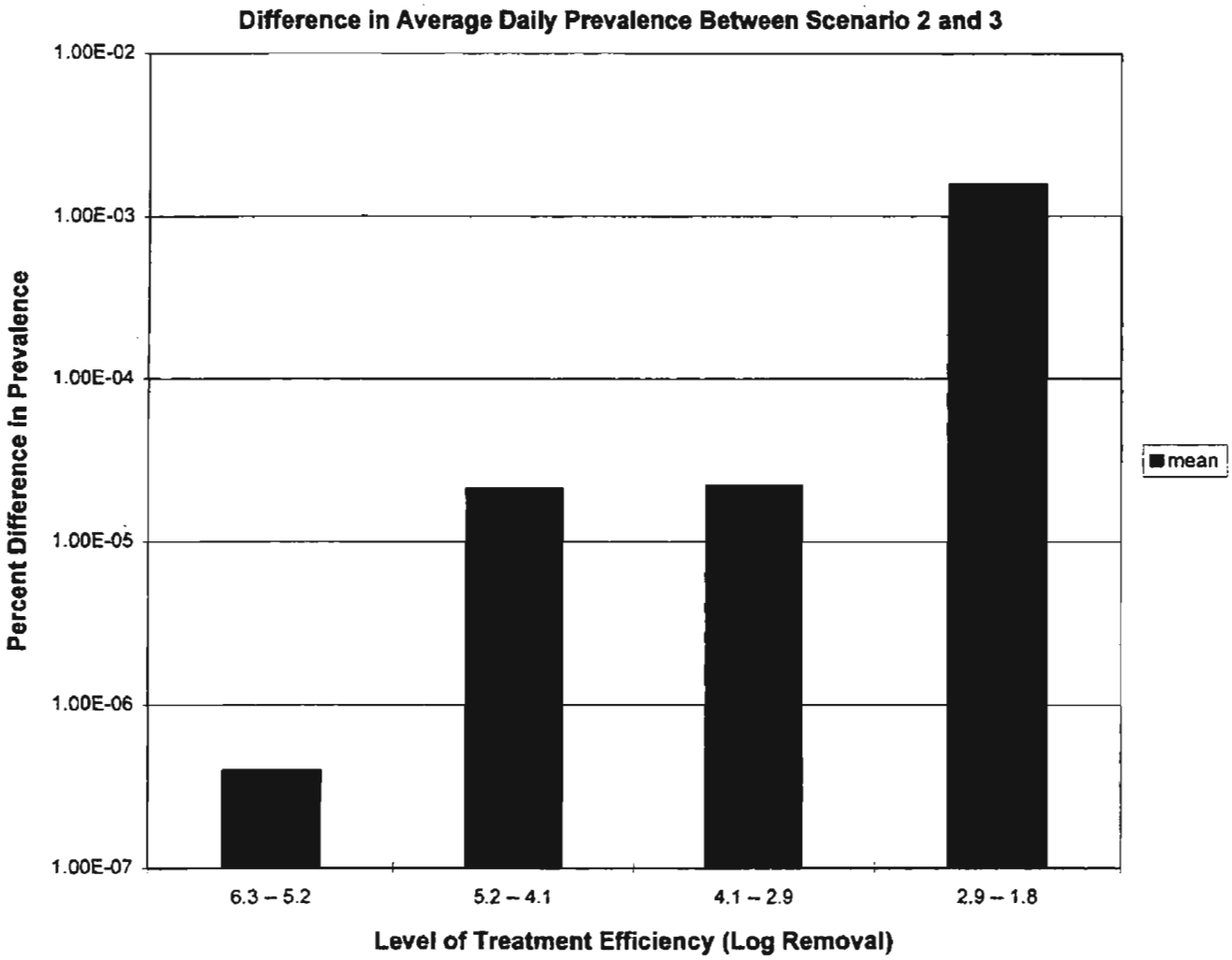

Difference in Average Daily Prevalence Between Scenario 2 and 3
1.00E-02
1.00E-03
1.00E-04
1.00E-05
1.00E-06
1.00E-07
Percent Difference in Prevalence
mean
6.3 – 5.2
5.2 – 4.1
4.1 – 2.9
2.9 – 1.8
Level of Treatment Efficiency (Log Removal)

Figure 2.4

Relationship Between Average Daily Prevalence and Rate of Shedding for Swimming Impoundment (Scenario 3)

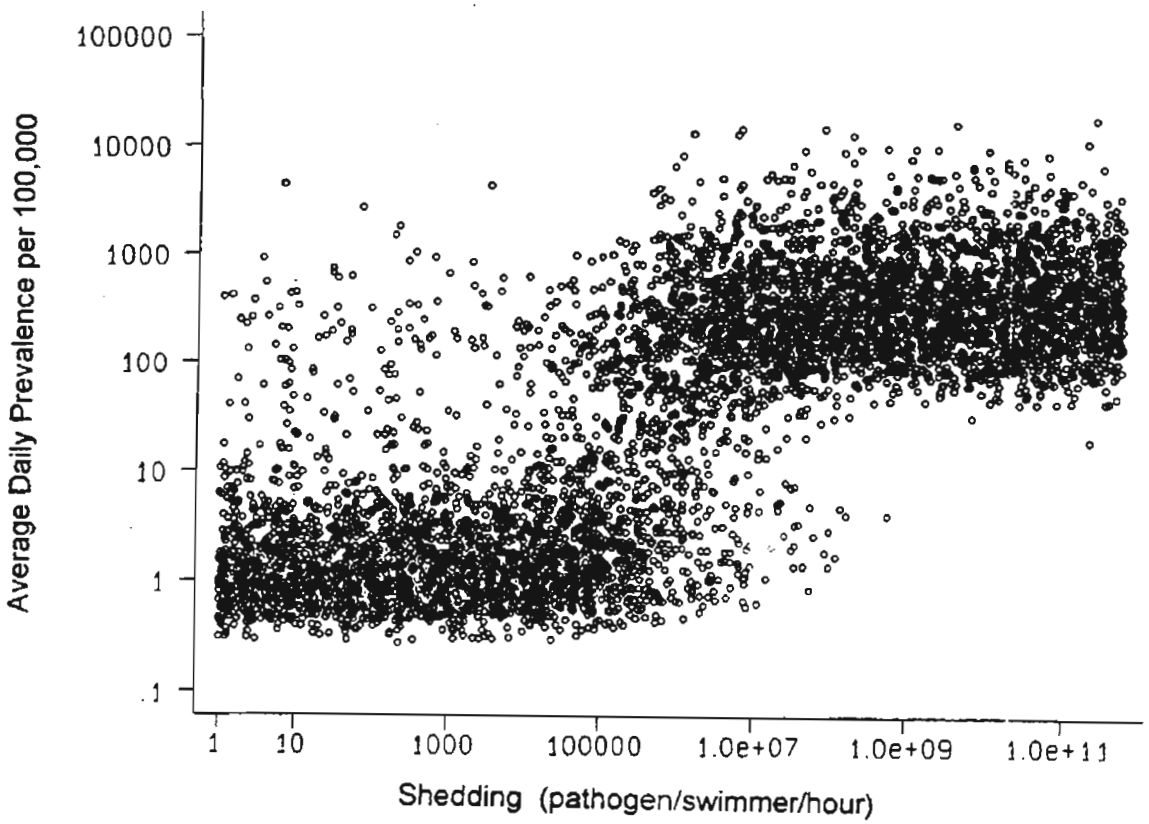
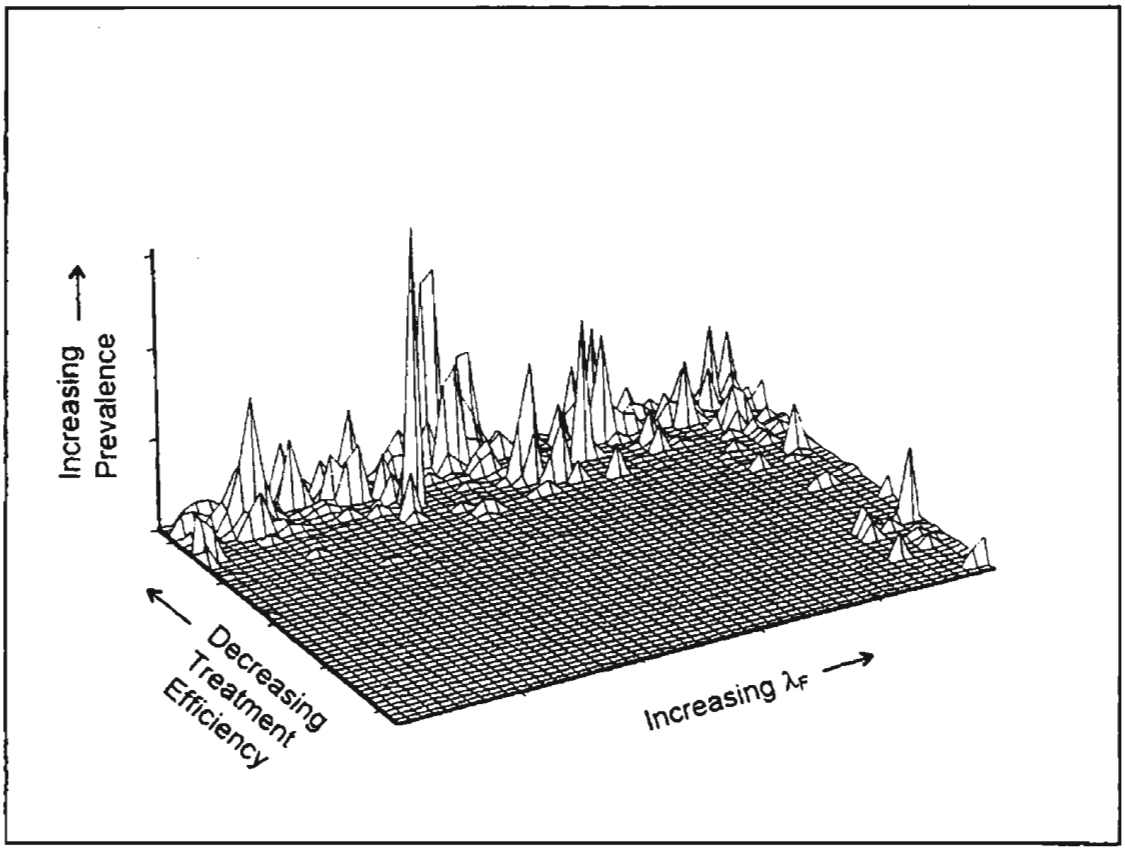


Figure 2.5

Relationship Between Average Daily Prevalence and Shedding and Treatment Efficiency for Shedding Rate Range of 1 - 10^5 Pathogen/Swimmer/Hour



Treatment efficiency range in the above figure is 1.8 to 6.3 log removal of pathogen.

3.0 MICROORGANISMS AND WATER RECLAMATION ALTERNATIVES MODELED

This section describes the identification and selection of the microorganisms included in the risk assessment, the literature review performed for these microorganisms, and the water reclamation alternatives selected for modeling and associated exposure assumptions.

3.1 Identification and Selection of Microorganisms Included in the Risk Assessment

The first step in selecting appropriate pathogens was to consider communicable diseases in humans that may be transmitted by water. Table 3.1 presents a list of the significant water-related pathogens and their associated diseases. Because of the limited data available on the microorganisms in Table 3.1, only specific representative species were selected for inclusion in the risk assessment. The list of pathogens selected is as follows:

1. *Giardia lamblia*
2. *Cryptosporidium* spp.
3. *Shigella* spp.
4. *Salmonella* spp.
5. *Vibrio cholerae*
6. Pathogenic *E. coli*
7. Enteroviruses
8. Hepatitis A virus
9. Rotavirus

The criteria used to select representative pathogens from the pathogens associated with waterborne diseases included:

- the pathogen is known to exist in the general population;
- some data are available regarding the duration of symptoms, incidence, case-fatality, incubation period and immunological aspects of the disease associated with the pathogen; and
- some data are available regarding the infectious dose and environmental persistence of the pathogen.

In addition, all of the selected organisms are shed in feces and could potentially be found in municipal wastewaters; the risk assessment model was designed for such organisms. A model application using the first microorganism, *Giardia lamblia*, was already presented in Chapter 2.0.

3.2 Literature Review

A literature review was performed on the above selected microorganisms to establish ranges of values for appropriate model parameters. The first step in the review was to review a literature search performed previously as part of the microbial risk assessment for the U.S. Army described in Chapter 1.0.

The next step was to update the Army review by collecting new relevant data. The emphasis was on recent literature (1980 to the present). An information retrieval service was used to access four literature data bases: Medline, Water Resources Abstracts, SciSearch and Ei Compendex. These data bases were selected following a review of readily accessible data bases and the relevant journals which they include. Medline was

the primary data base used; the other three data bases were used to search for articles in two journals not included in Medline but deemed important to this study (Journal of the Water Pollution Control Federation and the American Water Works Association Journal). Selected relevant key words and the microorganism's name were searched for in titles and author's key word lists, and a list of titles was generated. The selected key words were:

virulence	dose	outbreak	mortality
persistence	pathogenicity	latency	review
immune response	indicator organism	morbidity	prevalence
shedding	infectivity	epidemiology	vaccine
infection	occurrence	incubation	risk assessment

Relevant abstracts were selected for review from the titles. The selected abstracts were reviewed and selected articles were then obtained. The articles were read and data relevant to performing the risk assessment were recorded and summarized. Table 3.2 summarizes the numbers of journal titles, abstracts and articles reviewed as part of the literature review update. Appendices A - I contain write-ups that summarize the results of the literature review for each organism. Ranges for appropriate model parameters were selected for each microorganism using the available data found during the literature review. The selected ranges are presented in Tables 3.3 through 3.10.

Although rotavirus was one of the microorganisms originally selected and the literature search was completed for this organism (Appendix I), it was not included in the risk assessment. The primary reason was that the literature search revealed that gastroenteritis caused by rotavirus primarily afflicts six-month to two-year-old babies; adults are generally not susceptible. The model assumes that the population is heterogeneous with respect to susceptibility. Modeling rotavirus would require a structural change in the model to account for age-related variations in susceptibility of the population. Refining the model in this way is discussed as a topic for future research in Chapter 6.0.

The range of the sampled parameter β_{Exp}, the fraction of ingested microorganisms that survive, was determined using a maximum likelihood estimator (MLE) approach, using the dose response data provided for each microorganism in Appendices A - I. For a discussion of the use of the MLE method see Chapter 2.0, Section 2.3.[c]

It should be noted that dose response data was not found for Hepatitis A during the literature search; the dose response data for polio virus (Appendix G) was used for this microorganism.

It should also be noted that the rate of pathogen die-off was calculated by taking the reciprocal of the environmental persistence of the pathogen in water, i.e., the period of time a pathogen remains viable in water. However, since survival time depends on the initial concentration of pathogens in the water, a more meaningful type of data is rate of pathogen die-off. We used survival time data because it was generally available in the literature for each of the organisms, whereas die-off rates were not always available.

[c]Recent dose-response data for *Cryptosporidium* was published too late for incorporation into this report (Dupont, Herbert L. et al, "The Infectivity of *Cryptosporidium parvum* in Healthy Volunteers," New England Journal of Medicine, 332(13), 855-9 (March 30, 1995). The data in this article indicates a lower infectivity in humans for this microorganism than the extrapolated animal data used in this report.

3.3 Reclaimed Water Alternatives and Human Exposure Assumptions

We identified seven wastewater reclamation alternatives and corresponding levels of wastewater treatment for inclusion in the study. The seven reclamation alternatives selected were:

1. Non-Restricted (Swimming) Recreational Impoundment
2. Golf Course Irrigation
3. Restricted Recreational Impoundment
4. Park Irrigation
5. Industrial Cooling Tower
6. Irrigation of Food Crops
7. Groundwater Recharge

An application of the first alternative, the swimming impoundment, was already presented in Chapter 2.0. One alternative not included in this study but proposed for use in California is indirect potable reuse. It may be desirable to include this alternative in future risk assessment studies.

Recent literature and reports were reviewed to develop estimates of the amount of reclaimed water that could potentially be ingested at the above water reclamation sites. The results of the review are summarized for each alternative below.

Levels of water treatment for each reclamation alternative are also discussed below. The levels of treatment given are specified in the version distributed for public comment dated March 1994 of the proposed revised Title 22 Water Reclamation Criteria.

The uses selected require disinfected tertiary, disinfected secondary-2.2 (2.2 MPN/100 mL) or disinfected secondary-23 reclaimed (23 MPN/100 mL) water. Disinfected tertiary is the highest level of conventional Title 22 treatment and is defined as an "essentially" virus free water suitable for all uses except food preparation and potable reuse. In addition to secondary treatment, disinfected tertiary (2.2 MPN/100 mL) requires contact/direct filtration with coagulation (if secondary effluent turbidity is greater than 5 NTU) plus 90 minute modal chlorine contact time with minimum 5 mg/L residual. Disinfected secondary-2.2 does not require filtration. Disinfected secondary-23 reclaimed water represents typical secondary treatment plant effluent widely used for restricted access golf course irrigation and landscape impoundments.

For the following scenarios, exposure is assumed to be 100% oral ingestion. Indirect ingestion through spray inhalation of irrigation or cooling tower aerosols, other exposure pathways, and factors such as microorganism attenuation are not addressed.

1. **Non-Restricted (Swimming) Recreational Impoundment** - This water reclamation alternative is a recreational impoundment filled with disinfected tertiary reclaimed water. Human exposure occurs via incidental ingestion of water during swimming, with estimates ranging from 10 to 130 mL/day of swimming. Estimates of swimming days ranged from 1 to 40 per year. Parametrix[1] assumed 130 mL/day for children (50 mL/hour, 2.6 hours swimming/day, 7 days swimming/year) and 25 mL/day for adults (50 mL/hour, 0.5 hours swimming/day, 5 days swimming/year). The USEPA[2] gives 7 days swimming/year and 2.6 hours swimming/day as national averages. Haas[3] assumed 100 mL/day and 0.924 swimming days per person per year (for the Lake Michigan Basin). Asano and Sakaji[4] proposed a range of 10 mL to 100 mL/day for ingestion and 40 swimming days per year (weekends over a five month summer period). Asano et al.[5] and Tanaka et al.[6] assumed 100 mL/day and

40 days/year. The 40 day/year estimate may be appropriate for warmer climates such as California.

2. **Golf Course Irrigation** - This water reclamation alternative is a golf course irrigated with disinfected tertiary, secondary-2.2 or secondary-23 reclaimed water, depending on the degree of public access. Asano and Sakaji[4] and Asano et al.[5] assumed that 1 mL/day for 2 days per week year round is ingested by golfers handling and cleaning golf balls. For exposure to joggers/golfers or children playing via incidental ingestion of spray, Parametrix[1] applied the swimming ingestion rate assumption of 50 mL/hour for 10 minutes/day (i.e., ~8 mL/day) and 61 days per year exposure (irrigation every third day for six months per year). It is estimated that the golfer population in the U.S. was 24.5 million people in 1993.[7]

3. **Restricted Recreational Impoundment** - This water reclamation alternative is a restricted recreational impoundment filled with disinfected secondary-2.2 reclaimed water. Fishing and boating are allowed in this impoundment, but not swimming. Exposure occurs via incidental ingestion of spray or incidental ingestion following handling of wet objects such as a fishing line or boat tiller. A highly conservative spray exposure estimate that has been applied is the swimming exposure rate assumption of 50 ml/hour.[1] For handling of wet objects, 1 ml/day could be assumed to be ingested, similar to golfers handling and cleaning golf balls at a golf course irrigated with reclaimed water, as described above. Both exposure categories could be assumed to occur 4 hours/day and 14 days/year (professional judgement).

4. **Park Irrigation** - This water reclamation alternative is a public park irrigated with disinfected tertiary reclaimed water. Exposure occurs to adult joggers via accidental contact with spray and to children via contact with wet grass while playing. For joggers or children playing exposed to spray, the above assumptions for golf course irrigation could be applied. For children playing on wet grass, a 1 mL/day exposure rate, analogous to handling and cleaning golf balls, and 14 days per year exposure (professional judgement) could be assumed.

5. **Industrial Cooling Tower** - This water reclamation alternative is a cooling tower using disinfected tertiary, secondary-2.2 or secondary-23 reclaimed water. Exposure occurs via incidental ingestion of reclaimed water during routine maintenance of the cooling tower's heat exchangers and piping. Industrial or commercial cooling or air conditioning with cooling towers, evaporative condensers, or spraying that creates a mist requires disinfected tertiary reclaimed water. Equivalent uses that do not create a mist can use disinfected secondary-2.2 or secondary-23 reclaimed water.

   For exposure, Parametrix[1] applied the swimming ingestion rate of 50 ml/hour for 4 hours/day (i.e., 200 mL/day) and 6 days/year. This is an extremely conservative assumption, since direct immersion is highly unlikely, unless a pressurized pipeline were to burst. A more likely exposure route is ingestion of residue on hands, analogous to the golf ball handling scenario (1 mL/day). Indirect ingestion due to inhalation of mist downwind of cooling towers is not evaluated here.

6. **Irrigation of Food Crops** - This water reclamation alternative is the irrigation of food crops with disinfected tertiary reclaimed water. Exposure occurs via daily direct consumption of food crops with 10 mL reclaimed water assumed left on the portion of the crop eaten raw.[5]

7. **Groundwater Recharge** - This water reclamation alternative is a groundwater basin recharged with 50% by volume disinfected tertiary reclaimed water (assumed to meet all other proposed Title 22 regulations). Exposure occurs when an individual consumes well water at an assumed rate of 2 L per day,[2] equivalent to 1 L of reclaimed water (given the minimum 50% dilution requirement).

An assumed range for the rate of ingestion of reclaimed water was selected for each reclamation alternative using the results of the above review and, when data were unavailable, professional judgement. These selected ranges were used for appropriate model parameters, as summarized in Tables 3.11 through 3.17. Parameters that do not apply to certain exposure scenarios are designated "Not Applicable" in these tables. This will be discussed further in Chapter 5.0.

Tables 3.11 through 3.17 also give the Title 22 level of wastewater treatment selected for each reclamation alternative. Parameterization of wastewater treatment, which is dependent upon both the reclamation alternative and the microorganism modeled, is described in Chapter 5.0.

Chapter 3.0 - References

1. Metro Effluent Reuse Baseline Risk Assessment, Volume II: Human Health (Parametrix, Inc., Kirkland, Washington, 1993).

2. Superfund Exposure Assessment Manual (Office of Remedial Response, US Environmental Protection Agency, Washington, DC, 1988).

3. Haas, C.N., "Effect of Effluent Disinfection on Risks of Viral Disease Transmission Via Recreational Water Exposure," JWPCF, 55:8, 1111-6 (1983).

4. Asano, T., and R.H. Sakaji, "Virus Risk Analysis in Wastewater Reclamation and Reuse," Chemical Water and Wastewater Treatment, H.H. Hahn and R. Klute, Eds., 483-496 (1990).

5. Asano, T., L.Y.C. Leong, M.G. Rigby, and R.H. Sakahi, "Evaluation of the California Wastewater Reclamation Criteria Using Enteric Virus Monitoring Data," Water Science and Technology 26:7-8, 1513-1524 (1992).

6. Takana, H., T. Asano, E.D. Schroeder, G. Tchobanoglous, "Estimating the Reliability of Wastewater Reclamation and Reuse Using Enteric Virus Monitoring Data," Water Environment Federation 66th Annual Conference and Exposition Proceedings, 105-118 (1993).

7. Golf Participation in the United States, (National Golf Foundation, Jupiter, Florida, 1994).

Table 3.1

Infectious Agents Potentially Present in Untreated Domestic Wastewater

Pathogen	Disease
Protozoa	
Entamoeba histolytica	Amebiasis (amebic dysentery)
Giardia lamblia	Giardiasis
Balantidium coli	Balantidiasis (dysentery)
Cryptosporidium	Cryptosporidiosis, diarrhea, fever
Helminths	
Ascaris lumbricoides (roundworm)	Ascariasis
Ancylostoma duodenale (hookworm)	Ancylostomiasis
Necator americanus (roundworm)	Necatoriasis
Ancylostoma (spp.)(hookworm)	Cutaneous larva migrams
Strongloides stercoralis (threadworm)	Strongyloidiasis
Trichuris trichiura (whipworm)	Trichuriasis
Taenia (spp.) (tapeworm)	Taeniasis
Enterobius vermicularis (pinworm)	Enterobiasis
Echinococcus granulosus (spp.) (tapeworm)	Hydatidosis
Bacteria	
Shigella (4 spp.)	Shigellosis (dysentery)
Salmonella typhi	Typhoid fever
Salmonella (1700 serotypes)	Salmonellosis
Vibrio cholerae	Cholera
Escherichia coli (enteropathogenic)	Gastroenteritis
Yersinia enterocolitica	Yersiniosis
Leptospira (spp.)	Leptospirosis
Legionella	Legionnaire's disease
Campylobacter jejune	Gastroenteritis
Viruses	
Enteroviruses (72 types) (polio, echo, coxsackie, new enteroviruses)	Gastroenteritis, heart anomalies, meningitis, others
Hepatitis A virus	Infectious hepatitis
Adenovirus (47 types)	Respiratory disease, eye infections
Rotavirus (4 types)	Gastroenteritis
Parvovirus (3 types)	Gastroenteritis
Norwalk agent	Diarrhea, vomiting, fever
Reovirus (3 types)	Not clearly established
Astrovirus (5 types)	Gastroenteritis
Calicivirus (2 types)	Gastroenteritis
Coronavirus	Gastroenteritis

Source: EPA Guidelines for Water Reuse (EPA/G25/R-92/004), 1992

Table 3.2

Literature Review Statistics

Organism	Titles Reviewed	Abstracts Reviewed	Papers Reviewed
Giardia lamblia	844	113	55
Cryptosporidium spp.	723	125	48
Shigella spp.	660	80	30
Salmonella spp.	460	180	52
Vibrio cholerae	649	90	31
Pathogenic *E. coli*	150	79	57
Enteroviruses	200	150	50
Hepatitis A virus	584	100	55
Rotavirus	844	200	30

Table 3.3

Microorganism-Dependent Parameters for *Giardia lamblia*

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	3 - 60	days	3-60 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.2 - 0.7		20 - 70% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	5.6e-3 - 0.033	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-6 months)	γ
σ	Rate of movement from state D to state Z	0.01 - 0.2	day^{-1}	Reciprocal of duration of symptoms (5-100 days)	σ
δ	Fraction in state D who die due to disease per day	0	day^{-1}	0% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1e3 - 1e5	pathogen/ mg	Concentration of 1e6 - 1e8 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	4.2e-3 - 0.01	day^{-1}	Reciprocal of survival time of pathogen in fresh water (100-240 days)	ζ
β_0	Background transmission rate	0 - 0.00021	day^{-1}	Calibration simulations using yearly incidence of disease of 20-60 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	0.008 - 0.04		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	1e8 - 4e10	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.4

Microorganism-Dependent Parameters for *Cryptosporidium* spp.

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	2-14	days	2-14 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.8 - 1		80 - 100% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	5.6e-3 - 0.033	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-6 months)	γ
σ	Rate of movement from state D to state Z	0.033 - 0.5	day^{-1}	Reciprocal of duration of symptoms (2-30 days)	σ
δ	Fraction in state D who die due to disease per day	0	day^{-1}	0% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	10 - 1e4 (log uniform)	pathogen/ mg	Concentration of 1e4 - 1e7 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	5.6e-3 - 0.033	day^{-1}	Reciprocal of survival time of pathogen in fresh water (1 - 6 months)	ζ
β_0	Background transmission rate	0 - 3e-5	day^{-1}	Calibration simulations using yearly incidence of disease of 20-60 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	0.07 - 0.12		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	1e9 - 4e11	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.5

Microorganism-Dependent Parameters for *Shigella* spp.

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	1-7	days	1-7 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.5 - 0.9		50 - 90% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	5.6e-3 - 0.033	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-6 months)	γ
σ	Rate of movement from state D to state Z per day	8.33e-3 - 0.5	day^{-1}	Reciprocal of duration of symptoms (2-120 days)	σ
δ	Fraction in state D who die due to disease	0.01 - 0.02	day^{-1}	1-2% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1e2 - 1e6 (log uniform)	pathogen/ mg	Concentration of 1e5-1e9 cysts per gram of feces	R, λ
ζ	Rate of pathogen die-off	0.033 - 0.2	day^{-1}	Reciprocal of survival time of pathogen in fresh water (5-30 days)	ζ
β_0	Background transmission rate	0 - 6e-5	day^{-1}	Calibration simulations using yearly incidence of disease of 100-200 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	0.001 - 0.0022		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	8e6 - 5e9	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.6

Microorganism-Dependent Parameters for *Salmonella* spp.

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	3-22	days	3-22 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.03 - 0.76		3 - 76% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	1.37e-3 - 2.74e-3	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-2 years)	γ
σ	Rate of movement from state D to state Z	0.033 - 0.33	day^{-1}	Reciprocal of duration of symptoms (3 - 30 days)	σ
δ	Fraction in state D who die due to disease per day	0.01 - 0.1	day^{-1}	1-10% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1e2 - 1e6 (log uniform)	pathogen/ mg	Concentration of 1e5 - 1e9 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	0.07 - 0.1	day^{-1}	Reciprocal of survival time of pathogen in fresh water (10-14 days)	ζ
β_0	Background transmission rate	0 - 5e-4	day^{-1}	Calibration simulations using yearly incidence of disease of 10-30 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	3.6e-6 - 4.8e-5		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	8e6 - 5e9	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.7

Microorganism-Dependent Parameters for *Vibrio cholerae*

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	0.5-5	days	0.5-5 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.1 - 0.3		10 - 30% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	5.6e-3 - 0.033	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-6 months)	γ
σ	Rate of movement from state D to state Z	0.2 - 1	day^{-1}	Reciprocal of duration of symptoms (1-5 days)	σ
δ	Fraction in state D who die due to disease per day	0 - 0.02	day^{-1}	0-2% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1 - 1e4 (log uniform)	pathogen/ mg	Concentration of 1e3-1e7 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	0.011 - 1	day^{-1}	Reciprocal of survival time of pathogen in fresh water (1-90 days)	ζ
β_0	Background transmission rate	0 - 0.00001	day^{-1}	Calibration simulations using yearly incidence of disease of 0-10 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	3.3e-6 - 5.6e-6		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	2e9 - 5.1e10	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.8

Microorganism-Dependent Parameters for Pathogenic *E. coli*

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	0.5-12	days	0.5-12 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.5 - 0.9		50 - 90% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	5.6e-3 - 0.033	day^{-1}	Reciprocal of time for an immune person to become susceptible (1-6 months)	γ
σ	Rate of movement from state D to state Z	0.0833 - 0.2	day^{-1}	Reciprocal of duration of symptoms (5-12 days)	σ
δ	Fraction in state D who die due to disease per day	0.01 - 0.05	day^{-1}	1-5% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1e2 - 1e6 (log uniform)	pathogen/ mg	Concentration of 1e5-1e9 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	3.85e-3 - 0.143	day^{-1}	Reciprocal of survival time of pathogen in fresh water (7-260 days)	ζ
β_0	Background transmission rate	0 - 3e-6	day^{-1}	Calibration simulations using yearly incidence of disease of 1-6 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	1e-8 - 5e-8		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	8e6 - 5e9	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.9

Microorganism-Dependent Parameters for Enteroviruses

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	2-3	days	2-3 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0 - 0.02		0 - 2% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	4.21e-5 - 5.48e-4	day^{-1}	Reciprocal of time for an immune person to become susceptible (5-65 years)	γ
σ	Rate of movement from state D to state Z	0.14 - 0.33	day^{-1}	Reciprocal of duration of symptoms (3-7 days)	σ
δ	Fraction in state D who die due to disease per day	0 - 0.01	day^{-1}	0-1% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1e3 - 1e6	pathogen/ mg	Concentration of 1e6-1e9 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	5.71e-3 - 0.14	day^{-1}	Reciprocal of survival time of pathogen in fresh water (7-175 days)	ζ
β_0	Background transmission rate	0 - 0.22	day^{-1}	Calibration simulations using yearly incidence of disease of 2,000-4,000 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	0.1 - 0.5		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	1e7 - 4e9	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.10

Microorganism-Dependent Parameters for Hepatitis A Virus

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
ρ_T	Incubation period	8-60	days	8-60 day incubation period	ρ
ρ_P	Fraction of state Y that moves to state D	0.1 - 0.3		10 - 30% infected develop symptoms	ρ
γ	Rate of movement from state D to state X	4.21e-5 - 5.48e-4	day^{-1}	Reciprocal of time for an immune person to become susceptible (5-65 years)	γ
σ	Rate of movement from state D to state Z	0.017 - 0.07	day^{-1}	Reciprocal of duration of symptoms (14-60 days)	σ
δ	Fraction in state D who die due to disease per day	0.001 - 0.005	day^{-1}	0.1-0.5% case-fatality due to disease	δ
P_F	Concentration of pathogen in feces	1e3 - 1e5	pathogen/ mg	Concentration of 1e6-1e8 pathogen per gram of feces	R, λ
ζ	Rate of pathogen die-off	0.025 - 0.143	day^{-1}	Reciprocal of survival time of pathogen in fresh water (7-40 days)	ζ
β_0	Background transmission rate	0 - 2e-4	day^{-1}	Calibration simulations using yearly incidence of disease of 5-15 per 100,000 (see text)	β_0
β_{Exp}	Disease transmission function parameter	0.1 - 0.5		Result of fitting transmission function to dose response data (see text)	β
λ_F	Rate of pathogen shedding per infectious swimmer	1e8 - 4e10	pathogen/ hour	Most probable range of shedding distribution (see text)	λ

Table 3.11

Water Reclamation Alternative-Dependent Parameters for Non-Restricted Recreational Impoundment

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	1		Assumption that 100% of population swims	β, λ
β_I	Rate of water ingestion	0.03 - 0.05	liters/hour	Assumption that 30 - 50 ml/hour of water is ingested during swimming	β
β_{TsHour}	Number of hours per day exposure occurs	0.5 - 3	hours /day	National average of 2.6 hours for children and 0.5 hours for adults swimming per day	β
β_{TsDay}	Number of days per year exposure occurs	1 - 40	days/year	Assumption that individuals swim 1-40 days per year	β
T_L	Dilution factor	1e-3 - 1.6e-3		Assumed 5 - 7.5 ft/year evaporation rate for hypothetical impoundment with 10.4 acre surface area and volume of 1.7e8 liters	T
W_V	Volume of impoundment	5e7 - 5e8	liters	Hypothetical impoundment volume	W
P_{VS}	Fraction of impoundment used for swimming	0.05 - 0.15		Assumption that 5 - 15% of impoundment volume is used by swimmers	W
λ_F	Rate of pathogen shedding per infectious swimmer	See Tables 3.3 - 3.10		Most probable range of shedding distribution (see text)	λ

Assumed Title 22 Water Treatment: Tertiary

Table 3.12

Water Reclamation Alternative-Dependent Parameters for Golf Course Irrigation

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	0.05 - 0.15		Assumption that 5-15% of population uses golf course	β, λ
β_I	Rate of water ingestion	0.0005 - 0.0015	liter/hour	Assumption that 0.5 - 1.5 milliliters of water is ingested per day	β
β_{TsHour}	Number of hours per day exposure occurs	1		Not Applicable	β
β_{TsDay}	Number of days out of year exposure occurs	80 - 120	days/year	Assumption that golf course is used 2 days per week	β
T_L	Dilution factor	1		Not Applicable	T
W_V	Volume of impoundment	1		Not Applicable	W
P_{VS}	Fraction of impoundment used for swimming	1		Not Applicable	W
λ_F	Rate of pathogen shedding per infectious swimmer	0		Not Applicable	λ

Assumed Title 22 Water Treatment: Secondary-2.2 to Tertiary

Table 3.13

Water Reclamation Alternative-Dependent Parameters for Restricted Recreational Impoundment

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	0.001 - 0.1		Assumption that 0.1 - 10% of population uses impoundment	β, λ
β_I	Rate of water ingestion	0.0002 - 0.0003	liters/hour	Assumption that 1 ml/day water is ingested	β
β_{TsHour}	Number of hours per day exposure occurs	2 - 6	hours/day	Assumption that impoundment is used 4 hours per day	β
β_{TsDay}	Number of days per year exposure occurs	10 - 20	days/year	Assumption that impoundment is used 14 days per year	β
T_L	Dilution factor	1e-3-1.6e-3		Assumed 5 - 7.5 ft/year evaporation rate for hypothetical impoundment with 10.4 acre surface area and volume of 1.7e8 liters	T
W_V	Volume of impoundment	5e7-5e8	liters	Hypothetical impoundment volume	W
P_{VS}	Fraction of impoundment used for swimming	1		Not Applicable	W
λ_F	Rate of pathogen shedding per infectious swimmer	0		Not Applicable	λ

Assumed Title 22 Water Treatment: Secondary-2.2

Table 3.14

Water Reclamation Alternative-Dependent Parameters for Park Irrigation

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	0.001 - 0.1		Assumption that 0.1 - 10% of population uses park	β, λ
β_I	Rate of water ingestion	0.045 - 0.055	liters/hour	Assumption that 50 ml/hour water is ingested	β
β_{TsHour}	Number of hours per day exposure occurs	0.0833 - 0.25	hours/day	Assumption that park is used 0.0833 - 0.25 hours/day	β
β_{TsDay}	Number of days per year exposure occurs	50 - 70	days/year	Assumption that park is used 61 days per year	β
T_L	Dilution factor	1		Not Applicable	T
W_V	Volume of impoundment	1		Not Applicable	W
P_{VS}	Fraction of impoundment used for swimming	1		Not Applicable	W
λ_F	Rate of pathogen shedding per infectious swimmer	0		Not Applicable	λ

Assumed Title 22 Water Treatment: Tertiary

Table 3.15

Water Reclamation Alternative-Dependent Parameters for Industrial Cooling Tower

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	0.0001 - 0.001		Assumption that 0.01 - 0.1% of population performs maintenance of cooling tower	β, λ
β_I	Rate of water ingestion	0.045 - 0.055	liter/hour	Assumption that 50 ml/hour water is ingested during maintenance	β
β_{TsHour}	Number of hours per day exposure occurs	2 - 6	hours/day	Assumption that maintenance occurs 4 hours per day	β
β_{TsDay}	Number of days per year exposure occurs	4 - 8	days/year	Assumption that maintenance occurs 6 days per year	β
T_L	Dilution factor	1		Not Applicable	T
W_V	Volume of impoundment	1		Not Applicable	W
P_{VS}	Fraction of impoundment used for swimming	1		Not Applicable	W
λ_F	Rate of pathogen shedding per infectious swimmer	0		Not Applicable	λ

Assumed Title 22 Water Treatment: Secondary-2.2 to Tertiary

Table 3.16

Water Reclamation Alternative-Dependent Parameters for Irrigation of Food Crops

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	1		Assumption that 100% of population ingests food crops irrigated with reclaimed water	β, λ
β_I	Rate of water ingestion	0.005 - 0.015	liters/hour	Assumption that 10 ml/day reclaimed water on food crops is ingested	β
β_{TsHour}	Number of hours per day exposure occurs	1		Not Applicable	β
β_{TsDay}	Number of days per year exposure occurs	365	days/year	Assumption that food crops irrigated with reclaimed water are ingested 365 days per year	β
T_L	Dilution factor	1		Not Applicable	T
W_V	Volume of impoundment	1		Not Applicable	W
P_{VS}	Fraction of impoundment used for swimming	1		Not Applicable	W
λ_F	Rate of pathogen shedding per infectious swimmer	0		Not Applicable	λ

Assumed Title 22 Water Treatment: Tertiary

Table 3.17

Water Reclamation Alternative-Dependent Parameters for Groundwater Recharge

Sampled Parameter	Definition	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
P_S	Fraction of population exposed	1		Assumption that 100% of population uses recharged groundwater	β, λ
β_I	Rate of water ingestion	1.5 - 2.5	liters/hour	Assumption that 1.5 - 2.5 liters of water are ingested per day	β
β_{TsHour}	Number of hours per day exposure occurs	1		Not Applicable	β
β_{TsDay}	Number of days per year exposure occurs	365	days/year	Assumption that recharged groundwater is used as drinking water every day of the year	β
T_L	Dilution factor	0.5		Assumption that groundwater is recharged 50% by volume	T
W_V	Volume of impoundment	1		Not Applicable	W
P_{VS}	Fraction of impoundment used for swimming	1		Not Applicable	W
λ_F	Rate of pathogen shedding per infectious swimmer	0		Not Applicable	λ

Assumed Title 22 Water Treatment: Tertiary

4.0 APPLICATION OF RISK ASSESSMENT MODEL TO CRYPTOSPORIDIOSIS OUTBREAK IN MILWAUKEE

In Chapter 2.0 we calibrated the model using data from a Vermont study that assessed the incidence of giardiasis during non-outbreak conditions. Based on these data we developed a model of a closed community that used reclaimed water to fill a recreational swimming impoundment. We explored the relative risk of using reclaimed water and ran a sensitivity analysis. In this chapter we apply the model to an outbreak of cryptosporidiosis which occurred in Milwaukee in 1993. As with the *Giardia* study, the model was calibrated to background conditions. We then performed simulations of the outbreak scenario and analyzed the parameter ranges required to recreate the outbreak.

4.1 Description of Milwaukee Outbreak

During March and April 1993 a massive outbreak of *Cryptosporidium* infection associated with potable water supplies occurred in the Milwaukee area, as described in a study by Mac Kenzie et al.[1] Investigation of the water treatment facilities suggested that from March 23 to April 9 one of the two plants serving the area failed to adequately remove *Cryptosporidium* oocysts. It was estimated that over 400,000 cases of cryptosporidiosis were attributed to the plant failure. Figure 4.1 shows the period of the plant failure and the increase in cases of watery diarrhea associated with the outbreak.

From Mac Kenzie's description of the outbreak, the following information was obtained and used to parameterize the model:

- The total population of the greater Milwaukee area was 1,610,000.
- The plant failure lasted from March 23 to April 9.
- An estimated 403,000 cases of watery diarrhea associated with the plant failure occurred during a 2-month period.
- *Cryptosporidium* oocysts were detected at concentrations between 0.07 and 0.13 oocysts/L of water in samples of ice made from finished water during the plant failure.

4.2 Parameterization of Milwaukee Outbreak Simulations

The basic model used for the reclaimed water scenario of Chapter 2.0 was modified for this application. First, since this application models ingestion of treated surface water and wastewater is not involved, it was not necessary to model community wastewater. The effect on the output by this portion of the model was removed by setting the sampled parameters R_F, the weight of feces released into wastewater per day per individual and R_L, the volume of water produced per individual per day, to zero. Second, I, the number of pathogen from external sources per liter water entering the treatment plant, was used to represent the concentration of oocysts in the untreated water entering the treatment facility. Since R_L was set to zero, I_L, the flow rate of external wastewater (for this application untreated surface water) entering the treatment plant cancels out of the model mathematically; its value therefore has no effect on the output (see the equation for I in Table 2.4). Since R_L was set to zero and I_L cancels out, *I* became equivalent to the sampling parameter I_C. Finally, the route of exposure was ingestion of potable water. There was therefore no direct shedding of microorganisms into the treated water (unlike the swimming scenarios in Chapter 2.0), and the sampling parameter λ_F, the rate of pathogen shedding per infectious swimmer, was set to zero. Table 4.1 summarizes the

non-zero parameter values used to simulate the Milwaukee outbreak. Microbiological dependent-parameters were the same as the values previously described for *Cryptosporidium* in Chapter 3.0 (see Table 3.4). The range for β_I, the rate of water ingestion, was selected around a two liters/day rate obtained from the U.S. EPA.[2] Parameters listed as "Not Applicable" do not pertain to this application. These parameters are used as multipliers in model equations and were set to one, and therefore did not affect the model output. Parameter ranges derived using the data in Mac Kenzie et al.[1] are described below.

X_0 The susceptible population was 800,000 individuals. (It was assumed that approximately half the population of the greater Milwaukee area was exposed to the inadequately treated water).

T_E Treatment efficiency was a function of time. From day zero (January 1) through day 82 (March 23) of the simulation it was assumed that the plant operated at a normal treatment efficiency. Normal treatment (T_{EN}) was defined as 1 to 3 log removal of oocysts. This range was selected in part based on the proposed modifications to the Surface Water Treatment Rule in Volume 59, No. 145 of the Federal Register (July 29, 1994) which state that "conventional water treatment may not reliably achieve more than 2.5 to 3 log *Cryptosporidium* removal " and "2 log removal of *Cryptosporidium* is feasible using current conventional treatment methods." At day 82 of the simulation, it was assumed that the plant changed from operating at a normal treatment efficiency to an inadequate treatment efficiency. Inadequate treatment (T_{EI}) was defined as 0 to 1 log removal. At day 99 of the simulation (April 9), it was assumed that the plant changed from inadequate treatment back to normal treatment, and remained at the normal treatment efficiency until the end of the one-year simulation. Figure 4.1 shows the periods of normal and inadequate treatment.

I_C The concentration of oocysts in the untreated water during the plant failure is unknown. However, data do exist for the concentration of oocysts in ice made from the finished water during the failure, as described previously. Assuming that this was the concentration of the treatment plant effluent during the failure, it is possible to estimate the concentration of oocysts in the treatment plant influent. Given an oocyst concentration in the effluent of 0.07 to 0.13 oocysts/L and a treatment level of 0 to 1 log removal during the failure, the concentration of oocysts in the untreated water would range from 0.07 to 1.3 oocysts/L. The parameter I_C was sampled from a slightly larger range, 0.01 to 10 oocysts/L.

β_0 Calibration simulations were performed to obtain sets of parameter values that produced results consistent with non-outbreak conditions, as was done for the background scenario described in Chapter 2.0. As in Chapter 2.0, the background scenario used only those 10 parameters not related to exposure to reclaimed water (potable water for this application). As did Mac Kenzie et al.[1], we define a case of probable *Cryptosporidium* infection as the onset of watery diarrhea and use a non-outbreak incidence rate of watery diarrhea of 0.5 percent per month. This monthly rate is equivalent to an annual incidence rate of 6%. For the calibration simulations an acceptance criterion of annual incidence between 0.06 to 6 percent was selected. Simulations that produced incidence results within that range were produced only by values of β_0 less than 0.003. Hence, β_0 was sampled between 0 and 0.003.

4.3 Simulation Approach

Figure 4.2 is a flow diagram that describes the simulation procedure used to study the Milwaukee outbreak.

First, simulations were performed to obtain sets of parameter values that produced results consistent with non-outbreak conditions, as described above. Simulations that resulted in an incidence between 0.006 and 6% were saved. The simulation process was repeated until 500,000 such parameter sets were obtained.

These 500,000 parameter sets were then combined with the remaining sampling parameters to run simulations modeling a plant failure between day 82 (March 23) and day 99 (April 9) of the simulations.

Each "outbreak" simulation was considered a pass if its output met the following criteria: 1) the number of new cases for the two-month period beginning March 23 was between 300,000 and 500,000; and 2) the number of new cases had fallen to a level between 500 and 5,000 cases per day by the end of the year. This range was based on data from Mac Kenzie et al.[1] indicating that between one and ten cases of watery diarrhea occurred per day during the non-outbreak period from March 1 to March 23 for 1,663 Milwaukee residents surveyed; this was extrapolated to the total simulation population (see Figure 4.1).

4.4 Results and Analysis of Simulations

Only 124 of the 500,000 simulations passed the outbreak criteria, a 0.0025% pass rate. The parameter sets associated with these 124 simulations, each one a point in parameter space, were each labeled a pass. The parameter sets associated with the remaining 499,876 simulations were each labeled a fail. Using these data, cumulative distributions that represent the probability of a particular parameter value being associated with a pass, $F(\tau|P)$, or a fail, $F(\tau|F)$, were plotted (Figure 4.3 a and b). For a hypothetical parameter τ, the function $F(\tau|P)$ is defined as a standard cumulative distribution, where for any given value τ, $F(\tau|P)$ is the probability that the parameter values equal to or less than τ are associated with a pass. In each of the graphs in Figures 4.3 a and b the straight line represents a uniform distribution and represents the cumulative distribution of the parameters associated with a fail. Whenever the pass rate is below 1%, the distribution of the parameters associated with a fail can be approximated by a uniform distribution.[3]

By comparing $F(\tau|P)$ and $F(\tau|F)$, information on the importance of a given parameter in eliciting the criteria-defined outbreak may be obtained from the model. If both of these distributions are the same, then the univariate analysis provides no information on whether or not the given parameter has any influence on the classification of an output as either a pass or a fail. Only a multivariate analysis can provide further information. However, the greater the difference between the two distributions, the more sensitive the behavior of the model is to the parameter value. Therefore, parameters may be ranked by their importance in eliciting a specific response for the model by establishing a quantitative or statistical measure of the amount by which the pass/fail distributions differ.

Statistically, two independent samples can be tested to see if they are drawn from the same population distribution function. Given that the distributions are continuous, the best statistical test of this null hypothesis is the Kolmogorov–Smirnov (K-S) test.[4] The statistic in this test is D, the maximum value of the absolute difference between the two

cumulative distribution functions:

$$D = \max \mid F(\tau|P) - F(\tau|F) \mid$$

The probability that the distance measure, D, is greater than the actual distance is:

$$2 \cdot \sum_{j=1}^{\infty} (-)^{j-1} \cdot e^{-2j^2\lambda^2}$$

where

$$\lambda = \sqrt{\frac{N_1 \cdot N_2}{N_1 + N_2}}$$

N_1 and N_2 are the number of parameter sets eliciting a pass or a fail, respectively.[4] This probability measures the level of significance of the statistic D.

Initially, the D-statistic was used to determine whether there were any statistical differences between the pass and fail distributions. Once D was shown to be statistically significant, the D value was used as a measure of sensitivity. The determination of whether an output response will be classified as a pass or a fail becomes very sensitive to the fluctuations of a particular parameter when the associated D statistic becomes large. Therefore, parameters were ranked by their respective D statistics, reflecting their importance in a particular simulation output classified as a pass.

Table 4.2 shows the statistically significant ($P \leq 0.05$) D statistics obtained from the K-S test. Of the nine parameters associated with a statistically significant result, two contained large distance statistics because they did not span the full range from which they were originally sampled (see Figure 4.3a). The parameter I_C, the concentration of oocysts in the untreated water, was never larger than 2.9 oocysts/L in the parameter pass-set, and the parameter β_0 was never larger than 0.0008 per day. One other parameter associated with a large distance statistic, T_{EN}, was only larger than 0.025 for one of the simulations. The next two largest distance statistics were associated with σ, the rate of recovery from the symptomatic state (state D) to state Z and ζ, the fraction of oocysts in water which become non-viable per day. Both of these distributions were skewed to the right. For both parameters most of the pass simulations occurred for the top half of the originally sampled range, 88% for σ ($\sigma > 0.25$) and 82% for ζ ($\zeta > 0.15$). The four other parameters that were statistically significant (ρ_T, γ, α_{RAND}, and β_{EXP}) had relatively small D-statistics, showing no obvious trend in the distribution plots (Figure 4.3a).

The remaining parameters were associated with distributions that resulted in statistically insignificant K-S tests.

4.5 Conclusions

Using data obtained from a retrospective study, we produced a parameterized model that describes the epidemiology of a cryptosporidiosis outbreak in Milwaukee. As with previous modeling of *Giardia* (Chapter 2.0), the parameterization process resulted in a multivariate parameter distribution where any parameter set within that distribution produced an output consistent with the data. This parameter space defines the model. The low pass-rate means that the criteria used in this application constrained the pass-space to a relatively small volume compared with the *a priori* volume without constraining criteria. To be classified as a pass, a simulation was required to produce an output that

could describe the Milwaukee outbreak both with respect to the outbreak and background incidence of disease and the timing of the onset of the outbreak and subsequent decrease back to background incidence. For the parameter ranges used, these turned out to be strict criteria, i.e., results of the Monte Carlo simulation suggested that few parameter sets could reproduce the outbreak incidence and subsequently return to background incidence.

Exploration of the pass-space through univariate analysis indicated that three parameters were highly constrained. The parameter I_C, the concentration of oocysts in the untreated water, did not produce any passes above 2.9 oocysts/L. Approximately 80% of the passes were below 1.3 oocysts/L, the high end of the range calculated for I_C (the sampled range was 0.01 to 10 oocysts/L). The background transmission parameter, β_0, did not produce passes above 0.00074 per day, suggesting that the 6% per year background incidence rate may have been too high. Finally, the criteria constrain the normal treatment parameter, T_{EN}, away from 1 log removal towards 2 to 3 log removal. This suggests that a 2 to 3 log normal removal rate is more consistent with the data than is a 1 log removal.

Two other parameters that are of interest are biological parameters σ, the rate of recovery from the symptomatic state (state D) to state Z, and ζ, the fraction of oocysts in water that become non-viable per day. Given the ranges that were originally sampled, both of these parameters were skewed to higher values. That is, given a range for σ of 0.003 per day to 0.5 per day, the criteria biased the values toward faster recovery rates. Likewise, the parameter ζ, whose range was originally set at 0.0056 to 0.33, was biased toward higher rates of loss of viability.

Analysis of the results of these simulations pointed toward parameters that in the context of the model's structure and the parameter ranges selected require better definition. That is, given the parameter ranges that were sampled, the D statistic ranked each parameter as to the importance of its specific value in determining whether or not a simulation could reproduce outputs consistent with the data. This ranking helped identify the parameters that require better definition to decrease the uncertainty of the model outputs.

Chapter 4.0 - References

1. Mac Kenzie, W.R., et al. "A Massive Outbreak in Milwaukee of *Cryptosporidium* Infection Transmitted through the Public Water Supply," The New England Journal of Medicine, 331:3, 161-7 (1994).

2. Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part A) (Office of Emergency and Remedial Response, US Environmental Protection Agency, Washington, DC, 1989).

3. Hornberger, G.M., and R.C. Spear, "Eutrophication in Peel Inlet: I. The Problem: Defining Behavior and a Mathematical Model for the Phosphorous Scenario," Water Research, 14, 29-42 (1980).

4. Press W.H., B.P. Flannery, S.A. Teukolsky, and W.T. Vetterling, Numerical Recipes in C: The Art of Scientific Computing, (Cambridge University Press, Cambridge, 1992).

Table 4.1

Parameterization for Milwaukee *Cryptosporidium* Outbreak

Sampled Parameter	Description	Range of Sampled Parameter	Units of Sampled Parameter	Dependent Parameter
Biological Parameters:				
ρ_T	Incubation period	2 - 14	days	ρ
ρ_P	Fraction of state Y that moves to state D	0.8 -1		ρ
α_{Rand}	Fraction of state Y that does not move to state D that moves to state Z per day	0 - 1		α
γ	Rate of movement from state Z to state X	5.6e-3 - 0.033	day^{-1}	γ
σ	Rate of movement from state D to state Z	0.033-0.5	day^{-1}	σ
a	Rate of migration of new susceptible individuals into population	6.85e-5 - 9.59e-5	day^{-1}	a
μ	Rate of death due to natural causes	1.37e-5 - 4.11e-5	day^{-1}	μ
β_o	Baseline transmission rate	0 - 0.003	day^{-1}	β_o
β_{Exp}	Disease transmission function parameter	0.07 - 0.12		β
ζ	Rate of oocyst die-off	5.6e-3 - 3.3e-2	day^{-1}	ζ
Community Scenario Parameters:				
X_o	Initial number of individuals in state X	8e5		X
P_S	Fraction of population exposed	1		β
β_I	Rate of water ingestion	1.5 - 2.5	liters/hour	β
β_{Tshour}	Number of hours per day exposure occurs (Not Applicable)	1	hours/day	β
β_{Tsday}	Number of days per year exposure occurs	365	days/year	β
I_C	Concentration of oocysts in raw water entering treatment plant	0.01 - 10	oocysts/liter	I
W_V	Volume of impoundment (Not Applicable)	1	liters	W
P_{VS}	Fraction of impoundment used for swimming (Not Applicable)	1		W
Water Treatment Scenario Parameters:				
T_{EN}	Fraction of oocysts remaining after normal water treatment	0.001 - 0.1		T
T_{EI}	Fraction of oocysts remaining after inadequate treatment	0.1 - 1		T
T_L	Fraction of impoundment volume which evaporates per day (Not Applicable)	1	day^{-1}	T

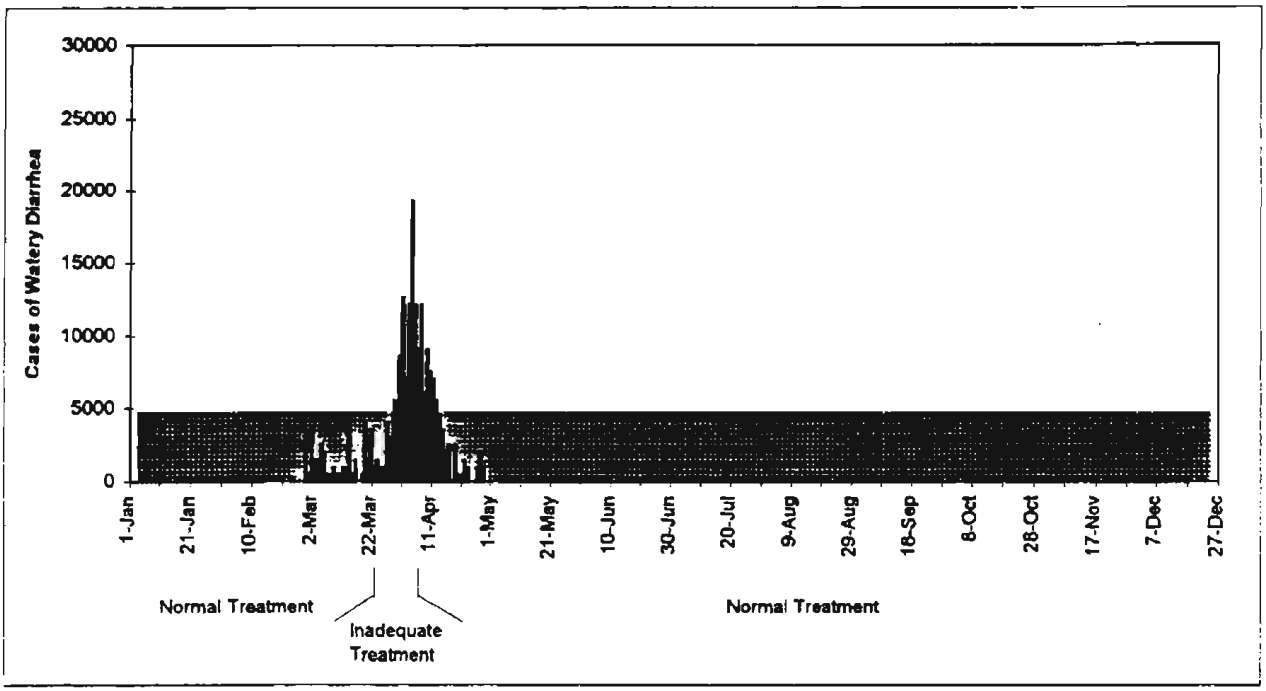
Table 4.2

Kolmogorov-Smirnov Test Results

Parameter	Distance
I_C	0.80
T_{EN}	0.79
β_0	0.75
σ	0.42
ζ	0.33
ρ_T	0.24
γ	0.20
α_{RAND}	0.17
β_{EXP}	0.15

Figure 4.1

Milwaukee *Cryptosporidium* Outbreak
(Data Extrapolated from Mac Kenzie et al., 1994)



The gray shaded area represents the extrapolated background data.

Figure 4.2

Milwaukee Outbreak Simulation Methodology

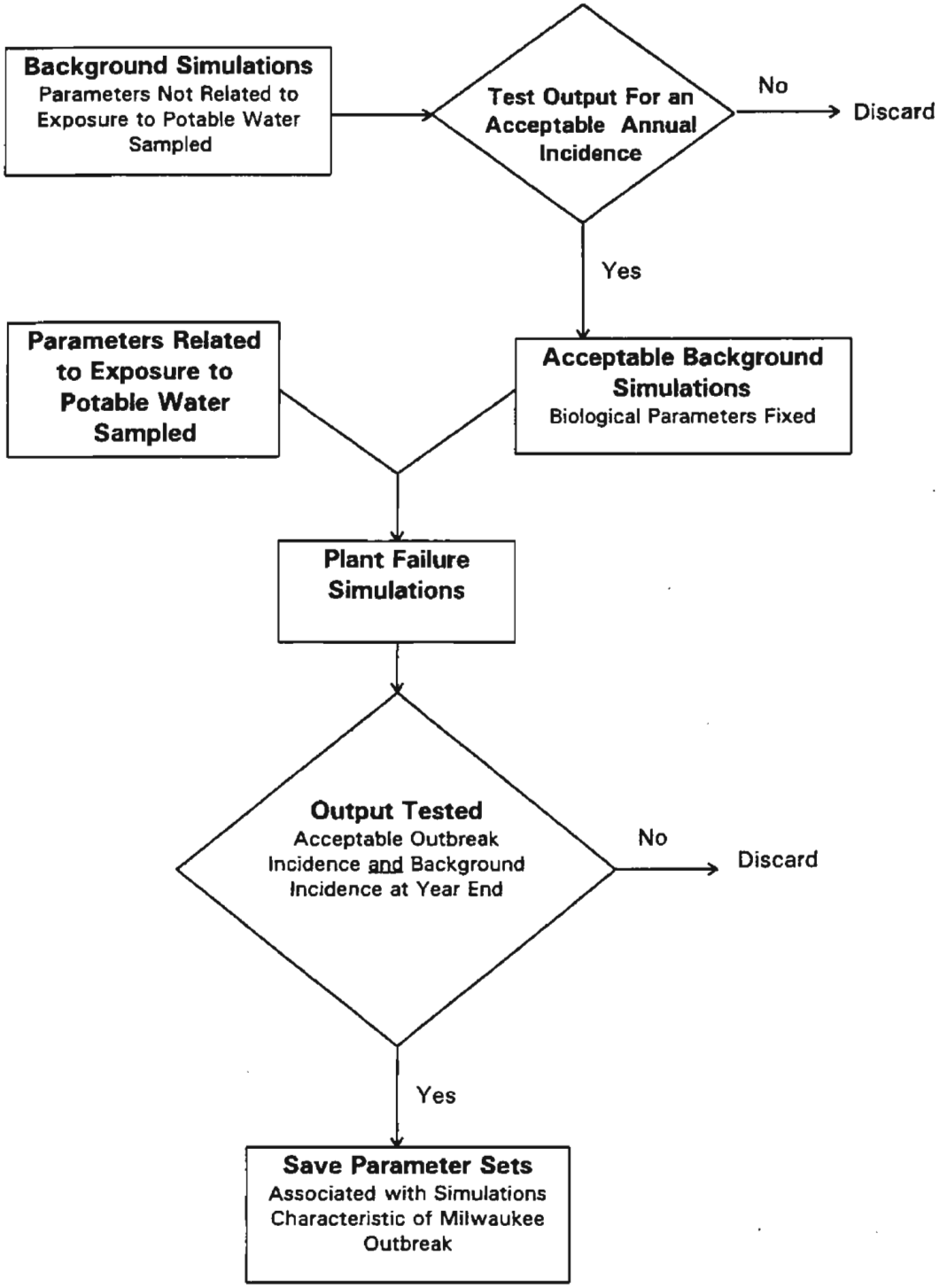


Figure 4.3a

Cumulative Distributions for Milwaukee *Cryptosporidium* Outbreak Parameters
(Statistically Significant Parameters)

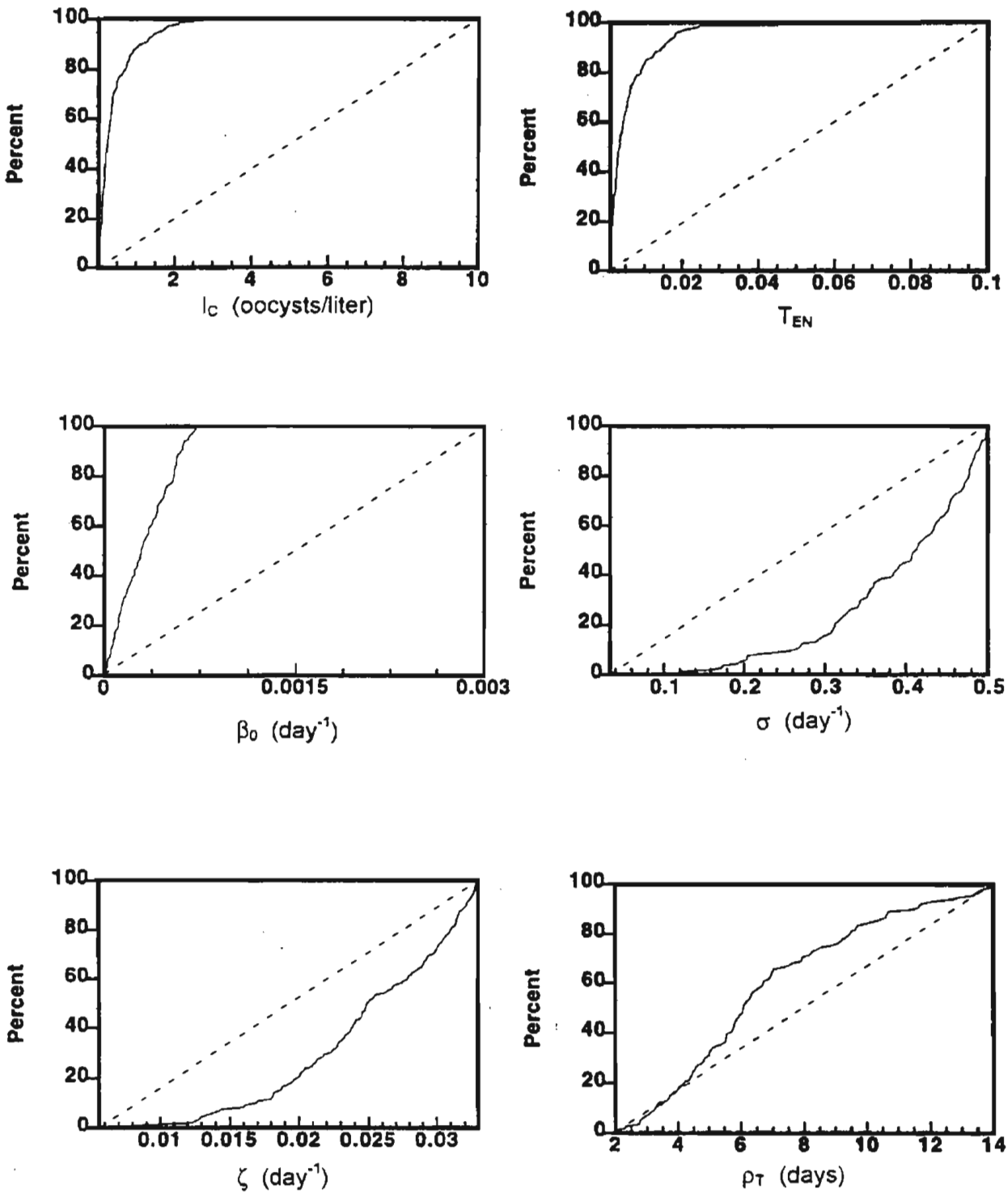
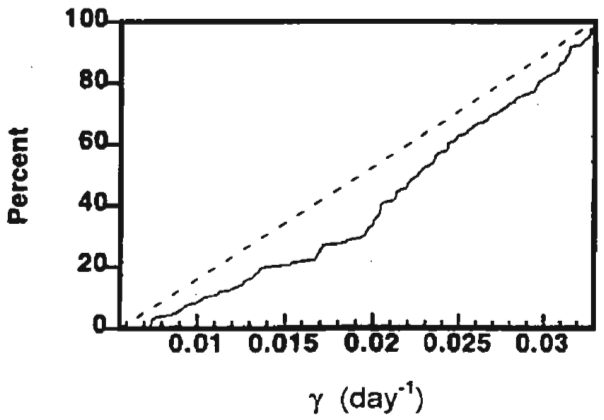
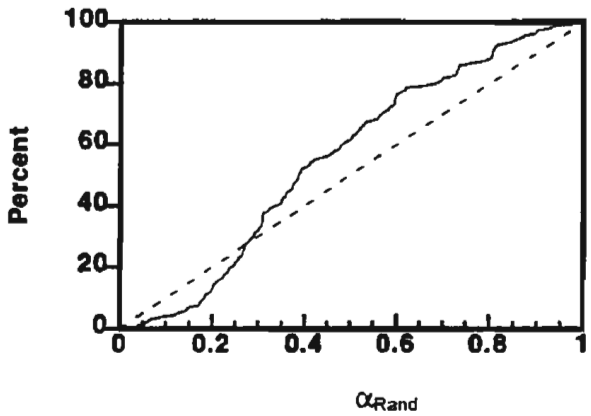


Figure 4.3a (Continued)

Cumulative Distributions for Milwaukee *Cryptosporidium* Outbreak Parameters
(Statistically Significant Parameters)





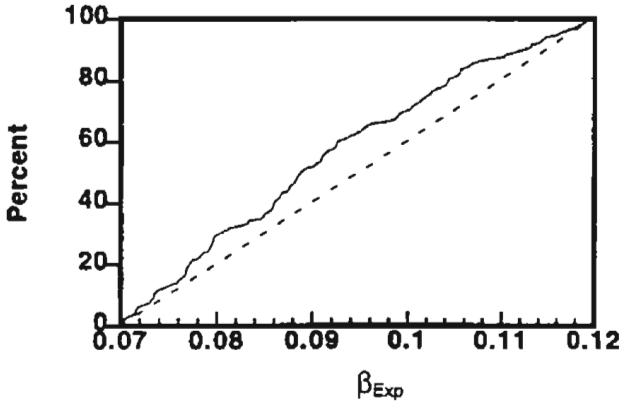
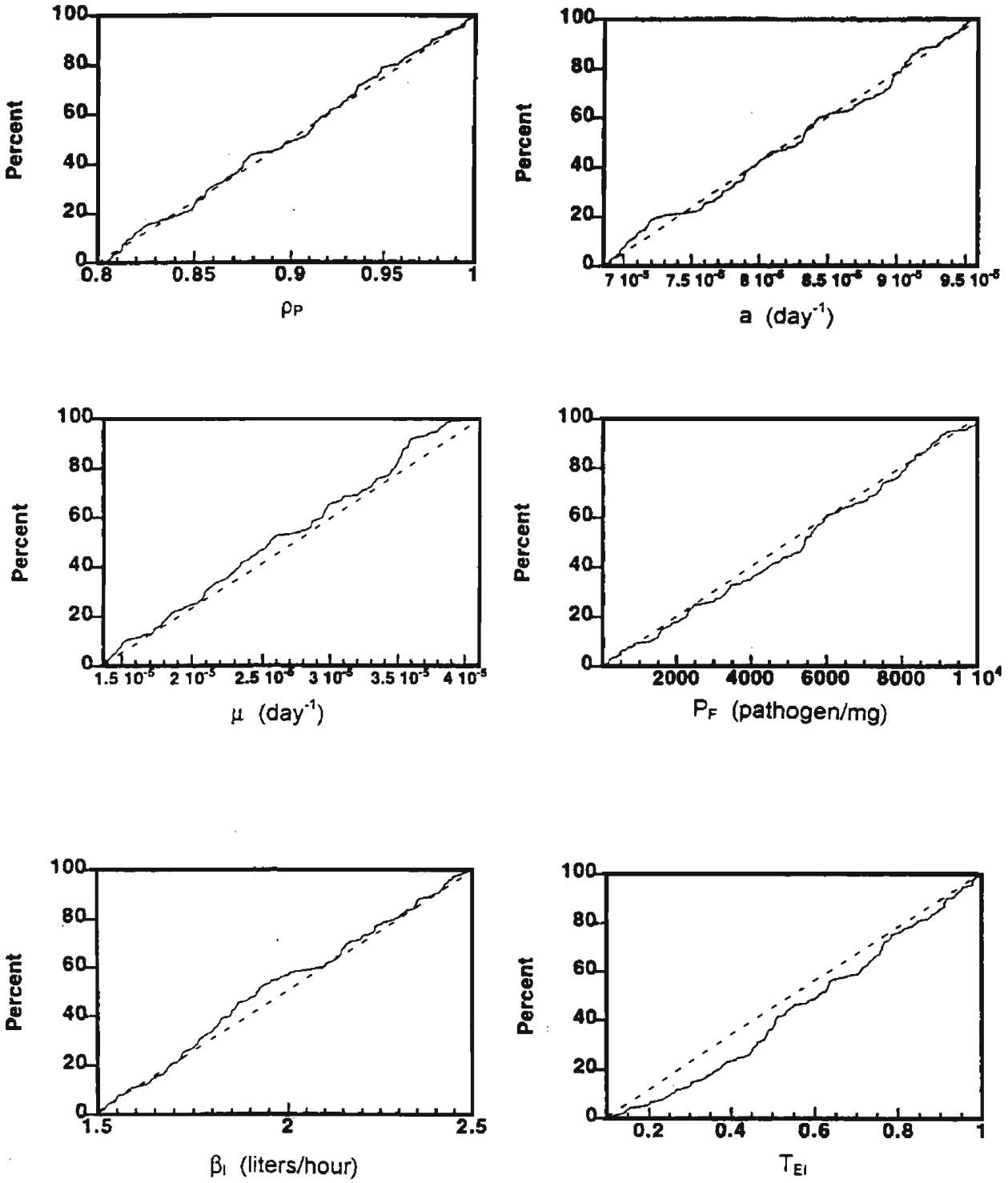


Figure 4.3b

Cumulative Distributions for Milwaukee *Cryptosporidium* Outbreak Parameters
(Not Statistically Significant Parameters)



5.0 MODEL APPLICATION RESULT SUMMARIES FOR ADDITIONAL MICROORGANISM AND WATER RECLAMATION ALTERNATIVE SCENARIOS

In Chapter 2.0, we examined a case study of exposure to *Giardia lamblia* via ingestion of water during swimming in an impoundment. In this chapter, we apply the model to other microorganism and water reclamation alternative scenarios. Since a large number of other scenarios were modeled, we did not attempt to perform an in-depth case study for each scenario (as was done earlier for *Giardia* and the swimming impoundment) but instead briefly summarize the results for each of the scenarios here.

Simulations were performed for the combinations that result (including *Giardia* and a swimming impoundment) when one water reclamation alternative and one microorganism are selected from the following lists of selected microorganisms and alternatives from Chapter 3.0 (note that the microorganism rotavirus was also originally selected and included in the literature search, but was not included in the risk assessment, for reasons explained in Chapter 3.0).

Microorganisms:

1. *Giardia lamblia*
2. *Cryptosporidium* spp.
3. *Shigella* spp.
4. *Salmonella* spp.
5. *Vibrio Cholerae*
6. Pathogenic *E. coli*
7. Enteroviruses
8. Hepatitis A virus

Water Reclamation Alternatives:

1. Non-Restricted (Swimming) Recreational Impoundment
2. Golf Course Irrigation
3. Restricted Recreational Impoundment
4. Park Irrigation
5. Industrial Cooling Tower
6. Irrigation of Food Crops
7. Groundwater Recharge

For each microorganism, a background scenario assessing the prevalence of disease when reclaimed water is not the mode of transmission was first modeled. Subsequent simulations of the different water reclamation alternatives were then compared with this background scenario. For the swimming impoundment reclamation alternative, an additional scenario was modeled, which was swimming in an impoundment filled with water from a pathogen-free source.

The sections that follow describe the parameters used in the simulations, the simulation approach and the results.

5.1 Microorganism- and Water Reclamation Alternative-Independent Parameters

Of the 26 sampled parameters in the model (see Chapter 2.0), 8 are independent of both the microorganism and the water reclamation alternative. The microorganism- and water reclamation alternative-independent parameters are α_{Rand}, a, μ, X_0, I_L, I_C, R_L and R_F. The

ranges sampled for these parameters are given in Table 5.1. Note that some of these values were set to zero for the background scenario, as described in Section 5.6.

5.2 Microorganism Parameterization

Assumed sampling ranges for each of the microorganism-dependent parameters were selected for each microorganism using the results of the literature review, as summarized in Chapter 3.0 (see Tables 3.3 through 3.10). Method 1 described in Section 2.3 was used to calculate λ_F, the number of pathogen shed per hour swimming by an infected person.

5.3 Water Reclamation Alternative Parameterization

Each of the water reclamation alternatives is described in Chapter 3.0, Section 3.3. Each alternative is parameterized separately. This parameterization is described in Tables 3.11 through 3.17, which give the sampled ranges for each parameter. Parameters listed as "Not Applicable" do not pertain to the particular alternative. These parameters are set to zero if used as an additive value in a model equation or set to one if used as a multiplier in a model equation, and therefore do not affect the model output.

5.4 Wastewater Treatment Parameterization

The wastewater treatment parameter T_E, the fraction of pathogen remaining after treatment, is dependent upon both the microorganism and the water reclamation alternative being modeled. Tables 3.11 through 3.17 in Chapter 3.0 specify the Title 22 level of treatment selected for each water reclamation alternative.

Table 5.2 summarizes the relationship assumed between Title 22 levels of treatment and microorganism-specific log removals used for model parameterization. This relationship is based on pathogen removals by treatment processes given by the USGA.[1]

Note that for some reclamation alternatives, the Title 22 level of treatment is given as "secondary-2.2 to tertiary" in Tables 3.11 through 3.17. For these alternatives the low end of the secondary-2.2 range to the high end of the tertiary range for log removal given in Table 5.2 was used for model parameterization.

T_E is calculated from the log removal as follows:

$$T_E = 10^{-t}$$

where t is the log removal.

5.5 Non-Restricted (Swimming) Recreational Impoundment with Pathogen-Free Water Source Parameterization

This scenario uses the parameters described in Chapter 3.0, Table 3.11 for the non-restricted (swimming) recreational impoundment. The swimming impoundment is filled with water from a pathogen-free source; T_E is therefore set to zero. Pathogen enter the water only via swimmers shedding directly into the swimming water.

5.6 Simulation Approach

We used the same general simulation approach described in Chapter 2.0. For each microorganism, simulations were performed until 6,000 sets of parameter values were produced consistent with the background incidence range of the disease (Tables 3.3

through 3.10) associated with that microorganism. Since none of the parameters related to exposure to reclaimed water was required for this scenario, these simulations used only 10 of the 26 sampling parameters (X_0, ρ_T, ρ_P, a_{Rand}, a, σ, γ, μ, β_0, δ). Five of the remaining 16 sampling parameters (I_C, R_F, T_E, P_S and λ_F) were set to zero, which removes their effect on the output of the model and results in the rest of these 16 sampling parameters mathematically canceling out of the model.

Of the above 10 sampling parameters, 6 are microorganism-dependent (ρ_T, ρ_P, σ, δ, γ, β_0). These parameters were sampled from the ranges presented in Chapter 3.0, Tables 3.3 through 3.10. The remaining 4 sampling parameters (X_0, a_{Rand}, a, and μ) are microorganism- and water reclamation alternative-independent and are therefore sampled identically for all microorganisms (see Table 5.1).

Once established for each microorganism, the parameter sets for which a background scenario simulation resulted in an acceptable incidence were used as a basis to perform simulations for each of the water reclamation alternatives in combination with that microorganism.

5.7 Results

For each microorganism, nine sets of 6,000 simulations were performed. The nine sets consisted of the background scenario, the non-restricted (swimming) recreational impoundment with water from a pathogen-free source scenario and each of the seven water reclamation alternative scenarios. The mean and standard deviation for average daily prevalence were computed for each set (Figures 5.1 through 5.8).

For each selected microorganism, Figures 5.1 through 5.8 can be used to compare the average daily prevalences calculated for each of the above nine scenarios. For example, in the case of *Giardia,* Figure 5.1 reveals that even in the presence of substantial uncertainty, exposures in restricted recreational impoundments and from industrial cooling towers result in public health risks indistinguishable from background levels. It should be noted that the prevalences calculated are case-specific and, while the information found in the literature relating to the values of the biological parameters can be used in more general analyses, the exposure-related parameters must be selected for specific sites, populations and water reuse applications.

In order to assess which parameters were important determinants of high prevalence for each microorganism and water reclamation alternative combination, the Kolmogorov–Smirnov (K-S) statistical test described in Chapter 4.0 was again performed. This statistical test is summarized below; Section 4.4 contains a more detailed discussion of this methodology.

Simulations were labeled high-prevalence if their output average daily prevalence was more than one standard deviation above the mean of the average daily prevalence of the 6,000 background simulations, hereafter referred to as the prevalence split point. Simulations which produced output prevalences below the prevalence split point were labeled low-prevalence simulations. Cumulative distributions that represent the probability of a particular parameter value being associated with a high-prevalence simulation, $F(r|H)$, or a low-prevalence simulation, $F(r|L)$, were calculated. These distributions were compared using the K-S test for the distance statistic (D-statistic). The D-statistic was initially used to determine if there were any statistical differences between the high- and low-prevalence distributions. Once D was shown to be statistically significant ($P \leq 0.05$), the D value was used as a measure of sensitivity. The determination of whether a simulation will be classified as high- or low-prevalence becomes very sensitive to the fluctuations of a

particular parameter when the associated *D*-statistic becomes large. Therefore, parameters were ranked by their respective *D*-statistics, reflecting their importance in a particular simulation classified as high-prevalence.

Parameters with significant *D*-statistics greater than 0.7 were defined as the *most important* determinants of high prevalence. Those between 0.5 and 0.7 were defined as *important* determinants of high prevalence. Those between 0.3 and 0.5 were defined as *medium* determinants of high prevalence. Those between 0.1 and 0.3 were defined as *less important,* and those smaller than 0.1 were defined as *least important* determinants of high prevalence.

Tables 5.3 through 5.9 summarize the results of the K-S tests for each microorganism, except pathogenic *E. coli*. K-S statistics were not calculated for pathogenic *E. coli* because the prevalence output for each reclamation alternative was the same as the background prevalence. A summary of Tables 5.3 through 5.9 listing the parameters that fell into the two highest importance categories is given below:

Giardia lamblia:

Water Reuse Alternative	Most Important Parameters	Important Parameters
Swimming Impoundment	β_{TSDAY}	
Golf Course Irrigation	T_E	
Restricted Recreational Impoundment	σ	
Park Irrigation		T_E
Industrial Cooling Tower		T_E
Irrigation of Food Crops	T_E	
Groundwater Recharge	P_F, T_E	σ, R_F

Cryptosporidium:

Water Reuse Alternatives	Most Important Parameters	Important Parameters
Swimming Impoundment		σ
Golf Course Irrigation		
Restricted Recreational Impoundment	σ	
Park Irrigation		σ
Industrial Cooling Tower		σ
Irrigation of Food Crops		T_E
Groundwater Recharge		P_F, T_E

Shigella:

Water Reuse Alternatives	Most Important Parameters	Important Parameters
Swimming Impoundment		β_{TSDAY}
Golf Course Irrigation	σ	
Restricted Recreational Impoundment	σ	
Park Irrigation	σ	
Industrial Cooling Tower	σ	
Irrigation of Food Crops	σ	
Groundwater Recharge		T_E

Salmonella:

Water Reuse Alternatives	Most Important Parameters	Important Parameters
Swimming Impoundment		
Golf Course Irrigation	σ	
Restricted Recreational Impoundment	σ	
Park Irrigation	σ	
Industrial Cooling Tower	σ	
Irrigation of Food Crops	σ	
Groundwater Recharge		σ

Vibrio cholerae:

Water Reuse Alternatives	Most Important Parameters	Important Parameters
Swimming Impoundment		
Golf Course Irrigation		σ
Restricted Recreational Impoundment		σ
Park Irrigation		σ
Industrial Cooling Tower		σ
Irrigation of Food Crops		σ
Groundwater Recharge		σ

Pathogenic *E. coli*

K-S statistics were not calculated for this microorganism because the prevalence output for each reclamation alternative was the same as the background prevalence.

Enteroviruses

There were no *most important* or *important* parameters for this microorganism.

Hepatitis A

Water Reuse Alternatives	Most Important Parameters	Important Parameters
Swimming Impoundment		β_0
Golf Course Irrigation	T_E	
Restricted Recreational Impoundment		σ
Park Irrigation		σ
Industrial Cooling Tower		T_E
Irrigation of Food Crops		T_E
Groundwater Recharge	T_E	

Examination of the above summary reveals that σ, the fraction of individuals in state D who move to state Z per day, was overall most often identified as *most important* or *important* for all the microorganism and water reclamation alternative combinations. This parameter was identified as *most important* a total of 12 times and *important* a total of 13 times. The second-most identified parameter was T_E, the fraction of pathogen remaining after water treatment. This parameter was identified as *most important* 5 times and *important* 7 times. All other parameters were identified as *most important* or *important* at most once. It should be noted that this analysis is case-specific and the results may not be applicable to different populations and/or scenarios.

Chapter 5.0 - References

1. Wastewater Reuse for Golf Course Irrigation (USGA, Lewis Publishers, Boca Raton, FL, 1994).

Table 5.1

Water Reuse Alternative- and Microorganism-Independent Parameters

Sampled Parameter	Description	Range of Sampled Parameter	Units of Sampled Parameter	Basis of Sampled Parameter	Dependent Parameter
α_{Rand}	Fraction of state Y that does not move to state D that moves to state Z per day	0 -1		Randomly generated number from 0 to 1	α
a	Rate of migration of new susceptible individuals into population	6.85e-5 - 9.59e-5	day^{-1}	Birth rate	a
μ	Rate of death due to natural causes	1.37e-5 - 4.11e-5	day^{-1}	Death rate	μ
X_0	Initial number of individuals in state X	1e5		All output "per 100,000"	X
I_L	Flow rate in of external wastewater	0	liters/day	No external wastewater modeled	I
I_C	Concentration of pathogen in external wastewater	0	pathogen/ liter	No external wastewater modeled	I
R_L	Volume of wastewater produced per individual per day	400 - 600	liters/day	Assumption that average volume of wastewater produced by an individual per day is 500 liters	R
R_F	Weight of feces released into wastewater per day per individual	2.5e4 - 2e5	mg/day	Assumption that an average person produces 25 to 200 grams of feces per day	R

Table 5.2

Assumed Log Removal of Microorganisms by Title 22 Wastewater Treatment

	SECONDARY-2.2	TERTIARY
PROTOZOA	0.3 - 1.5	1.8 - 6.3
BACTERIA	1.9 - 4.4	4 - 10.3
VIRUSES	0.3 - 3.1	3.8 - 8.7

Note: Above table is based on pathogen removals by treatment processes given by the USGA.[1]

Table 5.3

Giardia : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T				--	--		0
ρ_P					--	--	
α_{RAND}	-	--		--	--	-	0
a							
σ	-	-	++	-	0	-	+
γ	-	--					
μ		--		--			
β_0			--	--	--	--	0
λ_F	0						
P_F		-		-	--	-	++
P_S		--	--	-	-		
β_I		--	--	--	--	-	
β_{EXP}	--	--	--	--	--	-	0
β_{TSHOUR}	0			--	--		
β_{TSDAY}	++	--		--			
R_F		--		--	--	-	+
R_L			--				
T_E		++		+	+	++	++
T_L							
ζ						--	
W_V	-						
P_{VS}							

SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for *Giardia* for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smirnov equality of distribution test. A discussion of the procedure can be found in Section 5.7. *Giardia* is described in Appendix A, and each of the water reclamation alternatives is described in Section 3.3.

Table 5.4

Cryptosporidium : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T	-						
ρ_P							
α_{RAND}	0	--		--	--	--	-
a							
σ	+	0	++	+	+	-	-
γ	-						
μ							
β_0	0		-	--	--	--	--
λ_F							
P_F		-		-	-	0	+
P_S				--	--		
β_1		--				--	
β_{EXP}							
β_{TSHOUR}				--			
β_{TSDAY}							
R_F		--		--	--	--	-
R_L							
T_E		0		0	-	+	+
T_L							
ζ		--		--	--	--	--
W_V							
P_{VS}							

SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for *Cryptosporidium* for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smirnov equality of distribution test. A discussion of the procedure can be found in Section 5.7. *Cryptosporidium* is described in Appendix B, and each of the water reclamation alternatives is described in Section 3.3.

Table 5.5

Shigella : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T							
ρ_P		--	--	--	--	--	
α_{RAND}	-	--				--	--
a							
σ	-	++	++	++	++	++	0
γ						--	
μ							
β_0							
δ							
λ_F	0						
P_F		--				-	0
P_S							
β_I	--						
β_{EXP}	--						
β_{TSHOUR}	0						
β_{TSDAY}	+						
R_F						--	--
R_L							
T_E		--				-	0
T_L	--						
ζ	-					--	--
W_V	-						
P_{VS}	--						

SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for *Shigella* for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smirnov equality of distribution test. A discussion of the procedure can be found in Section 5.7. *Shigella* is described in Appendix C, and each of the water reclamation alternatives is described in Section 3.3.

Table 5.6

Salmonella : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T							
ρ_P							
α_{RAND}	-	--	--	--	--		
a							
σ	-	++	++	++	++	++	+
γ							
μ							
β_0	--	-	-	-	-	-	--
δ	--	-	-	-	-	-	-
λ_F	-						
P_F							-
P_S							
β_1	--						
β_{EXP}	-						--
β_{TSHOUR}	-		--	--	--		
β_{TSDAY}	0						
R_F							--
R_L							
T_E							-
T_L							
ζ	--						
W_V	-						
P_{VS}	--						

SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for *Salmonella* for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smirnov equality of distribution test. A discussion of the procedure can be found in Section 5.7. *Salmonella* is described in Appendix D, and each of the water reclamation alternatives is described in Section 3.3.

Table 5.7

Vibrio cholerae : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T		--	--	--	--	--	--
ρ_P							
α_{RAND}	--						
a							
σ	-	+	+	+	+	+	+
γ							
μ		--	--	--	--	--	--
β_0	-	0	0	0	0	0	0
δ							
λ_F	-						
P_F							
P_S							
β_1							
β_{EXP}	--						
β_{TSHOUR}	-						
β_{TSDAY}	0						
R_F							
R_L							
T_E		--	--	--	--	--	--
T_L							
ζ	-						
W_V	-						
P_{VS}	--						

SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for *Vibrio cholerae* for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smirnov equality of distribution test. A discussion of the procedure can be found in Section 5.7. *Vibrio cholerae* is described in Appendix E, and each of the water reclamation alternatives is described in Section 3.3.

Table 5.8

Enteroviruses : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T	--	--	--	--	--	--	--
ρ_P	-	-	-	-	-	-	-
α_{RAND}	0	0	0	0	0	0	0
a							
σ	0	0	0	0	0	0	0
γ	0	0	0	0	0	0	0
μ	--	--	--	--	--	--	--
β_0	-	-	-	-	-	-	-
δ							
λ_F							
P_F							
P_S							
β_I							
β_{EXP}							
β_{TSHOUR}							
β_{TSDAY}							
R_F							
R_L							
T_E							
T_L							
ζ							
W_V							
P_{VS}	--						

SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for the enteroviruses for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smimov equality of distribution test. A discussion of the procedure can be found in Section 5.7. The enteroviruses are described in Appendix G, and each of the water reclamation alternatives is described in Section 3.3.

Table 5.9

Hepatitis A : Important Parameters

Water Reclamation Alternatives

Parameters	SWIM	GOLF	RRI	PARK	ICT	IFC	GNDR
ρ_T	0						
ρ_P	-	--		--	--		--
α_{RAND}	0	--		--	--	--	-
a							
σ	0	-	+	+	0	-	-
γ							
μ							
β_0	+	--	-	-	--	--	
δ			--	--	--		
λ_F							
P_F	--	--		--	--	-	-
P_S			--	--	--		
β_I		--				--	
β_{EXP}		--		--	--	--	-
β_{TSHOUR}							
β_{TSDAY}							
R_F		--		--	--	--	-
R_L							
T_E		++	--	-	+	+	++
T_L							
ζ		--		--	--	--	-
W_V							
P_{VS}							

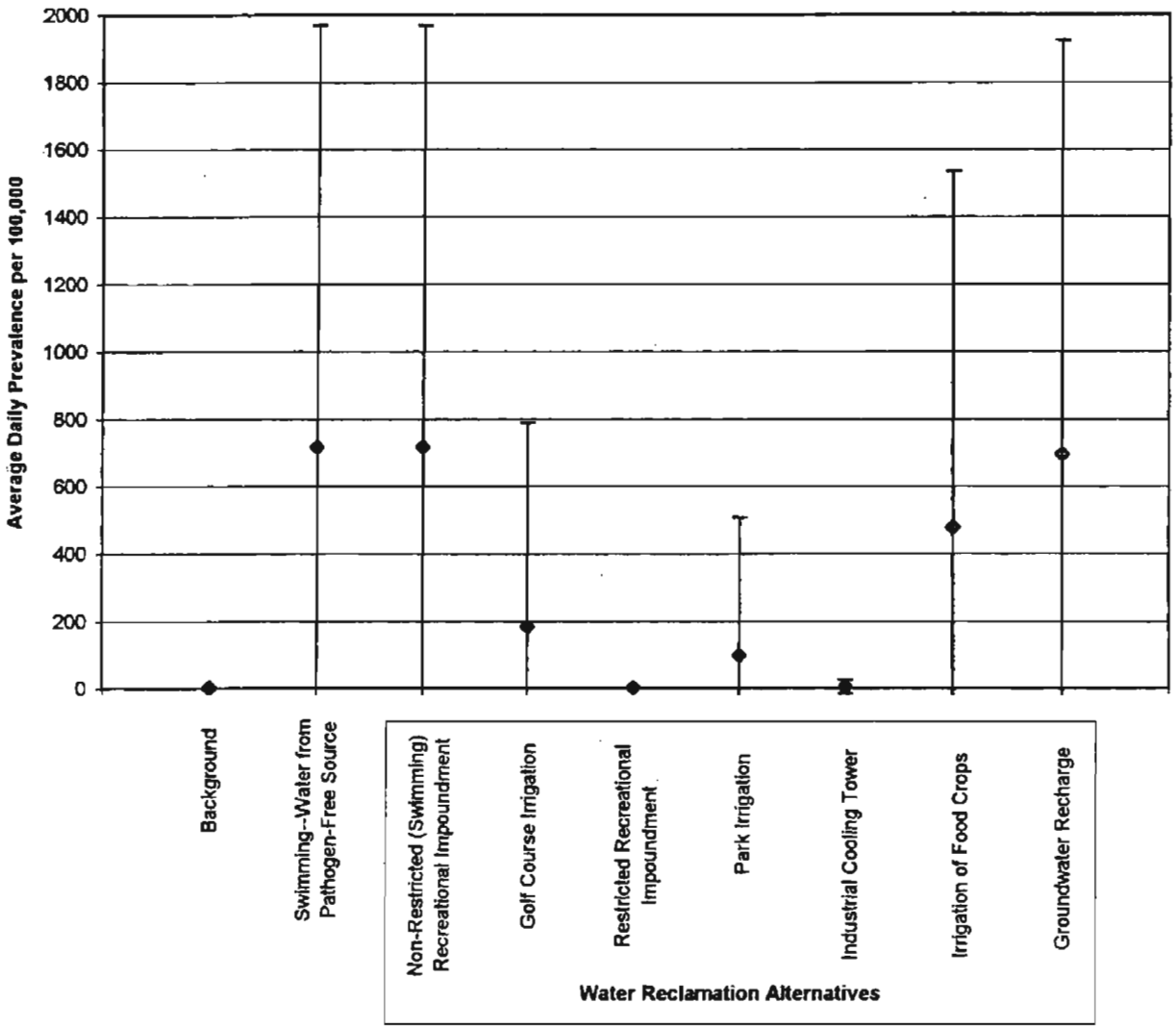
SWIM = Non-Restricted (Swimming) Recreational Impoundment
GOLF = Golf Course Irrigation
RRI = Restricted Recreational Impoundment
PARK = Park Irrigation
ICT = Industrial Cooling Tower
IFC = Irrigation of Food Crops
GNDR = Groundwater Recharge

++ = most important
+ = important
0 = medium importance
- = less important
-- = least important
Blank = importance was not significant
Gray = parameter not applicable to reuse alternative

The above table shows which parameters are important for Hepatitis A for each water reclamation alternative. Parameter importance was based on the Kolmogorov-Smirnov equality of distribution test. A discussion of the procedure can be found in Section 5.7. Hepatitis A is described in Appendix H, and each of the water reclamation alternatives is described in Section 3.3.

Figure 5.1

***Giardia lamblia* Average Daily Prevalence**

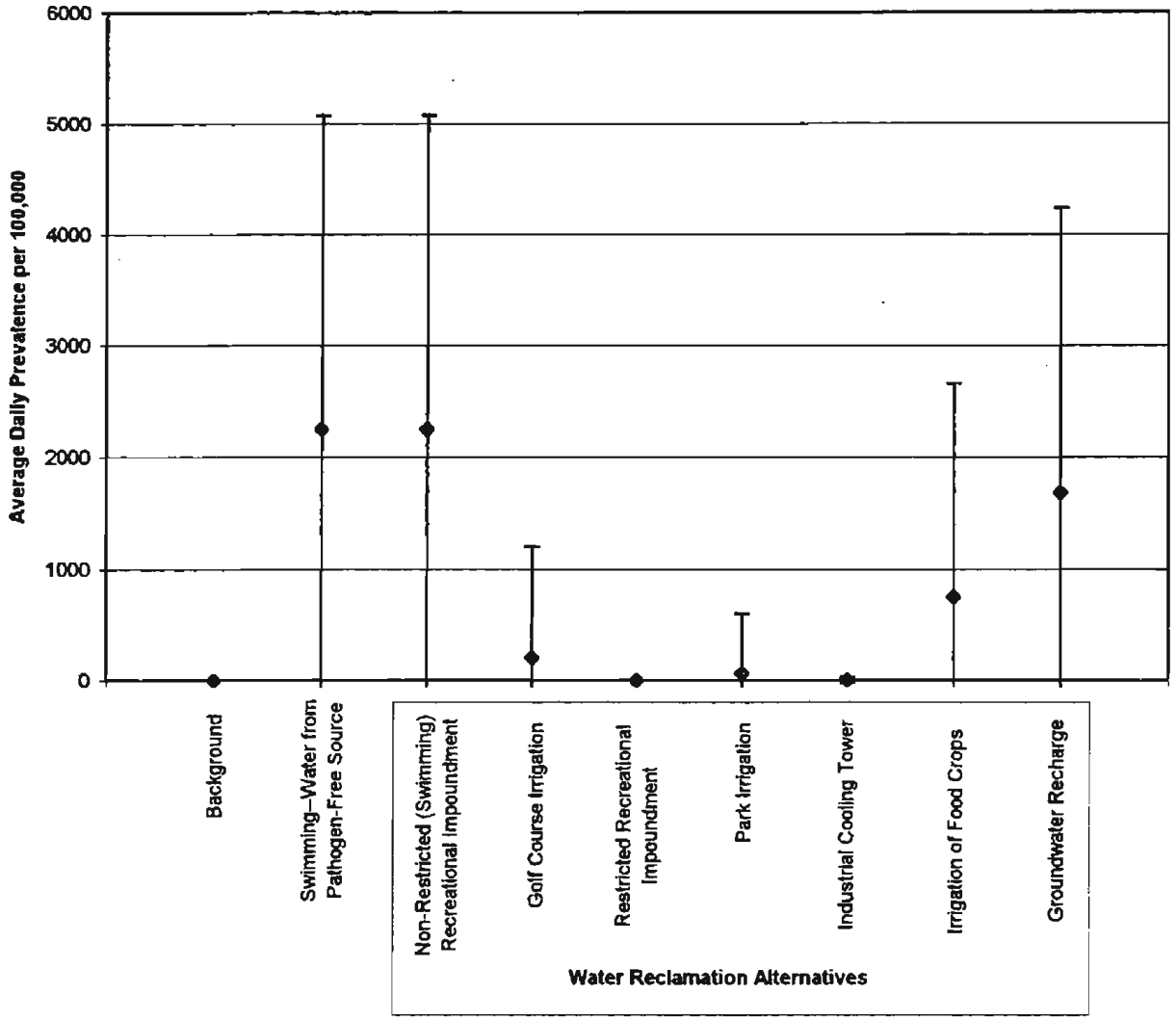


The figure above shows the results for *Giardia*. 6,000 simulations were performed for each water reclamation alternative. *Giardia* is described in Appendix A, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.2

***Cryptosporidium* Average Daily Prevalence**

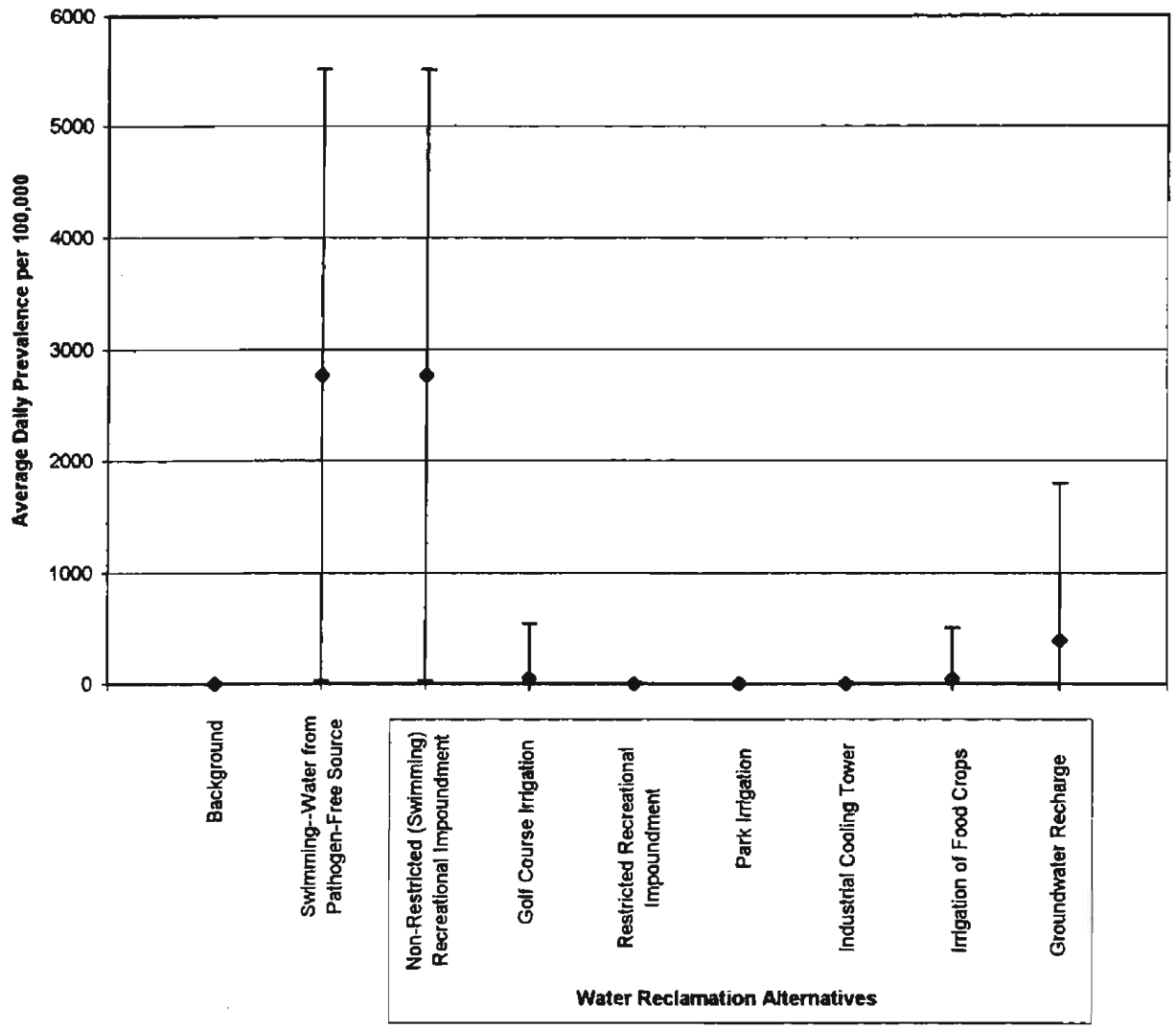


The figure above shows the results for *Cryptosporidium*. 6,000 simulations were performed for each water reclamation alternative. *Cryptosporidium* is described in Appendix B, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.3

***Shigella* Average Daily Prevalence**

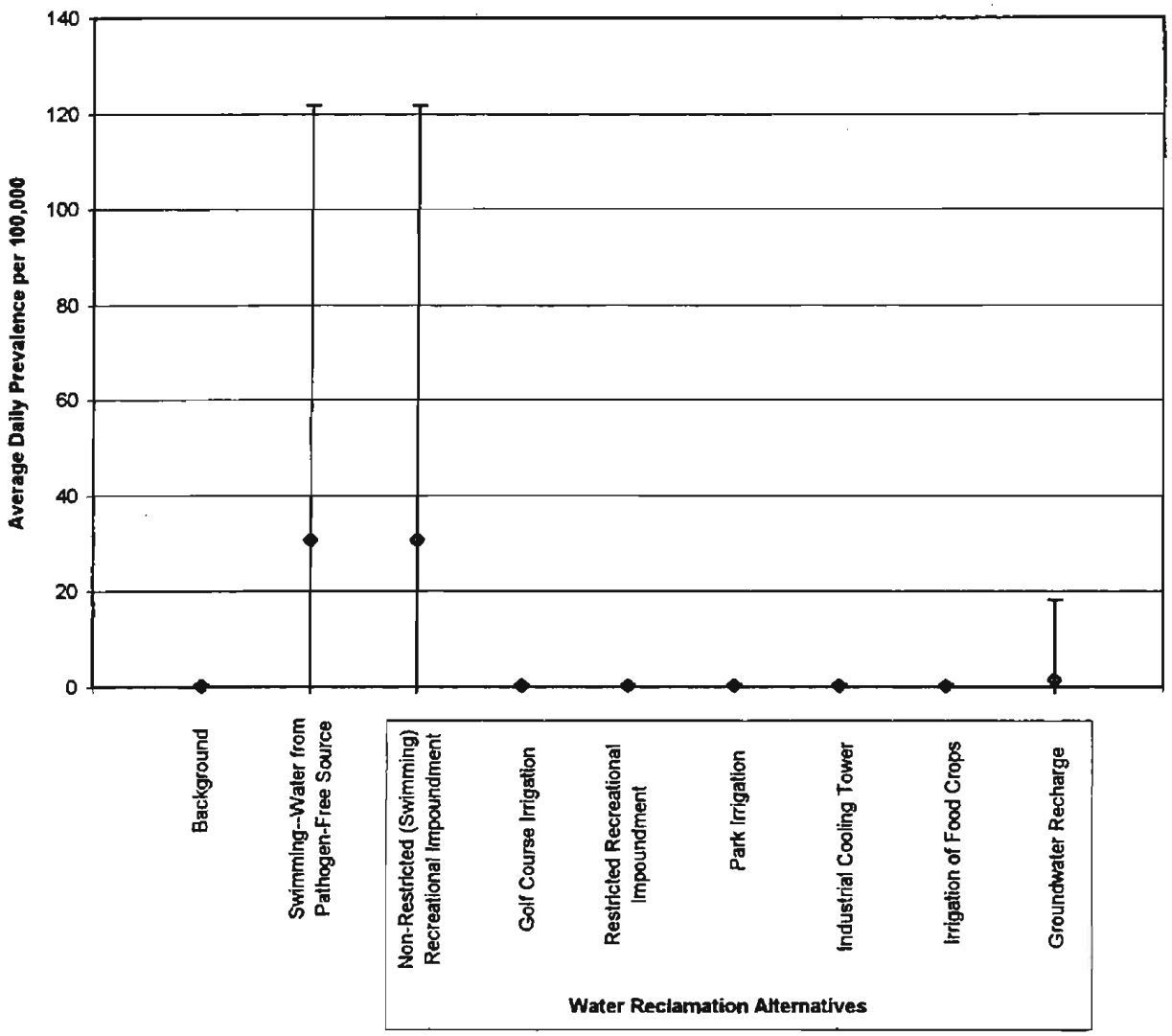


The figure above shows the results for *Shigella*. 6,000 simulations were performed for each water reclamation alternative. *Shigella* is described in Appendix C, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.4

***Salmonella* Average Daily Prevalence**

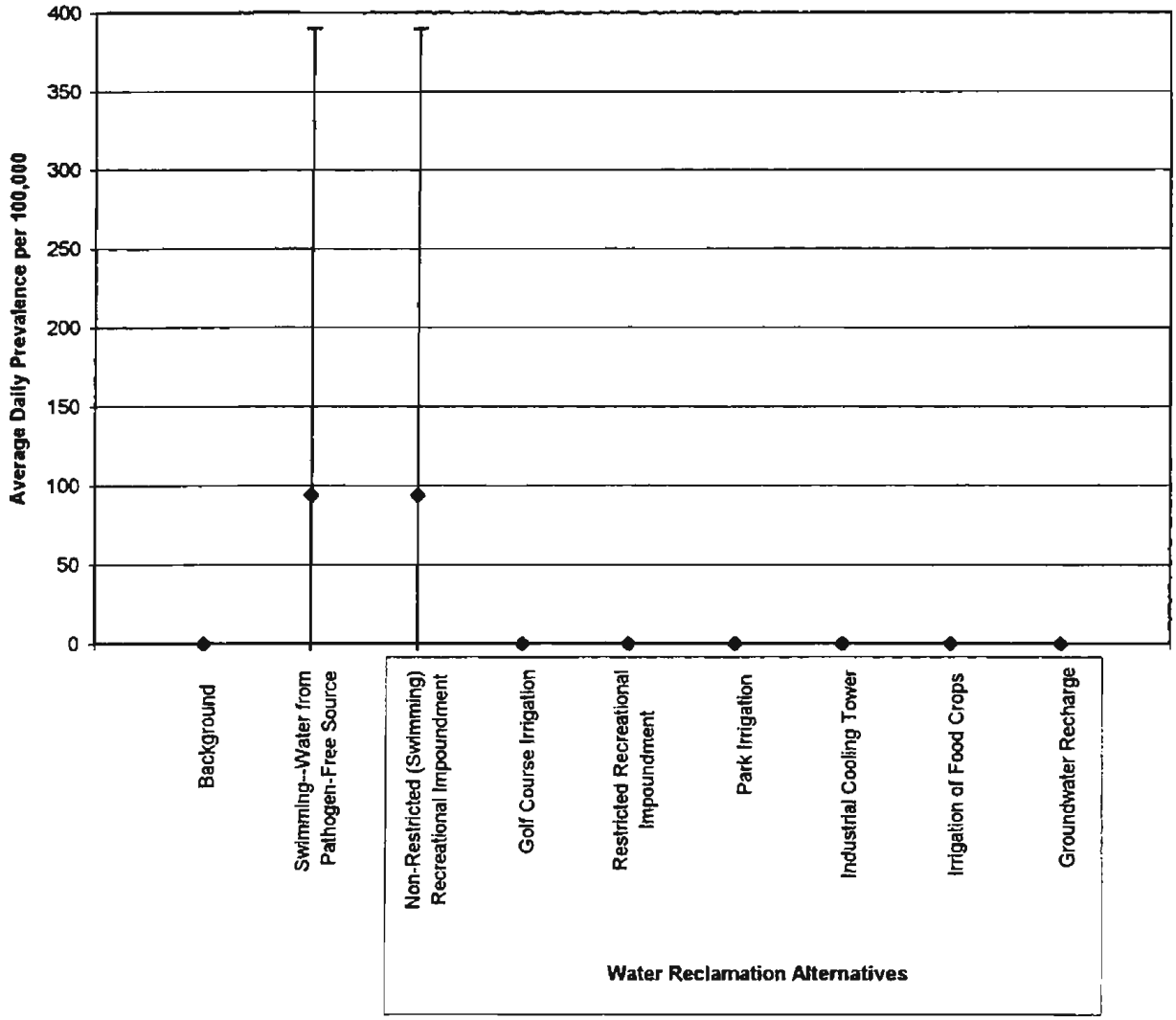


The figure above shows the results for *Salmonella*. 6,000 simulations were performed for each water reclamation alternative. *Salmonella* is described in Appendix D, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.5

***Vibrio cholerae* Average Daily Prevalence**

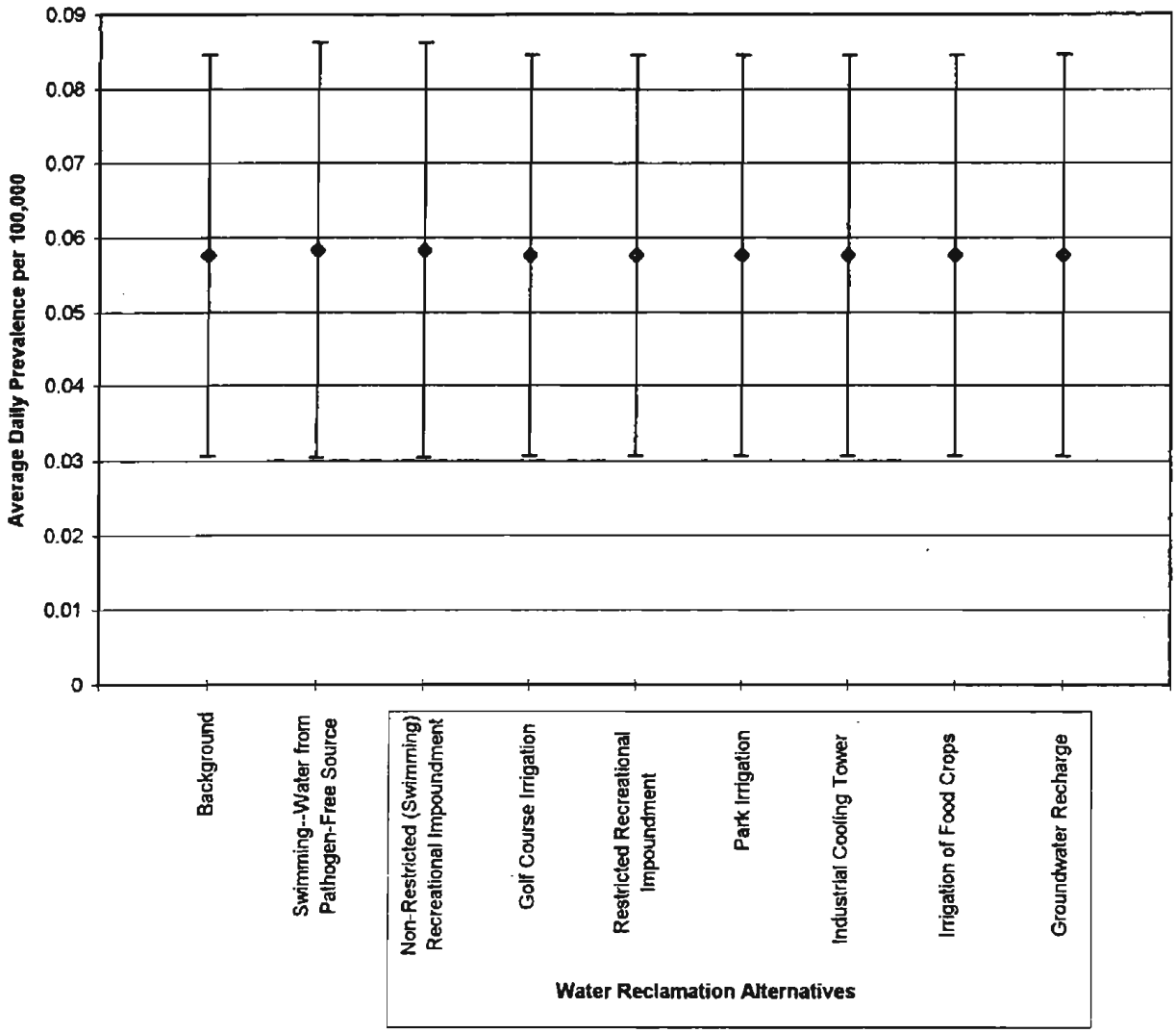


The figure above shows the results for *Vibrio cholerae*. 6,000 simulations were performed for each reclamation alternative. *Vibrio cholerae* is described in Appendix E, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.6

***E. coli* Average Daily Prevalence**

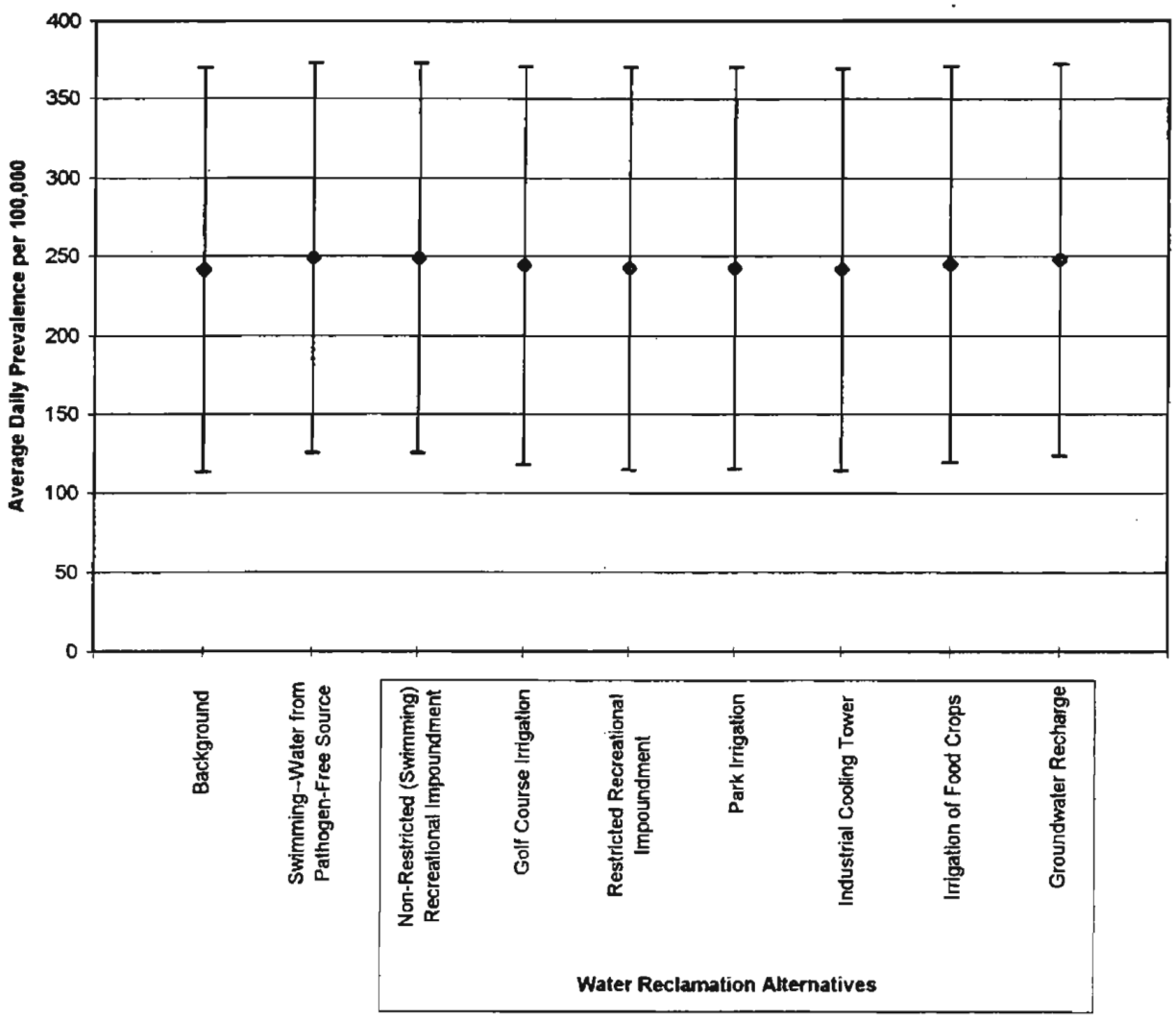


The figure above shows the results for *E.coli*. 6,000 simulations were performed for each water reclamation alternative. *E.coli* is described in Appendix F, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.7

Enteroviruses Average Daily Prevalence

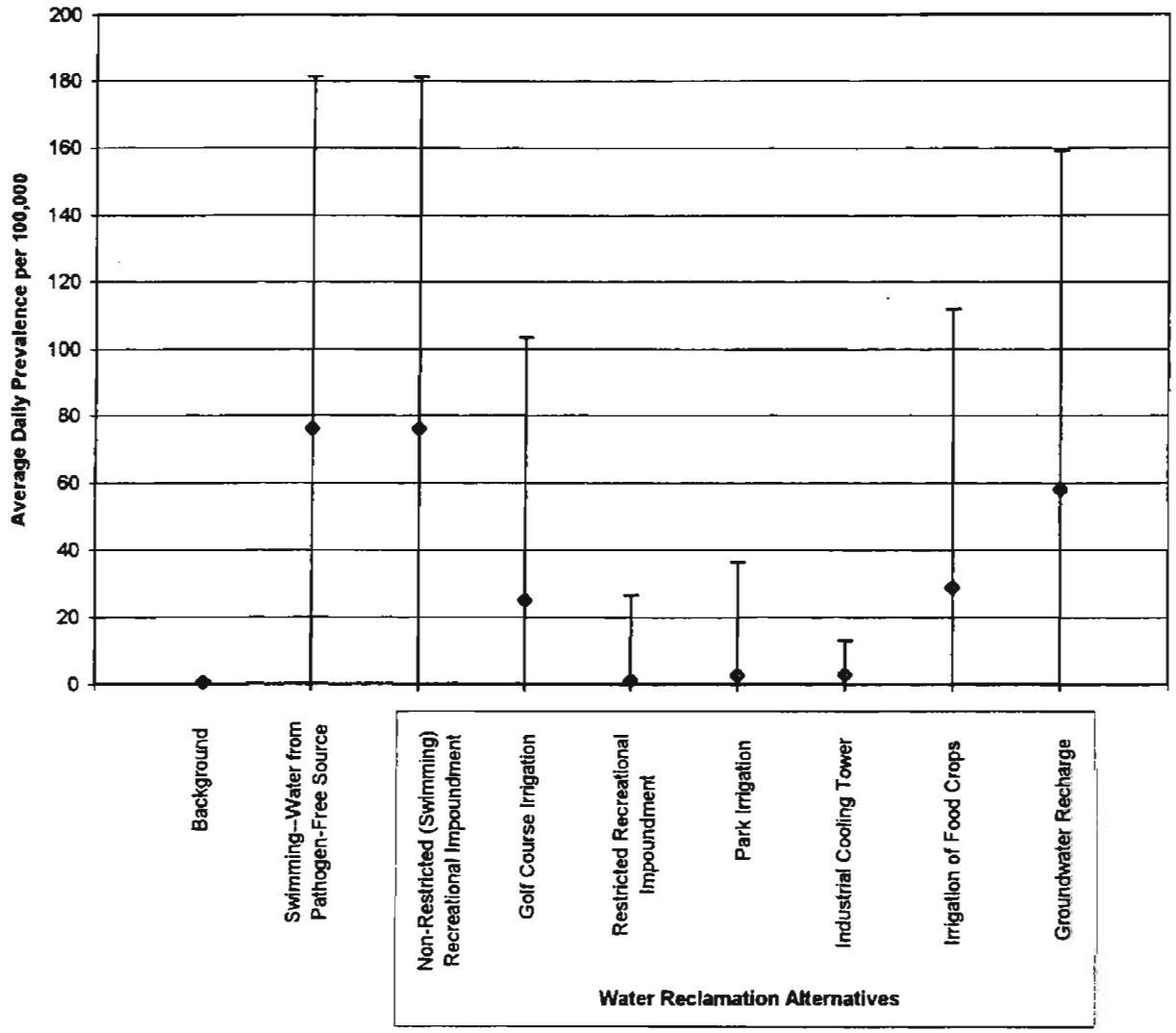


The figure above shows the results for the enteroviruses. 6,000 simulations were performed for each reclamation alternative. The enteroviruses are described in Appendix G, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

Figure 5.8

Hepatitis A Average Daily Prevalence



The figure above shows the results for Hepatitis A. 6,000 simulations were performed for each water reclamation alternative. Hepatitis A is described in Appendix H, and each of the water reclamation alternatives is described in Section 3.3.

For each alternative, the average daily prevalence is shown with the standard deviation indicated with error bars.

6.0 DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH

This chapter discusses the study and presents our conclusions and recommendations for future research.

6.1 Future Work

Because this work represents a first step in the development of a dynamic simulation approach that explicitly acknowledges uncertainty and variability, much work is needed in refining these techniques. Two areas discussed in this section pertain to the model structure and to the analysis.

Model Structure

There are various ways to expand the scope of the model depending on the specific application. Two such applications are the incorporation of seasonal patterns of exposure and susceptibility status within the population. The first application would transform the model, which to date has primarily been used for steady state analysis, to one in which no steady state exists. The dynamics of such a system contain information that could be valuable in a risk assessment. This is particularly true when seasonal variations of factors such as exposure are considered. The model currently assumes that human exposure to reclaimed water is uniform throughout the year. For many of the water reclamation alternatives presented in this report, such as swimming and irrigation, exposure is likely to vary during different seasons of the year. Preliminary studies could look at phenomenological variations in exposure, e.g., an exposure increase during the warmer months and a decrease in the cooler months. However, seasonal exposure data also would be needed.

Once the dynamics are explicitly incorporated into the model, failure analysis studies could provide interesting information on the effects of plant failures. The type of plant failure, as well as information on when and for how long the plant fails, are important factors in assessing risk. This new model structure would allow for a comparative study of various disinfection scenarios such as the difference in human risk between the presence of a constant low level of pathogen activity and an extremely low level of pathogen activity accompanied with periodic large but short lived releases of pathogens.

The second application modifies the model's current assumption that the population is homogeneous with respect to susceptibility to disease and other parameters related to disease transmission. It is clear that any population to some degree is heterogeneous. The heterogeneity most often described and accounted for in modeling studies is age. Because epidemiological factors, such as susceptibility, are age-dependent, a model could account for many factors by dividing the population into age groups, e.g., infants, children, adults, and elderly. The parameterization of this grouping could, given the lack of age-specific data, be approached phenomenologically, assuming that the young and the old are more susceptible then other age groups. However, there is a growing group of immuno-compromised individuals within the population which is not dependent on age. Patients receiving corticosteroid therapy or chemotherapy and AIDS patients are examples of immuno-compromised groups which have increased susceptibility to disease. In addition, individuals potentially having compromised immune systems include the young, the elderly, smokers, alcohol and/or drug abusers, and people living in poverty. Therefore, depending on the population of interest, a significant portion of this population will be immuno-compromised, leading to a heterogeneous group of individuals that have differing epidemiological properties. A more refined model would divide the population into

different susceptibility groups, each with a distinct set of epidemiological parameter values.

Analysis

Research in refining the methodology presented in this study is necessary to provide more detailed information. These refinements include expanding the analysis beyond parameter sensitivity to include structural sensitivity, as well as sensitivity to a specific criterion chosen. In this study we assumed that our model correctly described the system. However, it would be interesting to study the sensitivity of simulation output to specific mechanistic choices, e.g., changes in the dose-response description.

In addition to expanding the analysis, there is also a need to go beyond the standard univariate and linear multivariate statistical techniques used in this study. A potential utility of our modeling approach is to identify those processes that are not important in assessing risk. In general, complex systems such as the one we are studying contain a large number of factors that have been identified by various researchers in fields such as microbiology, epidemiology, and clinical medicine. Even though all these factors have some effect on the system, not all of them are important in determining the epidemiological trends that we observe. Therefore, there is a subset of factors that "drive" the system. From a modeling perspective it is important to find these driving factors, thereby simplifying the model. A major problem with a large model is the "curse of dimensionality", i.e., as the number of factors in a model increases linearly, the complexity of the model increases exponentially. The univariate and linear multivariate statistical techniques were useful in obtaining information on the most uncertain model parameters; however, when applying the Kolmogorov-Smirnov test, a small D-statistic does not provide sufficient evidence that a parameter is unimportant. The importance of parameters that are highly correlated may be masked by other parameters. Linear multivariate analysis is obviously limited to linear correlations. Recent advances in nonlinear multivariate techniques may be useful for this application.[1,2] In these studies a classification algorithm was used to study local structure within a highly nonlinear multidimensional parameter space.

6.2 Conclusions

The approach taken in this project to the characterization of the risk to humans of waterborne pathogens has several distinctive features which have important implications from a public health perspective. First and foremost, public health deals with human populations rather than individuals, and that is the underlying perspective of the analyses conducted as a part of this project. We are not here concerned with worst-case analyses, but with estimating the probable number of cases of disease in a population of a given size. To illustrate this central point, suppose the distribution of the risk of disease across the population has a mean value of 1×10^{-4}. Then, the expected number of cases of disease in a population of 1,000 individuals is essentially zero, but in a population of 1,000,000 individuals, the expected number of cases is 100. Hence, population size is clearly a central issue in assessing the cost effectiveness of treatment alternatives, for example, in water reclamation scenarios. This form of risk analysis supports risk management decisions based on expected cases of disease rather than on the theoretical notion of individual risk. Moreover, the population view of risk is consistent with the way in which epidemiologists approach the issue and how they characterize risk based on direct population studies. The epidemiological notions of incidence and prevalence, for example, are inherently population-based concepts.

It has been argued that the differences between the maximum allowable individual risks that tend to be used as benchmarks by EPA and OSHA spring from an implicit, but

unstated, acknowledgment of the different population bases common to community versus occupational exposures. The 10^{-6} maximum individual risk number that EPA has often used in cancer risk assessments can be contrasted with the 10^{-4} number typical of many OSHA standards. Clearly, the level of exposure to almost any agent in occupational settings is usually higher than allowed in the community, but the population so exposed is almost invariably much smaller. The result, it can be argued, is that the expected number of cases of disease may be generally quite similar in these very different exposure situations. However, others have pointed out that this difference may be due to different safety margins used by the two agencies.

The second distinguishing feature of our approach to risk assessment is our use of mechanistic models to integrate and organize the diverse data bearing on disease risk. A review of the structure and information that went into the several models discussed earlier in this report will further emphasize the fact that some very specific information was required, not only about organisms and their transmission, but about the site and target population. While we argue that this is essential for a defensible assessment, it does not provide a simple formula for the use of regulators. Stated differently, the value of our approach does not rest on the particular model we have used, since that will be problem- and site-specific. Rather the approach demands that the risk assessment be explicit both in its general assumptions and in the specific details of a particular exposure scenario or other aspects of the local situation. The benefit of laying out the assumptions and the uncertainties allows for the identification of the source of controversial results and informs an explicit discussion of the strengths and weaknesses of the analysis.

The final aspect of our work worthy of comment is the explicit acknowledgment, at every stage of the analysis, of uncertainty and variability in the models parameter values. It is important to point out again that uncertainty can be reduced by new data and information, but variability, for example, in human susceptibility to infection, is with us always. This implies that risk assessments will always be probabilistic in nature. Because there is always some degree of uncertainty and variability associated with environmental phenomena, it is even more important to be explicit about what we know and what we do not. In this project, the large variability in the shedding parameter clearly precludes definitive assessments of risk in the case of *Giardia*. For the case of the *Cryptosporidium* simulation study, at least five parameters were identified as requiring better definition. Of these parameters, two were associated with the specific scenario understudy (I_c, the concentrations of cysts in the untreated water and T_{EN}, the normal treatment efficiency) and three were biological parameters (βo, the background transmission rate; σ, the rate of recovery from state D to state Z; and ζ, the pathogen die-off rate).

This result is of great value in setting a research agenda for the future. For example, we may ask if the true variability in the shedding parameter is as large as we have assumed or if there is an important component of uncertainty in these values due to the methods or study designs that have provided these data. Notwithstanding our present dilemma, the analysis has identified the parameters which have a major influence on the distribution of risk, and thereby provides us with a very clear idea of the nature of further data on processes and parameters that must be obtained before a more precise definition of the risk can be provided. There may also be cases where, as uncertain as the outcome may be, the risk information is sufficient to allow making a preliminary risk management decision. For example, the disease risk from the shedding of pathogens by swimmers may be greater for some organisms than for others. Therefore, focusing the concern on defining and regulating the shedding exposure pathway over the reclaimed water exposure pathway may be organism-dependent.

While most aspects of our analysis have analogs in the chemical risk assessment literature, the focus here on population risk is relatively unusual. Moreover, due to the paucity of work in the past relating to risks arising from exposure to pathogenic agents in any medium, there remains much to be done in refining these ideas in specific applications. The next step is to put these ideas before the scientific and regulatory communities for comment as well as to identify specific projects in which the ideas can be tested in real world applications.

Chapter 6.0 - References

1. Shang, N., "New Developments in Tree-Structured Methodology," PhD dissertation, University of California, Berkeley (1993).

2. Spear, R.C., T.M. Grieb, and N. Shang, "Parameter Uncertainty and Interaction in Complex Environmental Models," Water Resources Research, 30, 3159-69 (1994).

Appendix A - *Giardia lamblia*

Giardia lamblia

ETIOLOGY AND CLINICAL DISEASE

Giardia lamblia is a flagellate protozoan that principally infects the upper small bowel. Infection by *Giardia* is frequently asymptomatic, but can result in a variety of intestinal symptoms. Most commonly, these symptoms consist of chronic diarrhea, steatorrhea, bloating, abdominal cramps, frequent greasy malodorous stools, weight loss, and fatigue.[1] Malabsorption syndrome may occur, with impaired absorption of carotene, vitamin B-12, folate, and fats. Symptoms of this syndrome are flatulence, foul-smelling bulky stools, abdominal distension, anorexia, nausea, and weight loss. Certain immunodeficiency syndromes may also be associated with *Giardia* infection.[2] There is generally no tissue invasion beyond the bowel lumen, but damage to duodenal and jejunal mucosal cells may occur in severe disease.[1]

Illness can last from 1 day to 3 months or more,[3] up to several months.[4] Barbout et al.[5] reported 9 of 59 (15.2%) clinical cases relapsed within 3 months of treatment. Symptoms seldom last less than 1 week.[6] In one study, only 2 of 56 (3.5%) infected persons were ill less than 10 days.[7] The average duration of symptoms is reportedly 30 to 45 days [2,8] or 6 weeks[9], but may be as short as 10 to 15 days.[5,10] Chunge et al. reported a 73 day average duration of infection for children aged 10 to 28 months.[11] Rauch et al. reported a mean duration of *Giardia* infection of 2 weeks $\mp$1.5 weeks for children aged 1 to 24 months attending a day care center. Carriers can shed *Giardia* for years,[12] but usually self-cure occurs within 2 to 3 months.

INCUBATION PERIOD

The incubation period is variable. It has been reported to range from 3 to 56 days with an average of 7 to 10 days.[3,13,14,15,16,17,18] In a recent review of giardiasis, Wolfe reported that the incubation period generally varies from 9 to 15 days.[17]

SHEDDING

Giardia cysts are shed by humans in feces in widely varying numbers, may be shed by asymptomatic infected individuals and are often shed intermittently. In one study, some individuals excreted enormous numbers of cysts and others only an occasional cyst.[13] Another study indicated that a person with giardiasis may excrete 700 million cysts daily;[19] it has also been reported that up to 900 million cysts may be passed in the stools during one day[20] and that 1 X 10^6 cysts/gram of stool may be shed by infected persons.[21]

OCCURRENCE

Giardia is found worldwide. The prevalence of *Giardia* infection worldwide has been estimated to be about 7 percent, and infection is more common in children than adults.[20] Prevalence rates vary between less than 1 and 50% depending on the population sampled, being higher in under-developed than developed countries and in urban rather than rural areas.[4] In Europe and the United States, there is considerable variation from place to place in the likelihood of contracting the disease, and this appears to be related to the safety of the drinking water.[20] Prevalence rates between 2 and 7% are more common in these developed areas.[4] In the United States, a national average of 3.8% for *Giardia*-positive stools has been reported.[22] 7.2% of 215,275 stool specimens examined by state diagnostic laboratories in the United States in 1987 were positive for *Giardia*.[23] It should be noted that results for samples submitted to state diagnostic labs may show a positive bias if compared to samples collected randomly from a given population. In a review of Vermont's surveillance system between 1983 and 1986, giardiasis was the most common reportable disease in the state, with an average annual incidence rate of 45.9 cases per 100,000 population per year.[24] This incidence was adjusted to exclude outbreaks of disease. Incidence in this study and another study in Oregon was most common in the 1-4 years age group, followed by the 30-49 years group.[24, 25] In a study in two Washington counties, *Giardia* prevalence among 1 to 3 year old children was 7.1%.[26] In a study in Denver, Colorado, 16% of children aged 12 to 42 months attending day-care centers and 9% who had not were positive for *Giardia* in stool samples. Areas of the United States known to be associated with increased risk of infection are usually mountainous and include New England, the Pacific Northwest, and the Rocky Mountains. In the United Kingdom and the United States, a late summer/autumn peak in cases reported occurs.[4,22]

The illness to infection ratio is highly variable. The reported percentage of symptomatic infections for various groups ranges from 20% to 67%.[4,14,27,28,29,30] The percentage of persons asymptomatically excreting *Giardia* in the industrialized countries is estimated to be between 1% and 7%.[14] The development of symptoms may be influenced by a number of factors including strain differences in the organism, previous exposure and immune competence of the host, and age, with children generally becoming less ill than adults and frequently developing asymptomatic infections.[4] The populations with the highest rate of asymptomatic infection are often comprised of children attending day-care centers; however, it was estimated that 76% of the infections in an outbreak in Berlin, New Hampshire were asymptomatic.[27] One study indicated that asymptomatic excretion in children younger than 36 months in day-care centers is common and appears to be well-tolerated.[31]

For the two year period from 1991 to 1992, 11 outbreaks associated with water intended for drinking and with a known etiologic agent were reported in the United States. *Giardia* was the agent in four of these outbreaks. In addition, *Giardia* was identified as the etiologic agent in four of eleven outbreaks of gastroenteritis associated with recreational water.[32] Four food-borne outbreaks also have been reported in the United States.[4]

RESERVOIR

The major reservoir of *Giardia* is man, but their is evidence that man may acquire infections from other animals. Beavers may be a reservoir and have been implicated in waterborne outbreaks.[33,34,35] Dogs, gerbils, guinea pigs, beavers, raccoons, and bighorn sheep have been experimentally infected with *Giardia*,[36] and muskrats in the Detroit watershed were found to be infected.[37]

MODE OF TRANSMISSION

Modes of transmission for giardiasis include contaminated water supplies, food-borne outbreaks and person-to-person contact.[4] Person-to-person transmission is particularly prevalent and well documented in day-care centers.[4] Transmission also occurs among male homosexuals engaging in oral-anal sexual practices.

IMMUNOLOGY

Individuals with impaired immune function appear to have increased susceptibility to giardial infection. Attack rates of giardiasis observed in populations which are chronically exposed to this organism are lower than in other populations.[4] Thus prior exposure appears to impart a partial resistance to reinfection.[38]

A variety of antibody (secretory and serological) and cell-mediated responses occur in human giardiasis and experimental giardiasis in animals. However, the functional aspects of these responses remain poorly defined.[39] Evidence points to a role for intestinal antitrophozoite antibody in blocking adherence of trophozoites to intestinal epithelium.[40] It has been reported that secretory IgA plays an important role in resistance to subsequent infection.[41] The serological response appears to involve IgA, IgG and IgM antibodies. Several studies have indicated that those with current infections have higher levels of antibody than controls. One study found that serum IgM levels return to normal two to three weeks post-infection. Another study indicated that serum IgA levels remain elevated for some weeks post-infection.[4] Unpublished data cited in one study indicated that serum antibody levels decline steadily over six months following an initial infection.[42] For populations in Colorado and Thailand, serum levels of antibodies increased substantially during childhood.[42] It should be noted, however, that antibodies in serum do not appear to relate to the host's ability to eliminate the parasite.[43] The cellular response to infection may be responsible for some symptoms. There is a marked increase in numbers of lymphocytes in jejunal mucosa which decreases again following clearance of infection.[4] Data regarding the duration of protective immunity was not found in this literature search; it is probably unknown.

ENVIRONMENTAL PERSISTENCE

Giardia generally forms a resistant cyst before leaving the intestine; this is the form found

in the environment.[12] Trophozoites, which may be passed in severely diarrheic feces, do not survive.[44] *Giardia* cysts may remain viable for several months in water at 4-8°C;[4] 5°C appears to be optimal.[45] Cysts have survived for up to 10 months in fresh water at 8°C[4] and 1 month in fresh water at 21°C.[45] In one study, cysts survived 32 days in fresh water at room temperature.[46] In another study, cysts did not survive when exposed for 24 hours to artificial sea water at 4°C.[47] They cannot tolerate freezing. Cysts are at optimum viability at pH 6 to 8.[45]

DOSE-RESPONSE RELATIONSHIP

Rendtorff and Holt[46] and Rendtorff[48] performed a series of feeding studies on prison volunteers in 1954. The results are shown in Table 1. As few as 10 cysts were capable of initiating infection. It should be noted that Table 1 reports infection only, as detected by examination of stools for *Giardia* cysts. Of the infections produced, none resulted in outright disease, as is noted below. In one of these studies, 64.7% of men fed 100 cysts stored 0 to 16 days became infected[46] with no decrease in infectivity over cyst-storage time. A reexamination of Rendtorff's data, presented by the same author in 1978,[8] attributed the low infectivity of the 25-cysts dose to the suspected low infectivity of the cysts used. Cysts were recovered from feces of humans shedding *Giardia*, and the cysts used for the 25-cyst doses were from a different person than those used in the other tests. There were no clinical signs in any of these volunteers during the 5-1/2-month study, except for mild transient changes in frequency and consistency of stools in a few subjects. The dose size did not seem to be related to persistence of infection.

Rose, Haas and Regli[30] used Rendtorff's data to model the dose-response relationship. Using this data, the probability of an infection (P) resulting from ingestion of a single volume of liquid containing an average number of organisms (N) was modeled by the following exponential equation:

$$P = 1 - \exp(-rN)$$

where r, the fraction of microorganisms that are ingested which survive to initiate infections, was calculated to equal -0.01982. The authors acknowledge that this model may overestimate risk because it assumes that all cysts are viable and are species which infect humans and that 2 liters of tap water are consumed per day, which may be an overestimation of exposure. The authors also state that the underestimation of risk may be of greater concern due to the underestimation of exposure resulting from inefficient methods of detecting cysts in water. Glicker and Edwards[25] compared the annual incidence predicted using the above model to actual incidence in Multnomah County, Oregon. The incidence predicted by the model was much greater than the measured incidence. However, since infection rates are often underestimated due to asymptomatic or unreported infections, the authors conclude that the level of giardiasis in a community will likely fall between the incidence rates reported to health authorities and those predicted by the model.

INDICATOR-PATHOGEN RELATIONSHIP

Frequently there is no apparent correlation between coliform numbers and the presence of cysts.[39] This is particularly so in unfiltered but disinfected drinking water. Negative coliform tests do not provide assurance that water is free of *Giardia* cysts; however, positive coliform results often correlate with outbreaks.[15] Stream water associated with an outbreak in Utah contained 42 colonies of fecal coliforms/100 mL; a fecal coli count of <50/100 mL is considered normal (uncontaminated) for a stream of that size and elevation in Utah[5] (The coli counts may be from animal origin and do not necessarily indicate human fecal contamination). In a giardiasis outbreak involving treated water, samples of raw water upstream from treatment-system intakes showed ≤ 5 total and fecal coliforms/100 mL.[33]

Table 1

Dose response for *Giardia lamblia*[46,48]

Number Cysts Given	Number of Individuals Exposed	Number of Individuals Infected	Percent Infected
1	5	0	0
10	2	2	100
25	20	6	30
100	2	2	100
10,000	3	3	100
100,000	3	3	100
300,000	3	3	100
1,000,000	2	2	100
Total	40	21	52.5
Controls	21	0	0

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Appendix B - *Cryptosporidium* spp.

Cryptosporidium spp.

ETIOLOGY AND CLINICAL DISEASE

Cryptosporidium spp. is a coccidian protozoa primarily associated with gastrointestinal infection. Cryptosporidiosis was initially considered an opportunistic infection in immunocompromised patients, but is now recognized as a frequent cause of diarrhea in otherwise healthy humans and domestic animals. It is considered an important public health problem throughout the world.[1] The most obvious symptom of disease is a watery diarrhea, which may be accompanied by anorexia, nausea, weight loss, dehydration and abdominal pain.[2]

The infected intestinal mucosa usually remains intact, but mild to moderate villous atrophy, crypt hyperplasia and submucosal inflammatory infilitration do occur. Diarrhea may be malabsorptive; however, the profuse, watery diarrhea seen especially in immunocompromised patients suggests a secretory process. The exact mechanisms by which *Cryptosporidium* alters intestinal function remain to be clarified.[1]

There is considerable variability in the duration and intensity of symptoms. In immunocompetent individuals the duration of symptoms is generally 2 days to 1 month, but most individuals become asymptomatic within 2 weeks.[2,3,4] In immunologically compromised individuals the duration and severity of symptoms are generally much greater. Most patients with AIDS never clear the infection and die.[3]

Cryptosporidiosis is considered a self-limiting and non-fatal infection in immunocompetent individuals. However, in a recent study this disease was associated with excess mortality in West African children who had the infection in infancy. This excess mortality could not be explained by malnutrition, socioeconomic factors, hygienic conditions, or breast feeding.[5]

INCUBATION PERIOD

The incubation period is variable, but generally falls in the range of 2 to 14 days.[1,2,3]

SHEDDING

Cryptosporidium oocysts are shed by humans and animals in feces. Oocyst shedding may be variable and intermittent during the course of illness and may persist after clinical resolution.[3] Excretion usually ceases within 1-4 weeks after the cessation of symptoms, but may continue longer in some cases.[6] Shepard et al.[7] reported that oocysts were found in stool specimens up to 35 days after the onset of symptoms, but most patients had stopped shedding by 20 days. In another study, 73% of patients for which the end of

oocyst excretion could be determined accurately had positive stools after the cessation of symptoms for a mean perod of 6.9 days, with a range of 1 to 15 days.[8]

The number of oocysts excreted during the course of infection may fluctuate markedly and formed or semi-formed stools may be found to contain many oocysts while some fluid stools may contain few. During the acute diarrheal phase oocyst numbers may exceed $10^6/cm^3$ of stool.[6] In one study, shedding of up to 10^7 oocysts per gram of feces was reported for infected calves.[2] Blewett[9] found a mean excretion of 2.0×10^6 oocysts per gram of stool on calves with natural *Cryptosporidium* infections.

OCCURRENCE

Cryptosporidium is found worldwide. Human cryptosporidiosis has been reported in at least sixty countries on six continents, with widely varying prevalence among those seeking medical care for diarrhea.[1] The prevalence is highest in non-industrialized regions. In a review of 36 large-scale surveys of selected populations, such as children and adults seeking medical attention for diarrhea and other gastrointestinal symptoms, prevalence rates reported in Europe (1% to 2%) and North America (0.6% to 4.3%) were lower than those reported in Asia, Australia, Africa and Central and South America (3% to 20%). Seroprevalence rates in immunocompetent individuals in the United States are between 25% and 35% and are well over 50% in Latin America. Children generally have a significantly higher prevalence than adults, and infections are often seasonal, with a higher prevalence during warmer, wetter months.[4]

The importance of asymptomatic carriage in the epidemiology of cryptosporidiosis is not fully understood, but it may be of more significance than previously suspected.[2] Early childhood and persistent exposure may contribute to asymptomatic infection and acquired immunity to the illness.[1] In three surveys of children in day care centers (two in the United States and one in France), 50% to 100% (5 of 9, 6 of 6 and 6 of 9) of children excreting oocysts were asymptomatic.[10,11,12] However, in another day care center in Florida, only 1 of 27 children whose stools tested positive for *Cryptosporidium* was asymptomatic.[13]

A surveillance for waterborne disease outbreaks in the United States during 1991 and 1992[14] revealed that *Cryptosporidium* was implicated in three of eleven outbreaks associated with water intended for drinking for which the etiologic agent was identified. In addition, *Cryptosporidium* was identified as the etiologic agent in two of eleven outbreaks of gastroenteritis associated with recreational water.

RESERVOIR

The resevoir of cryptosporidiosis is infected animals; infected humans may also be a resevoir. *Cryptosporidium* readily crosses host-species barriers; human infections are often the result of zoonotic transmission. It is harbored by more than 40 mammals. Reservoir hosts include calves, dogs, cats and rodents.[4]

MODE OF TRANSMISSION

Modes of transmission for cryptosporidiosis include person-to-person contact, zoonotic transmission and contaminated food and water.[15] Person-to-person transmission is probably the most important mode[15] and has been documented among family/household members, sexual partners, health workers and their patients, and children in day care centers.[2] Several waterborne outbreaks have been reported in the United States where the filtration component of water treatment was suboptimal.[1] Cryptosporodiosis has also been associated with recreational use of swimming pools.[16,17]

IMMUNOLOGY

It appears that exposed immunocompetent humans and animals develop a partial acquired immunity to infection by *Cryptosporidium* which may account for adults generally having a lower prevalence rate than children.[15] Miller et al. demonstrated partial acquired immunity in a primate model during experimental infection studies.[18] Experimental and clinical evidence suggests that both cell-mediated and humoral immune mechanisms are involved in host defense against *Cryptosporidium* infection. Humans and animals infected with *Cryptosporidium* develop serum IgG, IgA, IgM and IgE and secretory IgA antibody responses.[1] Because the parasite appears to be confined to the microvillous region of the intestinal (sometimes biliary and respiratory) mucosa, it is unlikely that serum antibodies play a major role in acquired immunity, especially since such a role has not been established by extensive laboratory investigations of other species of coccidia. It is more probable that secretory antibodies (IgA) coupled with cell-mediated immune mechanisms are responsible for the clearance of parasites from the infected mucosa and for rendering the immunocompetent host resistant to reinfection.[19] Data regarding the duration of protective immunity was not found in this literature search; it is probably unknown.

ENVIRONMENTAL PERSISTENCE

Approximately 80% of *Cryptosporidium* oocysts form a hardy, thick wall and are passed into the environment in stools.[1] There is little information available on the viability of oocysts in the environment, but oocysts have the potential to survive months following excretion.[2]

DOSE-RESPONSE RELATIONSHIP

Little is known about the infectious dose size in humans for *Cryptosporidium*, but it is thought to be small.[6] In an experimental infection study using primate subjects (pigtailed macaques), Miller et al.[18] found that a dose of 10 oocysts resulted in clinical enteritis in all four subjects studied. Table 1 presents the data from an experimental infection study performed by Finch et al.[20] using neonatal CD-1 mice.

INDICATOR-PATHOGEN RELATIONSHIP

This literature search did not find any data directly relating concentrations of fecal indicator bacteria to *Cryptosporidium* in water or wastewater. It should be noted that it cannot be guaranteed that drinking from water supplies which meet bacteriological standards will not lead to *Cryptosporidium* infection.[6] During a waterborne outbreak of cryptosporidiosis due to contamination of a public water supply which affected an estimated 13,000 people in Georgia, routine samples from the water system met USEPA and State of Georgia standards for coliform bacteria.[21] In addition, coliforms were not detected in samples of treated water during another public water supply-associated cryptosporidiosis outbreak leading to an estimated 403,000 cases of diarrhea in Milwaukee.[22] It should be noted that effective and reliable water treatment practices are generally recognized to remove or inactivate *Cryptosporidium* oocysts.

Table 1

Dose Response Data for *Cryptosporidium* in Neonatal CD-1 Mice[20]

Number of Oocysts Given	Number of Animals	Number of Animals Infected	Fraction of Animals Infected
18	9	0	0.00
25	16	2	0.13
40	11	3	0.27
43	5	1	0.20
50	14	1	0.07
53	4	1	0.25
55	9	4	0.44
64	4	1	0.25
73	7	5	0.71
75	13	9	0.69
81	12	10	0.83
85	5	5	1.00
88	8	8	1.00
100	24	3	0.13
110	9	9	1.00
120	5	5	1.00
130	11	11	1.00
150	23	5	0.22
160	9	6	0.67
180	6	4	0.67
200	30	22	0.73
220	8	8	1.00
250	6	6	1.00
300	8	8	1.00
310	9	6	0.67
330	5	5	1.00
400	11	11	1.00

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Appendix C - *Shigella* spp.

Shigella spp.

ETIOLOGY AND CLINICAL DISEASE

The genus *Shigella* is made up of Gram-negative, facultatively anaerobic, nonmotile rods. Four species exist which are all pathogenic in humans and other primates. Most other animals are resistant to *Shigella* infection and disease.[1] The four species are divided into groups: Group A, *S. dysenteriae* (10 serovars); Group B, *S. flexneri* (17 serovars); Group C, *S. boydii* (15 serovars); and Group D, *S. sonnei* (1 serovar).

Shigellosis, an acute bacterial disease primarily involving the large intestine, can be characterized by diarrhea, fever, nausea, cramping and sometimes vomiting. In severe cases, stools may contain blood and mucus. Shigellosis can also be mild and self-limited and asymptomatic infections occur.[2,3] Data regarding the percentage of infections which are symptomatic was not found in this literature search. Shigellosis differs from salmonellosis in that *Shigella* organisms rarely invade beyond the mucosal epithelial cells or submucosa lining of the intestine. In severe cases of dysentery, mucosal destruction and ulceration occur but do not extend beyond the intestinal tract.[4] The ability of the bacterium to penetrate the colonic epithelial cell is plasmid-mediated in association with some chromosome-encoded determinants. *S. dysenteriae* type 1 produces a heat-labile cell-associated toxin that affects both the gut and the vascular system of the brain and spinal cord resulting in neurological symptoms.[5,6,7] The severity of illness and the fatality rate are functions of age, nutrition, and dose of organism. Almost all fatal cases of shigellosis occur in developing countries.[8] For *S. dysenteriae* (Shiga bacillus) infection, case-fatality rates approach 20%; for *S. sonnei* infection, a short clinical course results in an almost negligible fatality rate, except in a compromised host.[2]

The symptoms of shigellosis may last from 48 hours to several months.[9,10] However, carrier states lasting longer than one year have been reported.[11] Treatment with ampicillin and tetracycline shortens the clinical disease and shedding stage of *Shigella*.[12]

Shigellosis occurs most frequently in the summer,[13] and is most frequent in children six months to five years of age. For unknown reasons it is rare in children under three months of age.[3] The disease is frequently more severe in young children than adults, among whom many infections may be asymptomatic. The elderly, debilitated individuals, and persons of all ages suffering from malnutrition, are particularly susceptible to severe disease, including death.[2] The case-fatality rate for children less than 15 years old at a diarrhea treatment center in Dhaka, Bangladesh from 1974 through 1988 was 19.1%.[14]

INCUBATION PERIOD

The incubation period for shigellosis typically ranges from 1 to 7 days.[2] The disease symptomology is usually rapid once the organisms have become established in the gut.

SHEDDING

Shigella organisms are excreted in feces. Infected patients with diarrhea typically shed 10^5 to 10^9 organisms per gram of feces.[15,16] Most infected persons who have not been treated excrete the organism for one month or less; treatment shortens the period of shedding.[13] The infectious agent is usually not present in feces within 4 weeks after illness.[2]

OCCURRENCE

Shigellosis occurs worldwide. Outbreaks of this sometimes serious disease are common under conditions of crowding and poor sanitation, such as in jails, institutions for children, mental hospitals, crowded camps and ships. Attack rates from 3 to 90% have been reported for outbreaks of shigellosis in different locations around the world.[17] It is endemic in both tropical and temperate climates, and its habitat is the gut of humans and other primates.

From 1967 to 1988, annual isolation rates of *Shigella* reported to the Centers for Disease Control varied between about 5 to 10 per 100,000 persons. It has been estimated that 5% of all symptomatic cases of shigellosis are reported to the national surveillance system.[18] The most common species of *Shigella* found in this country is *S. sonnei*.[3] Mixed infections have been reported to occur in up to 39% of shigellosis patients.[19,20]

This literature search revealed that *Shigella* spp. had in the past been the most common bacterial pathogens implicated in waterborne outbreaks in the United States.[21-23] However, *Shigella* was implicated in only one of the eleven outbreaks associated with water intended for drinking reported from 1991 to 1992 in which the etiologic agent was identified.[24] This outbreak occured when an untreated spring-water source was contaminated with surface water. Shigellosis has also been implicated in outbreaks associated with recreational swimming.[16,25,26]

RESERVOIR

The only known significant reservoir is man, but outbreaks have been reported in primate colonies.[2]

MODE OF TRANSMISSION

Shigellosis is transmitted via the fecal-oral route, directly or indirectly. Modes of transmission include person-to-person contact and contaminated food and water. It is believed that most cases of shigellosis in the United States are a result of person-to-person contact. In developing tropical regions, where general sanitation is lower, food and probably water play a greater role in transmission, and flies have been shown to be important in the epidemiology of the disease.[13]

IMMUNOLOGY

Protective immunity has been demonstrated to occur after naturally occurring *Shigella* infections and infections with live oral vaccines, but the protection is incomplete and the duration is unknown. One study, which documented increasing isolation rates of *Shigella sonnei* in the United States, found that the greatest percentage increases occurred in populations that previously had the lowest isolation rates, suggesting that they were particularly susceptible due to low exposure and therefore low levels of immunity to this organism.[18]

What constitutes the basis of serotype-specific immunity is unknown[27] and the contributions of the humoral and cellular parts of the immune system have not been well defined.[28] Despite this poor understanding of immunological basis of protection, it is likely that secretory IgA on mucosal surfaces and mucosal lymphocytes play important roles.[29] Elevated levels of serum antibody has been shown to be associated with protective immunity to shigellosis,[30] but induced immunity has not been demonstrated to correlate strictly with with their presence.[27]

Mucosal IgA antibody to *Shigella* appeared to be short-lived and was no longer detectable one month after challenge in a study of children and adults from Peru with shigellosis.[27] Serum antibody levels appear to remain elevated for longer periods of time. For example, serum IgG levels have been shown to remain elevated for 6 months or more after infection.[31,32,33]

ENVIRONMENTAL PERSISTENCE

The survival of *Shigella* in water depends upon factors such as temperature and the concentration of other bacteria, nutrients, and oxygen.[15] A study by Wang et al.[34] showed that Shigella survived: (1) longer when fecal coliform numbers were high; (2) poorly when total plate counts were high ($\geq 10^6$ heterotrophic organisms/mL); and (3) longer at lower temperatures (i.e., 15 to 17°C vs 20°C). In clean waters, survival times are typically less than 14 days at warm temperatures (>20°C), but organisms may survive a few weeks below 10°C. At warm temperatures, 99% reduction in organism numbers is likely to occur in <5 days.[15] In one study, *Shigella* survived 22 days in well water; however, die-off reportedly began within 1 hour.[35] In another study, *Shigella* displayed the same basic survival pattern as most other enteric organisms in fresh water except that die-off occured more steadily; the organisms completely disappeared within 14 days.[36]

Shigella has been shown to survive for 47 days in a frozen river in Siberia and 135 days in associated soil. It is thought that the permafrost may maintain a reservoir of *Shigella* around Siberian settlements.[37]

Shigella can be resistant to increases in salt concentration, but this phenomenon is temperature-dependent. For example, in an estuarine environment, *Shigella* may persist for 25 days at 13°C, but only 4 days at 37°C. In seawater, *Shigella* survival is strain-dependent; organisms persist from 15 to 70 days.[37] *Shigella flexneri* strain 6 was

found to grow in stored water contaminated with seawater on a cruise ship.[38] A study by Mitchell showed that *Shigella* die-off in seawater is about 90%/day.[39]

DOSE-RESPONSE RELATIONSHIP

Shigella is considered the most highly communicable of the bacterial diarrheas.[13] The dose-response data presented in Table 1 are based on human-volunteer feeding studies with *Shigella dysenteriae* type 1 (strains M131 and A-1), *S. flexneri* 2A, and *S. sonnei* (strain 53G). Table 1 reveals that as few as 10 organisms have been reported to cause clinical illness.

Secondary attack rates can be high, especially under crowded conditions.[21,40] Secondary transmission rates of *S. sonnei* often exceed 50% in households with young children.[18] A waterborne *Shigella* epidemic in an Iowa school and adjacent buildings resulted in a secondary attack rate of 9%.[41]

INDICATOR-PATHOGEN RELATIONSHIP

Based upon the data collected in this literature search, the current enumeration techniques for *Shigella* spp. in water samples are inadequate; thus, a direct comparison with coliform numbers was not found. McFeters et al.[42] have shown that Shigella survived longer than coliforms in well water at 9 to 13°C. This information may raise some doubt about the validity of coliforms as indicators of the presence of *Shigella*.[43] However, this literature search did not identify any *Shigella* outbreaks associated with water that definitively met coliform standards at the time of exposure.

Table 1

Dose-Response for *Shigella* spp.

Dose (Number of organisms)	Fraction Ill	Number of Subjects	Species	Reference
10	0.1	10	S. dysenteria 1, M-131	44
10	0.1	131	S. dysenteria 1, M-131	45
10^2	0.39	36	S. flexneri 2a	44
1.8×10^2	0.25	36	S. flexneri 2a	44
1.8×10^2	0.18	33	S. flexneri 2a	46
2×10^2	0.38	8	S. dysenteria 1, A-1	44
2×10^2	0.5	4	S. dysenteria 1, M-131	45
2×10^2	0.25	4	S. dysenteria 1, A-1	45
5×10^2	0.35	20	S. sonnei, 53G	44
5×10^2	0.5	38	S. sonnei, 53G	44
2×10^3	0.7	10	S. dysenteria 1, M-131	45
5×10^3	0.67	49	S. flexneri 2a	46
10^4	0.56	103	S. flexneri 2a	44
10^4	0.76	87	S. flexneri 2a	46
10^4	0.25	4	S. flexneri 2a	46
10^4	0.33	6	S. dysenteria 1, A-1	45
10^4	0.83	6	S. dysenteria 1, M-131	45
10^5	0.44	34	S. flexneri 2a	46
10^5	0.75	4	S. flexneri 2a	46
10^6	0.86	8	S. flexneri 2a	46
10^7	0.68	19	S. flexneri 2a	46
10^8	0.75	8	S. flexneri 2a	46

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Appendix D - *Salmonella* spp.

Salmonella spp.

ETIOLOGY AND CLINICAL DISEASE

The genus *Salmonella* consists of Gram-negative, facultatively anaerobic, rod-shaped, motile bacteria that are usually pathogenic to man and other animals. There are approximately 2,000 known serotypes (serovars) of *Salmonella*, some of which are host--adapted, or found in specific reservoirs or geographic locations.[1] Infection resulting from ingestion of *Salmonella* usually manifests itself as gastroenteritis, enteric fever, and/or septicemia.[1,2] The two major disease syndromes associated with *Salmonella* are salmonellosis (gastroenteritis) and typhoid fever (enteric fever). The major vehicle of salmonellosis is food; however, there are many documented waterborne outbreaks with this symptomology. *Salmonella typhi*, the etiologic agent of typhoid fever, is primarily waterborne. Because the focus of this report is on waterborne pathogens, the following discussion will center on *S. typhi* and those *Salmonella* spp. (e.g. *S. typhimurium*, *S. paratyphi* B) that have been implicated in waterborne outbreaks.

Typhoid fever is characterized by sustained fever, headache, malaise, anorexia, a relative bradycardia, enlargement of the spleen, rose spots on the trunk, nonproductive cough, constipation more commonly than diarrhea, and involvement of the lymphoid tissue. Ulceration in the ileum can result, producing intestinal hemorrhage or perforation in untreated cases. The case-fatality rate can reach 10% if symptoms go untreated; there are approximately 500 fatalities per year (0.2 per 100,000 deaths year) in the United States.[3] Treatment with antibiotics will lower the case-fatality rate to 1%. The drug of choice is chloramphenicol; however, due to widespread use of antibiotics, drug-resistant *S. typhi* may occur, and other antibiotics may be needed.[2] In the United States, chloramphenicol is not generally used because of its idiosyncratic (and potentially fatal) bone marrow suppression (aplastic anemia). Most practitioners use amoxicillin, cotrimoxazole or ciprofloxacin. Milder forms of this disease may occur in populations native to endemic areas.

The carrier state for *S. typhi* may last for up to 1 year, with some individuals becoming permanent carriers.[2,4] Carrier rates in the U.S. have been estimated to be 0.12% in adults and 0.24% in children.[5] Roberts and Wilkins[6] surveyed stool specimens from pregnant women and found an asymptomatic carrier rate of 0.14%. The gall bladder is the focus of infection in long-term carriers, and cholecystectomy may eradicate the carrier state.[2]

Clinical symptoms of salmonellosis include acute abdominal pain, diarrhea, nausea, sometimes vomiting, fever, and dehydration. Anorexia and looseness of bowels may persist for several days.[2] Septicemia may develop with or without fecal infection, which may on occasion lead to the localization in any body tissue, producing abscesses and causing arthritis, cholecystitis, endocarditis, meningitis, pericarditis, pneumonia, pyoderma or pyelonephritis.[2] Death is not common except in the very young, the very old, or the debilitated.[2] The duration of symptoms is from 3 days to 4 weeks.[2]

Antibiotic treatment may not be curative and can lead to prolonged excretion of *Salmonella*

and/or the development of antibiotic-resistant strains.[7] Treatment of salmonellosis is supportive therapy, i.e., rehydration and electrolyte replacement.[2]

INCUBATION PERIOD

A summary of incubation period data based on dose is shown in Tables 1 and 2. Review of the typhoid fever incubation period data indicates a dose-dependent relationship with an incubation period of 3 to 22 days. Review of the incubation period data for salmonellosis indicates an incubation period ranging from 6 hours to 3 days, with less noticeable dose-dependency.

SHEDDING

Fecal excretion of *Salmonella* spp. usually persists for several days or weeks following acute gastroenteritis. Shedding of *Salmonella paratyphi* B may persist from carriers and diseased individuals for 1 year and rarely as long as 20 years.[4] The concentration of Salmonella shed in the stool of carriers and diseased individuals ranged from 10^4 to 10^{11} organisms per gram stool in various studies cited by Feacham.[8]

OCCURRENCE

Waterborne outbreaks of *Salmonella* occur worldwide, and are associated primarily with fresh water. It has been estimated that 400,000 to 3.7 million cases (17.3 cases per 100,000) of salmonellosis (including foodborne and waterborne transmission) occur every year in the U.S.[5,9] However, about 70% of reported U.S cases are imported.[10]

RESERVOIR

The reservoir for typhoid fever is human (currently ill or chronic carriers). There are many zoonotic reservoirs for salmonellosis, including such domestic and wild animals as poultry, swine, cattle, rodents, dogs, cats, turtles, and tortoises. Man is a reservoir in the carrier state; human chronic *Salmonella* carriers are rare.[2]

MODE OF TRANSMISSION

Typhoid is transmitted via water or food contaminated by feces or urine of a patient or carrier. Shellfish, fruits, vegetables, and milk contaminated by sewage or from hands of carriers are also modes of transmission. Transmission of salmonellosis is most commonly fecal-oral (i.e., person-to-person), via food and less frequently, by water.[2]

IMMUNOLOGY

In the case of typhoid fever, man is the only affected host, and susceptibility in the population is general; however, susceptibility is increased in individuals with gastric achlorhydria. Approximately 5% of naturally infected persons fail to develop an immune response, while 9% of vaccinated persons also fail to respond.[11]

Recent studies have shown that *Salmonella* spp. may invade intestinal tissue within 30 to 60 minutes.[12-14] Immune resistance to typhoid fever follows recovery from clinical disease, from inapparent infection, or active immunization. Full immunity is acquired within 7-14 days.[15,16] However, this resistance may not be adequate to overcome the challenge of large doses of *S. typhi*, and repeated infections do occur. In endemic areas, attack rates usually decline with age.[2] Human susceptibility to salmonellosis is not limited to any population group and is usually increased by achlorhydria, antacid therapy, gastrointestinal surgery, neoplastic disease, immunosuppressive therapy, or other debilitating conditions. Goh *et al.*[17] described that 8.5% of patients relapsed after one month. The severity of the disease is related to the serotype, the number of organisms ingested, and host factors.[2] *Gonzalez et al.*[18] reported that 75% of *Salmonella* spp. tested were found to be toxic.

Recent surveys have reported that the current oral typhoid vaccine is about 70% - 90% effective,[19,20] and can last for about one to two years.[21]

ENVIRONMENTAL PERSISTENCE

Persistence of *Salmonella* in the environment is dependent upon species and environmental conditions. *Salmonella* follow the same general survival pattern as most enteric bacteria in fresh water. The characteristic pattern was described by Beard[22] and later by Andre et al.[23] as (1) rapid decrease of organisms in the first 2 to 3 days; (2) a leveling or lag period of 1 days; (3) regrowth for 2 to 3 days; and (4) death phase lasting more than 10 days. The death phase may or may not result in *Salmonella*-free water.

Salmonella spp. may persist for several (1 to 6) months and be associated with sediments long after they have disappeared from the water column.[24-27] *Salmonella* have been isolated from environments with wide ranges of pH (pH 5-8).[24,28,29] Die-off occurs within two weeks at pH 4.5 and just two days at pH 3.5.[28] Seasonality and temperature changes affect *Salmonella* survival, with a more rapid die-off during the summer months than in the winter.[24,25,29-32] At 20°C, 99% die-off occurs in 10 days, compared with 95% die-off at 10°C after 14 days.[31] Rhodes and Kator[32] reported that while >15°C in natural waters was detrimental to *Salmonella* spp., the increased level of microflagellates and bacteriolytic microorganisms significantly contributed to salmonellae densities. Fresh water has been the main source of waterborne outbreaks, although *Salmonella* have been isolated from estuaries with salinities approaching 17 parts per thousand.[24,33] In general, as salinities increase the presence of *Salmonella* decreases.[24,33-35] The organic load of the water is critical for bacterial survival; microorganisms can withstand changes in pH, salinity, and temperature more readily if they are provided with good nutritional supplements.[36] Review of the literature shows that *Salmonella* display better survival in water contaminated with sewage than in unpolluted water; for example, a die-off of 1 x 10^5 *Salmonella* organisms in

10 weeks with fecal pollution, compared with a die-off of 1 x 10^6 *Salmonella* organisms in 2 weeks without fecal pollution.[25,27,37]

DOSE-RESPONSE RELATIONSHIP

The attack rate of *Salmonella* in either typhoid fever or salmonellosis is dependent on the dose of the organism. Tables 3 and 4 summarize dose-response data for *Salmonella typhi* and known agents of salmonellosis. A major portion of the data is based on human feeding studies, while the remainder is based on estimates from disease outbreaks.

INDICATOR-PATHOGEN RELATIONSHIP

There has been much controversy as to the reliability of indicators and their relationship to the presence of pathogens. The purpose of enteric indicators is to act as a signal of possible contamination. Ideally, an enteric indicator should be present when there has been fecal contamination and pathogens are also present. Even if there are no pathogens, the indicator warns of possible fecal presence and the associated possibility of the presence of enteric-disease agents. These general conclusions can be drawn from the literature: (1) coliforms do not always signify the presence of *Salmonella* and are not always present when *Salmonella* are;[35,38,39] (2) common coliform indicators do not coincide with *Salmonella* presence in tropical zones;[36,40] (3) at higher temperatures, coliforms tend to die off much more rapidly than *Salmonella*;[31,41] and coliforms appear to be better indicators in more temperate zones.[4]

Kehr and Butterfield[30] reviewed several studies in England, Indonesia and California where enumeration of both coliforms and typhoid bacilli in sewage and polluted waters was carried out at the time of outbreaks of typhoid fever. They derived a relationship between the morbidity rates from typhoid fever and the ratio of *S. typhi* to total coliform in sewage and polluted waters. A relationship between these organisms was suggested based on correction of the data reviewed for recovery ratios. The relationship can be described by the following equation:

$$y = ar^n,$$

where

a and n are constants, a = 3 and n = 0.46
y = the number of pathogenic bacteria per 10^6 coliform organisms
r = morbidity (relative incidence/100,000 persons)

Based on a morbidity rate of 0.18 per 100,000 persons for typhoid in the United States, the number of *S. typhi* organisms per 10^6 coliforms would be approximately 1.4 using the above relationship.

Table 1

Incubation Period for Typhoid Fever

Dose	Incubation Period (days)	Reference
$<10^3$	15-22	42
10^3	7-14	2
10^5	9	42, 43
10^8	7-8	42
10^9	3-9	37, 42

Table 2

Incubation Period for Salmonellosis

Organism	Dose	Incubation Period (days)	Reference
S. typhimurium	10^3	0.5	44
S. typhimurium	10^4	0.04-4.0	45
Salmonella spp.	10^2-10^3	0.25-3.0	2

Table 3

Dose response for *Salmonella typhi*

Dose (organisms/mL)	Response (per 1,000)	Number of subjects	Reference
10^3	0.1	14	46
10^3	10.0	1,300	42
10^3	45.0	11,800	42
10^3	40.0	10,675	42
10^3	75.0	4,293	42
10^3	90.0	378	42
10^3	100.0	1.6×10^6	44
10^5	275.0	116	46
10^5	270.0	10^4	46
10^5	350.0	110	43
10^7	500.0	32	46
10^7	530.0	30	46
10^7	330.0	6	46
10^7	500.0	30	46
10^8	890.0	9	46
10^9	950.0	42	46
10^9	950.0	6	37
10^9	1000.0	4	46

Table 4

Dose Response for *Salmonella* spp.

Dose (organisms/mL)	Response (per 1,000)	Number of subjects	Reference
17	120	16,000	42, 44
2×10^9	1000	2	42
10^{10}	1000	1	42

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Appendix E - *Vibrio cholerae*

Vibrio cholerae

ETIOLOGY AND CLINICAL DISEASE

Vibrio cholerae is a short comma-shaped Gram-negative rod, varieties of which are responsible for the disease known as cholera. Several strains of *V. cholerae* are pathogenic to humans. *V. cholerae* serogroup 01, the most recognized pathogen of this group, includes the Classical and El Tor biotypes each of which may be identified as belonging to either the Inaba, Ogawa, or the rare Hikojima serogroup. With minor exception, all of these bacteria produce an enterotoxigenic exotoxin (CT toxin).[1,2,3] The production of this toxin in the gut causes a severe diarrhea that can result in profuse watery (rice water) stools, rapid dehydration, acidosis, and circulatory collapse. Death may occur within a few hours. The case-fatality rate in severe, untreated cases is >50%; however, with proper treatment the rate is <1%.[4] In an investigation of an outbreak in Piura, Peru, Ries et al.[5] reported age specific attack rates of 0-4 years, 4.1%; 5-14 years, 1.3%; and ≥ 15 years, 1.8%. The overall death to case ratio was 0.22%. In the United States, 3 of the 136 cases of cholera reported from 1965 through 1991 resulted in death (case-fatality rate of 0.02).[6] Higher case-fatality rates are reported in famine areas and areas where minimal medical care is available. One study cited a death to case ratio of 23% during an outbreak that occurred in Mali in 1984 during a famine.[7] Mild cases with only diarrhea are common, particularly in children. Asymptomatic infection is much more frequent than clinical illness, especially with organisms of the El Tor biotype.[4] Blood-type O individuals are at high risk for development of heavy purging.[8]

There are at least 100-0 antigen serotypes in the *V. cholerae* group. The varieties that do not contain the 01 antigen are commonly referred to as the non-01 or NAG vibrios.[9] These non-01 bacteria are frequently found in the estuarine environment associated with water, sediment and plankton. They may also be found in sewage but are most frequently found in non-polluted brackish and warm water.[10,11] These bacteria appear to be autochthonous to the estuarine environment. Non-01 *V. cholerae* have been isolated that are pathogenic to humans. About 87% of the strains isolated from Chesapeake Bay (67 isolates) were toxigenic as measured in the Y-1 adrenal cell, ileal loop (rabbit) and mouse lethality assays (ibid). Although a few strains of non-01 vibrios have been shown to produce the CT toxin, the majority do not. Shigella-like cytotoxins have been reported to be present in many non-01 isolates.[12] Non-01 *V. cholerae* have been the cause of gastroenteritis in humans usually associated with the ingestion of shellfish (ibid). The Classical and El Tor biotypes are associated with epidemics of cholera while the incidence of non-01 vibrio enteritis occurs in smaller outbreaks or as isolated cases.

Cholera is primarily a waterborne disease. The relatively large volume of water drunk probably reduces the effectiveness of stomach HCl as a barrier to infection.[13] Also, since the residence time of water in the stomach is short, *V. cholerae* can pass quickly into the gut. In normochlorhydric patients, gastric juice can kill 10^8 *V. cholerae* organisms/mL. Cholera can also be foodborne, particularly in association with shellfish and other seafood.

Cholera symptoms usually last 1 to 5 days in the absence of antimicrobial therapy.[14] Prompt fluid therapy is the recommended treatment for cholera in order to counterbalance

the massive loss of electrolytes and to correct for dehydration, acidosis, and hypokalemia.[4] Sugar and salt solutions have been designed for use in fluid therapy for cholera diarrhea (e.g., WHO diarrhea treatment solution and Dacca solution).[4]

INCUBATION PERIOD

The data collected on latency of *V. cholerae* indicate that, dependent upon dose, the incubation period for *V. cholerae* diarrhea ranges from a few hours to 5 days. Usually the incubation time is 2 to 3 days.[4,15,16]

SHEDDING

Median durations of excretion of *V. cholerae* by asymptomatic carriers are 1 - 8 days.[17] The maximum carriage time ranges from 5 - 43 days. Intermittent carriage has been reported for individuals for as long as 100 days in an individual from the Philippines. Comparative studies of the Classical and El Tor biotypes indicate that the El Tor biotype has a longer carriage time. Investigations of carriage time by age revealed that children under the age of 5 have a median carriage time of 3 days, compared to persons over the age of 15 who have a median carriage time of 1 day.[17]

Persons infected by *V. cholerae* but not sick can excrete 10^2 - 10^5 bacteria/gram of feces, whereas those with active and severe cases may excrete 10^6 - 10^9/ml of rice water stool.[18] The prevalence of excretion in the general healthy population is generally very low (under 1%), even in endemic areas.[18]

OCCURRENCE

The distribution and number of *V. cholerae* are associated with water salinity, and they appear to be autochthonous to estuarine and marine environments.[18,19,20,21] Pandemic cholera repeatedly spread from India to most of the world during the 19th century. During the first half of the 20th century, the disease was largely confined to Asia, except for a severe epidemic in Egypt in 1947. Cholera has been reported throughout the Mediterranean area (North Africa, Portugal, and Italy).[4,22] Also, several outbreaks have been reported from the South Pacific in the Gilbert Islands[23] and on Nauru.[24]

Early in 1991, a cholera epidemic began in several coastal cities in Peru. The outbreak spread to other parts of Peru and to Ecuador, Columbia, Chile, Brazil, Mexico and the United States.[25] Since January 1991, more than 533,000 cases and 4,700 deaths have been reported from the South American hemisphere.[26] In 1991, the rate of cholera was estimated to be 0.3 for every 100,000 air travelers returning to the U.S. from South America.[6]

The first report of cholera in the Western Hemisphere in over 60 years was a single case reported in the United States in Texas in 1973.[4] Since that time, there have been outbreaks and sporadic occurrences in the U.S., Canada, and Australia.[4,21,27-31] There have

been numerous isolated cases of *V. cholerae* from the three coastal areas of the U.S.: Chesapeake Bay, [27,28] the Gulf states,[27,29,30] and California.[21] These cases were mostly non-01 vibrio associated and not Classical or El Tor. One-hundred and thirty-six cases of cholera were reported in the United States from 1965 through 1991, with a range of 0 to 26 cases reported per year.[6] Forty-two (31%) of the reported cases were acquired outside of the country.

RESERVOIR

In the past, humans have been considered to be the single reservoir of Classical and El Tor cholera. Asymptomatic carriers are not thought to be an important reservoir of infection because 90% of acute cases no longer excrete vibrios in the feces by the end of the third week of infection.[32] Evidence is accumulating that non-human reservoirs exist.[4,21,27-30] The maintenance of cholera vibrios during interepidemic periods appears to be achieved by their survival in estuarine and brackish waters and associated plankton.[21,27-30] There is a very strong suggestion that *V. cholerae* is a natural inhabitant on the brackish water environment. In India, Sanyal et al.[33] have shown that 0.6% of household animals were carriers of *V. cholerae* serotype 1 and that 3.6% were positive for non-01 vibrios. The significance of this latter report to the epidemiology of cholera is uncertain.

MODE OF TRANSMISSION

The mode of transmission is primarily through the ingestion of water contaminated with feces or vomitus of cholera patients, or, to a lesser extent, feces of carriers. Seafood (especially shellfish) and food contaminated with feces via soiled hands or flies have also been found to transmit *V. cholerae*.[4,34]

IMMUNOLOGY

In endemic areas, clinical cholera usually is confined to the lowest socioeconomic groups. In epidemics, attack rates rarely exceed 2%. Increased resistance occurs following infection due to a rise in agglutinating, vibriocidal, and antitoxic antibodies against homologous types.[4] Persons in endemic areas acquire antibodies by early adulthood. Recently developed oral live vaccines protect volunteers for up to 6 months after immunization and only 5-25% excrete *V. cholerae* after vaccination.[35] These vaccines induce intestinal and serum antibacterial immunity. Data regarding the duration of naturally acquired immunity was not found in this literature search.

ENVIRONMENTAL PERSISTENCE

V. cholerae appear to be autochthonous to estuarine and marine environments.[21,27-30] There have also been numerous isolations from surface, well, and tap waters (Table 1). There have been many studies concerning the survival of *V. cholerae*, and some of these data are presented in Table 1. Some generalities are: 1) El Tor vibrios survive longer in fresh

water than Classical vibrios; (2) in sewage, both biotypes, as well as the different serotypes, show no difference in survival; (3) vibrios survive in low numbers in estuaries at lower temperatures (overwintering); and (4) best survival and growth appear to be in brackish environments during the summer months.

Laboratory investigations on survival of *V. cholerae* under different physiochemical conditions have confirmed observations from field studies. Miller et al.[36] found the optimal salt concentration for survival was 2.0%. The lowest salt concentration consistent with stable survival was 0.05%. Eighteen out of twenty *V. cholerae* strains were also found to survive in simple salts media without nutrients at 25°C for up to 3 years.

Referring to Table 1, it appears that *V. cholerae* generally does not survive well in fresh water; therefore, unless there has been recent contamination, *V. cholerae* may not be a major concern. *V. cholerae* survives longer in brackish waters, but these sources are less likely to be utilized for drinking. However, brackish water may present a threat if flooding leads to mixing with freshwater sources.

DOSE-RESPONSE RELATIONSHIP

Table 2 summarizes dose-response data for *V. cholerae* from volunteer studies. Previous studies have reported an infection-to-case ratio of 2:1 to 4:1 for Classical cholera, and almost 100:1 for El Tor cholera.[37] However, the data in Table 2 show that in volunteer feeding studies the attack rates for Classical and El Tor are similar (it should be noted, however, that only 4 data points for El Tor are included). Also, Khan and Shahidullah[37] surveyed cholera in Dacca, and found that the severity and attack rates of cholera due to the El Tor biotype were equal to those of the Classical biotype.

INDICATOR-PATHOGEN RELATIONSHIP

The literature shows that standard indicator coliforms are probably not good indicators for *V. cholerae*.[27,28,38] In the case of contaminated freshwater sources, coliforms will survive longer than *V. cholerae*; therefore, in this instance, standard indicators may be useful.[18,38] However, in estuarine and marine environments, the factors that increase *V. cholerae* survival are antagonistic to coliforms.[27]

Table 1

Environmental persistence of *Vibrio cholerae*

Location	Source	Salinity (ppt)	Temp. (°C)	Survival (days)	Reference
U.S.	Brackish	5	10	4	19
U.S.	Brackish	25	10	25 - 42[a]	19
U.S.	Brackish	< 3 - 31.7		Year-round	21
U.S.	Unknown			Several years	30
U.S.	Marine-brackish	≤ 32	Warm	~90[b]	29
U.S.	Marine-brackish	6 - 12		Year-round	28
U.S.	Marine-brackish		9 - 12	Die-off: 10^2/2 days	22
England	Surface water			~90[b]	39
Pakistan	Surface water		Warm	~90[b]	40
India	Well water		21	12 - 51	39
India	Well water		37	1 - 4	39
Bangladesh	Surface water			7 - 13	41
Bangladesh	Marine			10	42
Worldwide	Sewage			10	18
Worldwide	Sweaty clothes			7	18
U.S.S.R.	Sewage			400	18
(Classical)	Well water			3	18
(Classical)	Surface water			0.75	18
(Classical)	Marine			4	18
(Classical)	Tap water			0.91	18

Table 1 (continued)

Location	Source	Salinity (ppt)	Temp. (°C)	Survival (days)	Reference
(Classical)	Sewage			0.5	18
(El Tor)	Well water			5	18
(El Tor)	Surface water			2.2	18
(El Tor)	Marine			2.3	18
(El Tor)	Tap water			2.0	18
(El Tor)	Sewage			2.75	18
Worldwide	Harbor			81	43
Worldwide	Marine			64	43
Worldwide	Surface			< 32	43
Worldwide	Marine			10 - 47	43

[a] During winter months
[b] During summer months

Table 2

Dose response for *Vibrio cholerae*

Biotype or serotype of *Vibrio cholerae*	Attack rate[a]	Dose	Reference
O1	800 - 1000	10^6	2
O1 Classical Inaba	260	10^3 - 10^4	15
O1 Classical Inaba	0.0	10^4	15
O1 Classical Inaba	0.0	10^5	15
O1 Classical Inaba	830 (10/12)	10^6	3
O1 Classical Inaba	0.0	10^7	15
O1 Classical Inaba	500 (2/4)	10^8	15
O1 Classical Inaba	500 (1/2)	10^9	15
O1 Classical Inaba	0.0	10^{10}	15
O1 Classical Inaba	500 (1/2)	10^{11}	15
O1 Classical Ogawa	670 (4/6)	10^5	3
O1 Classical Ogawa	960 (22/23)	10^6	3
El Tor Inaba	600 (6/10)	10^5	3
El Tor Inaba	1000 (10/10)	10^6	3
El Tor Ogawa	600 (3/5)	10^5	3
El Tor	111 (31/274)	$\geq 10^4$	44
Classical	890 (24/27)	10^6	16
El Tor	860 (32/37)	10^6	16

[a] Attack rate per 1,000; number of individuals with cholera per total tested in parentheses.

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Appendix F - Pathogenic *E. coli*

Pathogenic *Escherichia coli* 0157:H7

ETIOLOGY AND CLINICAL DISEASE

Escherichia coli is a facultative anaerobic, nonspore-forming, Gram-negative rod and a member of the Enterobacteriaceae.[1] *E. coli* is a common gut organism in humans and all the warm-blooded animals; however, some strains have been found to be pathogenic to man. The severity and type of pathogenicity is strain-related; basic *Escherichia* enteropathies recognized include: (1) enteropathogenic *E. coli* (EPEC), (2) enterotoxigenic *E. coli* (ETEC), (3) enteroinvasive *E. coli* (EIEC)[1,2] and (4) enterohemorrhagic *E. coli*. Mechanisms of pathogenicity recognized in *E. coli* diarrhea include presence of Shiga-toxin,[3] enteroadherence (EPEC),[4] invasive *E. coli*, [5] cholera-toxin like (LT),[6] Sta-cyclic GMP toxin[7] and Stb (new anion secretion) toxin.[8]

Invasive strains cause disease primarily localized in the colon, manifested by fever and mucoid and occasionally bloody diarrhea (somewhat like *Shigella* spp.)[2] ETEC strains behave more like *Vibrio cholerae* in producing profuse watery diarrhea without blood or mucus, abdominal cramping, vomiting, acidosis, prostration, and dehydration. Fever may or may not be present.[2] Both EIEC and ETEC are usually associated with sporadic disease and occasionally are the cause of common source outbreaks.[2] EPEC strains belong to the "classical" EPEC serotypes that have been associated with outbreaks of acute diarrheal disease in nurseries for the newborn.[2]

The remainder of this appendix generally focuses on *E. coli* 0157:H7, which exhibits enterohemorrhagic pathogenicity. Clinical features of disease caused by *E. coli* 0157:H7 can include abdominal cramps, watery and/or bloody diarrhea, vomiting, and no to low fever.[9] The organism has been associated with sporadic cases and outbreaks of hemorrhagic colitis. *E. coli* 0157:H7 has been added to the list of organisms known to cause Hemolytic-uremic syndrome (HUS) or Thrombotic Thrombocytopenic Purpura as a secondary complication.[10] HUS in North America occurs principally in children younger than two years of age.

Disease caused by pathogenic *E. coli* 0157:H7 is self-limiting for most individuals. Duration of disease varies depending on age. Pai et al.[9] reported the duration of illness to be 9.1 ± 2 days for children and 6.6 days ± 1.1 days for adults. For preschool children, Spika et al.[10] reported 12.2 days duration for those with bloody diarrhea and 6.8 days duration for those with nonbloody diarrhea. Griffin et al.[11] reported an average duration of 7.8 days for patients who had been treated with an antimicrobial drug versus 9.7 days for patients who had not been treated.

Case-fatality rates and morbidity are strongly related to extreme ages. In outbreaks in a day care center and a kindergarten, the youngest children had the highest rate of illness, whereas in a nursing home outbreak, the highest rate of illness was in the very old.[11] Krishnan et al.[12] reported a case-fatality rate of 31% in a nursing home in Ontario, Canada. Investigations of nine outbreaks occurring between 1982-1986 showed that all deaths occurred in the age group >65 years of age.[11]

INCUBATION PERIOD

Pai et al.[9] reported fifteen of twenty patients (75%) had consumed hamburgers 12 hours to 5 days before the onset of symptoms caused by *E. coli* 0157:H7, with a mean interval of 2 days. Spika et al.[10] reported a day care center outbreak in which 5 days after an infected 3 year old had contact with classmates other members of his class developed diarrhea. U.S. Morbidity and Mortality Weekly Report[13] indicated that 372 of 425 (88%) individuals in a Washington State outbreak had eaten in one of the locations of a restaurant chain during the 9 days preceding onset of symptoms. Padhye and Doyle[14] reported a median incubation period of 4 days with a range of 3-9 days. Ryan et al.[15] reported that in a nursing home outbreak the median interval between infection and disease was 8 days (range 3-12 days).

SHEDDING

The shedding period of *E. coli* 0157:H7 in humans is relatively short. This may in part account for the low recovery of the organism from stools of infected individuals and environmental sources. Culturing of stool specimens from infected individuals indicates that the organism can be recovered in adults up to five days from onset of illness.[9,10] Remis et al.[16] reported that delayed collection of stool samples (>6 days after onset) among persons meeting case definition may have decreased the yield of *E. coli* 0157:H7. The organism can be recovered in children up to 7-13 days after onset of illness.[9] Griffin et al.[11] reported isolating the organism up to 30 days after onset of illness in an infant and an elderly patient.

OCCURRENCE

Padhye and Doyle[14] reported the 1988 annual incidence rate for *E. coli* 0157:H7 to be 2.1 cases per 100,000 for the age range 11 months - 78 years. The incidence rate for this same year increased to 6.1 cases per 100,000 for children under 5 years. In the years 1981-1986 the incidence rate was 1.74 cases per 100,000 people for individuals <15 years of age. Similar incidence rates were reported in Scotland for the years 1984-1992 with 1.4 per 100,000 for the age group 0-65 years and 6.0 per 100,000 for the age group 0-4 years.[17]

Martin et al.[18] reported incidence rates in 1988 for HUS due to *E. coli* 0157:H7 to be 2.0 per 100,000 children under the age of 18. Reports of children and adults contracting HUS from *E. coli* 0157:H7 infection indicate a low attack rate of 2-7%.[9,10]

E. coli 0157:H7 was implicated in an outbreak associated with a contaminated water supply in Cabool, Missouri in 1989-1990.[19,20] This outbreak was the first reported *E. coli* 0157:H7 outbreak in the U.S. associated with a municipal water supply. Between December 15, 1989 and January 1990, visitors and residents of Cabool experienced 243 cases of diarrhea (85 bloody) and four deaths.[19] Another waterborne outbreak in which *E.*

coli 0157:H7 was implicated was reported in Scotland in 1990,[21] but the organism was never isolated from the suspected contaminated water source.

The attack rate of *E. coli* 0157:H7 during the waterborne outbreak in Cabool, Missouri ranged from 3.3% (overall cases) to 8.2% (age >64).[20] Higher attack rates have been associated with person-to-person transmission and foodborne outbreaks. Belongia et al.[22] reported a median attack rate of 23% (range 3% - 38%) in children from a day care center in Minnesota where person-to-person contact was the suspected mode of transmission. Carter et al.[23] reported an attack rate of 33% in a nursing home in Ontario, Canada.

The first known outbreak of gastroenteritis in recreational water due to *E. coli* 0157:H7 was reported in July of 1991.[24] Eighty cases associated with swimming in a park lake were reported in Oregon. Poor water exchange was deemed to be a contributing factor and fecal coliforms in shallow lake water exceeded recommended state levels by several-fold.[24]

RESERVOIR

Cattle have been implicated as the reservoir of infection for outbreaks associated with *E. coli* 0157:H7.[25] A survey conducted on cattle herds implicated in human infection showed 2.0% - 6.0% of fecal samples taken from dairy cows were *E.coli* 0157:H7 positive. All the isolations were from heifers and calves; none was from adult cows. Surveys of healthy cattle not implicated in human infection show lower isolation rates (<1.0%) of *E. coli* 0157:H7 from fecal samples with slightly higher rates of isolation from heifers and calves.[26] *E. coli* 0157:H7 has also been isolated from other animals such as chicken, turkey, buffalo, and pigs.[11] However, cattle are the only animal with the possible exception of turkey[23] that have been associated with human infection.[26]

MODE OF TRANSMISSION

The organism is transmitted through the fecal-oral route. Foodborne (contaminated hamburger meat) and person-to-person transmission have been implicated as the primary sources of infection in most outbreaks since the disease was first reported in 1982.[10,15,23,27] Besser et al.[28] reported an outbreak of diarrhea from *E. coli* 0157:H7 due to consumption of contaminated fresh pressed apple cider.

IMMUNOLOGY

Because more children than adults are afflicted, it has been hypothesized that some immunity is conferred upon adults. In addition, there is evidence of asymptomatic carriage. Padhye and Doyle[14] reported that many adults are carriers of EPEC *E. coli* but seldom express symptoms of illness. Immunity to enterotoxin and surface antigens of *E. coli* has been demonstrated, but its duration is not known. Local secretory immunity is

probably the most important defense mechanism.[2]

ENVIRONMENTAL PERSISTENCE

Doyle et al. [29] found *E. coli* 0157:H7 grew well in tryptic soy broth between 30°C and 42°C, with 37°C being optimal. *E. coli* 0157:H7 did not grow well at 44°C to 44.5°C, the temperatures normally used for isolation of *E. coli*. Singh et al.[30] showed that strains of enterotoxigenic *E. coli* can revive themselves in the small intestine and maintain their entertoxigenic activity after sustaining injury with copper concentrations (0.8 -1.0 mg/L) and chlorine concentrations (0.4 - 1.6 mg/L) that are similar to those found in drinking water systems. Several studies have shown enterotoxigenic *E. coli* strains survive in the water environment for prolonged periods of time.

Bench scale microcosm studies have shown that *E. coli* can survive for up to 260 days at temperatures from 4°C to 25°C with no loss of viability in sterilized river waters.[31] Viability decreased dramatically in treated sewage water. Presumably this was due to competition by other microorganisms. Terzieva et al.[32] found 3 strains of enterotoxigenic *E. coli* to survive at temperatures of 6°C and 16°C for up to 14 days in the water environment. Swerdlow et al.[20] reported laboratory investigations where *E. coli* 0157:H7 isolated from a waterborne outbreak survived in the implicated water source for up to 7 days with less than a tenfold decrease in culturable organisms. These studies indicate that *E. coli* is capable of surviving for extended periods of time in a water environment and that contaminated water is a possible vehicle of transmission for this organism.

DOSE-RESPONSE RELATIONSHIP

This literature search did not find data from outbreaks or volunteer studies on the infectious dose of *E. coli* 0157:H7. Griffin and Tauxe[26] hypothesized that the general patterns of transmission in outbreaks suggest that the infectious dose is low. In outbreaks where meat was the implicated vehicle of transmission, the meat was only slightly undercooked and not subsequently held in warm temperatures that would have permitted rapid bacterial growth. Further, cold bulk vehicles such as municipal water are most likely to dilute any organisms without permitting rapid bacterial growth, yet this vehicle has been associated with fairly high attack rates.[26] Using data from past outbreaks, Geldreich et al.[19] estimated that the infectious dose ranged from 10-100 microorganisms with infectivity being more severe among infants and senior citizens. Because of the severe invasiveness of the disease and the limited therapy available, no human volunteer studies have been done to establish the exact infective dose for *E. coli* 0157:H7.

Table 1 presents dose-response data for other strains of pathogenic *E. coli* fed to human volunteers. The effect of buffering the stomach acid barrier on infective dose should be noted. The attack rate was six times as high when stomach acid was buffered for the same strain of *E. coli* at the same dose.

INDICATOR-PATHOGEN RELATIONSHIP

Little data was found regarding the ratio of pathogenic *E. coli* to nonpathogenic *E. coli* or coliforms. Some authors have reported that < 1% of total coliforms are pathogenic *E. coli;*[33] others reported that possibly as high as 3-4% of total coliforms may be pathogenic *E. coli.*[34]

Table 1

Dose-Response for Pathogenic *E. coli*

Dose (Number of organisms)	Fraction Ill[a]	Strain Type	Reference
10^4	0 (0/5)	4608 (EIEC)	35
10^4	0 (0/5)	1624 (EIEC)	35
10^6	0 (0/5)	4608 (EIEC)	35
10^6	0.11 (1/9)	1624 (EIEC)	35
10^6	0.67[b] (2/3)	1624 (EIEC)	35
7.0×10^6	0.64 (7/11)	0111 (ETEC)	36
10^8	0.20 (1/5)	B7A (ETEC)	35
10^8	0.40 (2/5)	B2C (ETEC)	35
10^8	0.60 (3/5)	1624 (EIEC)	35
10^8	0.625 (5/8)	4608 (EIEC)	35
1.4×10^8	0.75 (6/8)	055 (ETEC)	37
2.7×10^8	0.56 (9/16)	H10407 (ETEC)	38
5.3×10^8	0.67 (8/12)	0111 (ETEC)	36
1.7×10^9	0.625 (5/8)	055 (ETEC)	37
5.0×10^9	0.75 (6/8)	055 (ETEC)	37
6.5×10^9	1 (11/11)	0111 (ETEC)	36
9.0×10^9	1 (12/12)	0111 (ETEC)	36
10^{10}	0.60 (3/5)	B2C (ETEC)	35
10^{10}	0.80 (4/5)	B7A (ETEC)	35
1.6×10^{10}	0.875 (7/8)	055 (ETEC)	37

[a] Number of individuals ill per total in parentheses.
[b] Stomach acid buffered with $NaHCO_3$.

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Appendix G - Enteroviruses

ENTEROVIRUSES

ETIOLOGY AND CLINICAL DISEASE

Enteroviruses from the family Picornaviridae encompass poliovirus, coxsackie virus groups A and B, and echoviruses. These enteroviruses should not be confused with those termed enteric viruses (describing any virus disseminated by the fecal route).[1] The enteroviruses may cause a wide variety of disease symptoms, as summarized in Table 1, but generally produce asymptomatic infections.

Poliovirus can cause the most serious of the symptoms of the enterovirus as polio is the major permanently crippling disease of infectious origin ascribed within this group of agents.[2] Case-fatality among the paralytic cases of polio infection ranges from 2 to 10% and increases markedly with age.[3] Although most infections caused by enteroviruses have no lasting effect, some of the "new enteroviruses" (most recently discovered) can also cause permanent paralysis.[4] Group B coxsackie viruses also have the potential for causing serious, even fatal, disease. The mean period of infectivity for persons infected with enteroviruses is 50 days. Poliomyelitis is characteristically a disease of children and adolescents.[3] As stated previously, the severity of disease in a nonimmune host is directly related to the age of the host; the risk of serious disease is lower at an earlier age.[1] There does not appear to be any difference in infection or severity of disease between the sexes or different races. It is generally thought that the other enteroviruses are similar to poliovirus in these characteristics.

INCUBATION PERIOD

The incubation period for the minor illnesses caused by enteroviruses, including minor polio infections, is about 2 to 3 days. When the nervous system is involved (including paralytic polio), the average incubation period is 7 to 17 days, with a range of 3 to 35 days.[2,3]

SHEDDING

The average concentration of enteroviruses in feces is about 10^6 Plaque Forming Units (PFU)/g. Some virus have been measured in human feces in concentrations as high as 10^9 PFU/g.[2] The duration of shedding is about 1 week to 1 month.[5] Due to the nature of viruses, they can not multiply outside of the host cell, therefore, although excreted numbers are large the viruses can be quickly diluted and fall prey to various environmental pressures (e.g., pH, temperature, sunlight, oxidants).[6]

OCCURRENCE

The enteroviruses have worldwide distribution, and infections by them are common. There are local variations in virus types and virulence of strains.[2] It should be stressed that

infection does not equal disease; it is epidemiologically estimated that 1 out of 100 poliovirus infections and 1 out of 1000 coxsackie or echovirus infections result in clinical illness.[7]

In 1988, the World Health Organization (WHO) adopted a resolution to eradicate poliomyelitis by the year 2000.[8] By 1993 the overall occurrence of poliomyelitis decreased by 70%, with no reported cases in the Americas or Western Europe. However, WHO continues to warn polio-free countries that, until global eradication is acheived, wild-type poliovirus may be imported from polio-endemic reservoirs.[8]

In endemic areas with temperate climates there is an increase in poliovirus infections in late summer and early autumn. Tropical and subtropical areas show less fluctuation, but the trend is the same. Similar seasonal variations also occur among the other enteroviruses.[2]

The reported distribution and occurence of waterborne enteroviruses, with the exception of poliovirus, has increased over the last few years.[9,10,11] This has been due to the development of more sensitive detection methods.[9] It is estimated that about 2×10^{-6} cases/100,000 (about 500 per year) occur in the United States.[10,12] However, the causes of the majority of acute gastrointestinal illness (AGI) associated with drinking water have not been defined. Many public health experts believe that viruses may be the cause; however, they can be difficult to isolate and identify from environmental and clinical sources. Another obscuring factor in tracing waterborne viral gastroenteritis is that this disease is so common that the vast majority of cases go unreported.

RESERVOIR

The reservoir of enteroviruses is the infected human.[2] Asymptomatic infections probably play an important role, and children under the age of two are the most potent disseminators. Some enteroviruses have been isolated from pets and other animals associated with humans, but it is not certain that they were naturally infected. Nonhuman reservoirs have not been shown to be significant.[2]

MODE OF TRANSMISSION

Enteroviruses are frequently transmitted by the fecal-oral route[1] and may also be passed by the oral-oral route via nasal and pharyngeal secretion.[13] Strong evidence exists that person-to-person transmission is the primary route of contagion.[14] There is little epidemiological evidence available concerning the waterborne disease potential of the enteroviruses.[14,15] In the last ten years over half of all outbreaks of waterborne gastroenteritis has been defined as Acute Gastrointestianl Illness, or, AGI. Although no known cause(s) has been determined for AGI, the Centers for Disease Control suspect that the majority of these illnesses are likely viral in origin.[10,12,16]

Rarely are epidemiological methods sensitive enough to detect low-level waterborne transmission. A reported waterborne polio outbreak in Huskerville, NE, involved

contaminated tap water.[17] Two outbreaks, one of echovirus 16 and one of coxsackie B5, may have been at least partly from waterborne viruses.[18] Shellfish are known to harbor enteroviruses.[19-22] In rare instances, food has been implicated in polio transmission.[3]

IMMUNOLOGY

Small children are the most susceptible age group to polio infection, because most adults have acquired resistance through earlier infection or vaccination.[2] As mentioned above the WHO is putting forth a concerted effort to eradicate poliomyelitis. Practically all developed and western countries have done so. It is estimated that 90-95% of the current U.S. population is immune to poliovirus.[12,23] There are no antiviral agents for the prevention or treatment of other enteroviral infections.[24] This literature search did not discover any literature describing the development of vaccines for other enteroviral agents of gastroenteritis.

The typical immune reponse to infection by virus is seen with this group of viruses. Following infection, antibody rise (IgM) is seen within seven to ten days. Long-term immunity (via IgG) varies virus to virus, poliovirus immunizations can last to 40 years or longer. Infection by the other enterovirsus also confer type-specific resistence.[2]

ENVIRONMENTAL PERSISTENCE

Enteroviruses are capable of surviving for extended periods under certain environmental conditions. Tables 2, 3, and 4 summarize some data on survival of enteroviruses under various conditions. The survivability of viruses in the environment depends on the virus type, the flow rate of the water in question, climatic conditions (especially temperature), degree and type of pollution, and whether the viruses are free or associated with solids. Low temperatures and high levels of pollution are most favorable to virus survival.[19] Enteroviruses are more labile in summer than in winter in free-flowing ocean water. They cease to be viable within 7 days at 37°C in seawater, and are more labile in natural waters than in artificially prepared marine and estuarine waters.[25] There is some antiviral activity in natural waters.[26,27] Viruses can survive for more than 175 days in soil particles with a moist environment at neutral pH, and at low temperature.[13] Enteroviruses have been known to survive several weeks in pit latrines[14] and up to 130 days in sewage.[20] Berg et al.[28] demonstrated that enteroviral survival of up to 38 days in aeration basins and 17 days in oxidation ditch sludges at 5°C. The time required to reduce numbers of enteroviruses by 99.9% in the environment ranges from 2 to 160 days. They can last up to 14 to 16 days in the sea.[29]

DOSE-RESPONSE RELATIONSHIP

Available dose-response information is given in Table 4. In addition to the data included in this table, Westwood and Sattar[30] reported the minimal infective dose for polio 1 as two plaque-forming units (PFU), for polio 3 as 10 times the tissue-culture infective $dose_{50}$ ($TCID_{50}$), for Coxsackie A21 as 18 times the $TCID_{50}$, and for Coxsackie B4 as 1.3 times

the mouse median lethal dose (LD_{50}).

There is some controversy about whether one virus particle can establish infection or not, but the conservative estimate is that one tissue-culture infectious dose can cause human infection.[20,30]

INDICATOR-PATHOGEN RELATIONSHIP

The majority of researchers in this field believe that at present there is no reliable indicator organism for enteroviruses in waters.[15,19,21,31-36] It is currently accepted that the presence of indicator organisms raises the distinct possibility of virus contamination, but their absence does not guarantee the absence of viruses.[37]

The search for appropriate viral indicators is an active area of study. Gerba et al. reported that the number of viruses detected in water is related to rainfall, salinity, and total coliforms, but these only explain a variance of about 16%. This is not enough to be a reliable indicator.[31] La Belle et al.[3] found a correlation between fecal coliforms and presence of enterovirus in sediment but not in overlying seawater. The authors developed the following equation for expressing this relationship:

$$Y = 11.93 + 0.008\ X$$

where Y = number of viruses in sediment, and
X = number of fecal coliforms in sediment.

Payment et al.[38] found a correlation between virus isolations and water turbidity at between 10 and 30 nephelometric turbidity units (NTU). Berg and Berman[34] found that many indicator bacteria were present in samples of raw or digested sewage sludges where no viruses could be detected. They suggested that the smallest numbers of indicator bacteria present in samples from which viruses were not recovered may serve as a guidepost number for judging sludges to be free of viruses. Fattal et al.[39] suggested that since fecal streptococci displayed a die-off rate similar to enteroviruses in seawater, they may be a useful indicator there. Guy and McIver[40] proposed bacteriophages as indicators of enteric virus removal by water-treatment practices. Roy et al.[41] mention bovine parvovirus as a possible enteric virus indicator, and Scarpino[42] suggested the phage of *Serratia marcescens* as a poliovirus indicator, and the use of other phages to monitor efficiency of virus removal in water treatment. Knott et al.[43] thought the use of *E. coli* B bacteriophages provided a satisfactory measure of the quality of waters with respect to viruses.

Clarke et al.[44] found a coliform-to-virus ratio of 92,000:1 in sewage and 50,000:1 in polluted surface waters in 1969, but these ratios do not appear to be widely accepted.

Table 1

Disease Syndromes of the Enteroviruses

Virus	Disease Symptions
Polio	Paralysis, aseptic meningitis, fever, nonparalytic polio
Echovirus	Aseptic meningitis, respiratory disease, rash, diarrhea, fever
Coxsackie Virus A	Herpangina, respiratory disease, aseptic mengitis, fever
Coxsackie Virus B	Myocarditis, congential hear anomalies, rash, fever, aseptic mengitis, respiratory disease, pleurodynia
New Enteroviruses	Aseptic meningitis, encephalitis, respiratory disease, acute hemorrhagic conjunctivitis, fever, paralysis

Table 2

Survival of Enteroviruses in River Water (Tanana River, AK), 0°C[45]

Sampling Station	Distance from source (km)	Mean flow time (d)	Mean number of enteroviruses/380 L
Sewage-treatment plant	--	--	235
T700, Tanana River	0	0	6.33
T600, Tanana River	77	1.9	5.67
T400, Tanana River	179	4.2	1.8
T100, Tanana River	317	7.1	1.25

Table 3

Survival of Enteroviruses in Ocean Water (Days)[25]

Virus	Winter	Summer	Estuarine water, winter
Polio 1	26	65	51
Coxsackie B5	48	80	>>100
Echovirus 6	30	70	>100

Table 4

Effects of Salinity and Incubation Temperature on Virus Survival (Weeks)[25]

Virus (ppt NaCl)	4°C			15°C			25°C		
	10	20	34	10	20	34	10	20	34
Polio 1 (Mahoney)	40	40	46	46	20	20	6	4	6
Echo 6 (D'Amori)	40	46	46	22	24	24	8	6	4
Coxsackie B5 (Faulkner)	>53	>53	>53	>53	46	40	10	8	8

Table 5

Infective Doses of Enteroviruses[46]

Virus	Dose	Carrier rate	%	Comments
Polio 1	200 PFU[a]	4/4	100	Koprowski, 1955, as reported in Reference 46
Polio 1	20 PFU	4/4	100	Adults, oral route
Polio 1	2 PFU	2/3	67	
Polio 1	0.2 PFU	0/2	0	
Polio 3	10 $TCID_{50}$[b]	2/3	67	Premature infants, oral route
Polio 3	2.5 $TCID_{50}$	3/9	33	Premature infants, oral route
Polio 3	1 $TCID_{50}$	3/10	30	Premature infants, oral route

[a]PFU = plaque-forming unit
[b]$TCID_{50}$ = tissue-culture infective $dose_{50}$

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Appendix H - Hepatitis A virus

HEPATITIS A

ETIOLOGY AND CLINICAL DISEASE

The hepatitis A virus (HAV) is a 27-nm virus physically resembling an enterovirus. Symptoms of hepatitis A typically include fever, nausea, malaise, anorexia, and abdominal discomfort. Jaundice develops a few days after onset of symptoms. The disease ranges from mild with a duration of 1 to 2 weeks, to severely disabling and lasting several months, although the latter occurrence is rare. The recovery period is usually prolonged. The case-fatality rate has been reported to range from 0.04% in children of 5-14 years old to 2.7% in adults over 49 years old.[1] Typically the case-fatality rate ranges from 0.1 to 0.5%, and usually only occurs in older patients with a severe case. Generally there is complete recovery without sequelae or recurrences,[2] but relapse rates can reach up to 20%.[1]

Hepatitis A can be diagnosed by the detection of virus in the stool or by the presence of IgM antibodies against HAV, which are only present in the serum of persons acutely or recently ill. There is currently no specific treatment for HAV. Supportive therapy is given as needed. Isolation of cases is not considered necessary, but they should be restricted from certain occupations such as food handling while in the infective stage. Patients are infective prior to development of jaundice and for the first 2 weeks of illness.

INCUBATION PERIOD

The incubation period of HAV is related to the dose. The average incubation period is 28 to 30 days but ranges from 8 to 60 days in the references cited. The most common range is 15 to 50 days.[2]

SHEDDING

Shedding has been reported to be as great as 10^{10} per gram of feces.[3,4] About 30-70% of those symptomatic will shed HAV for up to one month.[4,5]

OCCURRENCE

Hepatitis A has a worldwide distribution. In the U.S., since 1920, there have been about 15 reported outbreaks of HAV associated with drinking water.[6,7,8] The incidence for viral hepatitis in the United States in 1980 was reported as 26.5/100,000, of which 48% was HAV.[9] In 1985, the rate had decreased to 9/100,000.[10] Hepatitis A is particularly prevalent in areas with poor sanitation. The areas of greatest risk are the Indian subcontinent, Africa (especially West Africa), the Mideast, and Asia.[11] Nearly 100% of Thais by age 15, Ethiopians by age 13, and Taiwanese by age 20 have antibody to the virus.[3,12,13] In Europe it has been estimated that there are >300,000 cases due to HAV per year.[1] Twenty-two percent of tested U.S. Army personnel stationed in Thailand and

25% of tested personnel stationed in Germany possessed antibodies to HAV.[14,15]

The disease typically occurs in persons 15 years old and younger; many of the infections in young people are asymptomatic or mild without jaundice. About 3 to 30% of infected persons are asymptomatic for HAV (depending upon age group affected).[16,17] In general, HAV increases in severity with age and decreases in incidence after age 35. Both sexes have comparable attack rates.[2,18]

In groups of previously unexposed persons traveling to endemic areas of hepatitis, 13 to 48% have been reported to sero-convert, but only about 20% of those infected display clinical illness.[17,19]

RESERVOIR

The normal reservoir of HAV is acute-phase humans, whose feces are infective from the last half of the incubation period to the first week of jaundice, and whose serum is infective for a short time during the acute phase.[20] There is no known carrier state. Rarely, chimpanzees, or even less frequently, other nonhuman primates may be reservoirs of the virus.[2,21]

MODE OF TRANSMISSION

Mode of transmission is via the fecal-oral route. Person-to-person transmission is most frequent. Common-source outbreaks are linked to water or food. In the U.S., the role of waterborne outbreaks has been estimated to contribute to 0.4 to 8% of all HAV incidence.[22-24] Mollusks may concentrate virus from areas with minimally polluted water and be a source of disease[25,26] (see Environmental Persistence, below). Mbithi et al.[27] demonstrated that HAV can survive for long periods on inanimate objects and as well as on human hands. Therefore, food can be easily contaminated by infective persons. Hepatitis A has been shown to be transmitted sexually in male homosexuals through the fecal-oral route.

The majority of waterborne outbreaks in the United States involve small private or semiprivate water supplies, with or without chlorination. Outbreaks can occur by plumbing-sewage cross-contamination or when the raw-water source is so grossly polluted with sewage that virus levels cannot be eliminated by a given drinking-water treatment.[28]

Not much is known about the role of food or water in developing countries, whereas other enteric agents are transmitted frequently by these routes. It is not unreasonable to assume that water transmission and foodborne transmission may be more pronounced in these areas than in developed countries. The high level of HAV among Americans and Europeans in developing countries suggests a non-person-to-person vehicle association.[28]

IMMUNOLOGY

Susceptibility to HAV is general. Infants and small children have a low apparent attack rate, probably due to the frequency of mild and anicteric infections.[2]

In children, anti-HAV IgM is present in the blood stream within 7 to ten days and can persist for up to six months in extreme cases.[29,30] Viremia can last for up to 7 days past the onset of IgM production.[20]

There have been reports that persons receiving prophylactic human immunoglobulin to prevent infection by HAV have an infectivity rate of 0 - 0.2%.[19,31]

Clinical illness may occur in about 20% hepatitis infections overall.[19,32] Homologous immunity after infection is generally lifelong.

ENVIRONMENTAL PERSISTENCE

There is very little information on the persistence of hepatitis A in the environment. HAV have been reported to resist 56° C for 1/2 hours.[33] Water collected from a well 9 weeks after the onset of a 6-week outbreak of HAV was stored at room temperature unprotected from light for 40 days before 7 L were ingested by each of five volunteers. Four of these developed hepatitis without jaundice.[34]

Nasser et al.[35] conducted environmental studies on HAV in water at various temperatures. It was reported that 1 to 2 log inactivation of HAV can occur in water at 10-20°C, with >2 log inactivation at 30°C within 30 days. After 90 days exposure at 10-20°C, a 4 log inactivation of HAV was observed.

Hepatitis A virus is thought to be retained by oysters for up to 2 months after contamination.[36] Enriquez et al.[37] reported that mussels accumulate HAV a hundred times greater than in the surrounding water column, and can persist for up to seven days.

DOSE-RESPONSE RELATIONSHIP

At the time that human volunteer studies were performed on HAV, the agent had not been isolated. For this reason, there are no available data on the number of organisms necessary to produce infection. In 1945, Neefe and Stokes[34] fed volunteers 3600 mL of a 55-mg/L solution of feces from a hepatitis patient, resulting in hepatitis with jaundice in two of five volunteers. Subsequently 2900 mL of another 55-mg/L solution resulted in 4 of 5 volunteers contracting hepatitis. This was about 1 gram feces per 18.5 L.

Two of three persons receiving 3 mL of serum from acutely ill persons orally developed the disease, as did 13 of 21 volunteers that were fed 1.5 to 5 mL of a 10% feces solution.[34] In a study to determine median infective dose in marmoset monkeys, virus was measured by fecal suspension, but no estimate was made of particle number.[38]

Hepatitis A is considered to be very much like enteric viruses in general behavior.[3] Enteric viruses are excreted in concentrations as high as 10^{10} virus particles/gram of feces, and concentrations as high as 4.6×10^5 infectious virus particles/L have been detected in raw sewage. One tissue-culture infectious unit of poliovirus and 10 tissue-culture infective dose units of a wild-type enterovirus have been shown to cause infection in volunteers.[39,40] Because hepatitis A is considered to be an enterovirus-like particle, it may well occur in similar concentrations in feces and wastewater.

INDICATOR-PATHOGEN RELATIONSHIP

There is no reliable direct correlation between HAV and indicator organisms such as coliform bacteria, fecal streptococci, acid-fast bacteria, or coliphage. However, some of these organisms, particularly coliphage, can be useful indicators of the virucidal properties of water-treatment processes.[41] Although this information pertains to viruses in general, it can be applied to hepatitis as well. It is currently considered that the presence of indicator organisms may indicate possible virus contamination, but the absence of indicators does not guarantee the absence of viruses.[35,42,43]

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Appendix I - Rotavirus

ROTAVIRUS

ETIOLOGY AND CLINICAL DISEASE

Rotaviruses are wheel-shaped, 68-nm viruses constituting a genus (Rotavirus), which is included with the reoviruses in the family Reovirudae. They are double-stranded RNA viruses. Rotaviruses have been found to be associated with gastroenteritis in a wide range of animal species as well as humans.[1] The human rotavirus, the main target of this literature search and review, has two serotypes.[2]

Rotavirus has been associated with up to 50% of hospitalized cases of diarrheal illness in infants and young children.[2] Clinical symptoms are vomiting with, or followed by, severe diarrhea with no blood or mucus. Fever is often present and dehydration is common, especially in younger children, and may occur in about half of cases.[1] The disease usually lasts 4 to 8 days,[3] but in rare cases has lasted a month.[4] Death may occur, usually with dehydration and associated electrolyte imbalance as complicating factors.[1] There are about 300 deaths per year in children of $\leq$ 5 years old in the United States resulting from complications from rotavirus infections.[5] In a study of adults with diarrhea in Nonthaburi, Thailand, only individuals with cholera passed more watery stools in 24 hours and were more dehydrated than adults with rotavirus infections.[6] Treatment is nonspecific and consists of supportive therapy including rehydration. Once a patient has recovered, there appear to be no secondary effects.

INCUBATION PERIOD

The incubation period for rotavirus is approximately 48 hours.[2] The reported range is 1 to 4 days.[4,7-9]

SHEDDING

In feces of acutely ill humans, rotavirus is usually found in amounts of about 10^8 particles per gram; up to 10^{10} particles per gram have been reported.[4] It was determined by Champsaur et al.[10] that 48% of non-diarrheic children shed rotavirus. Ward et al.[11] demonstrated that 73% of adults fed rotavirus shed virus while the remaining 27% of volunteers had increased serum antibody, but no viral shedding.

OCCURRENCE

Rotavirus gastroenteritis occurs worldwide both in sporadic and epidemic outbreaks. It affects males and females equally. The primary targets are infants and children, particularly in the 6- to 24-month age group. In the US, rotavirus infections are responsible for 100,000 hospitalizations per year.[5] A Canadian study found that 62% of infants in a

prospective study had at least one rotaviral infection by 2 years of age.[12] Older children, neonates, and adults can also be infected; these infections are usually subclinical[2] but can result in severe illness[6,13-15]

Although rotaviruses have been isolated from untreated drinking water, drinking water and various foods, the occurrence of infections from these sources has been rare.[16] There have been only two occurrences in the United States and these have been traced to improperly treated waters.[17]

In temperate zones, the incidence of rotavirus infection peaks in winter; as many as 80% of the hospitalized gastroenteritis cases aged 6 to 24 months can be from this agent, with few or none in summer.[1] In subtropical and tropical areas there may be no or at best a slight seasonal peak.[1,3,18] Rotavirus accounts for 20 to 40% of all acute diarrheas in developing countries.[19] During the epidemic year 1979 in Washington, DC, 3.7/1000 children under 1 year old and 2.2/1000 children 1 to 2 year old were hospitalized for rotavirus gastroenteritis.[20] Champsaur et al.[21] reported that 40 to 98% of children (dependent upon age) that are infected with rotavirus (as determined by serum antibody levels) developed disease. Likewise, Matson et al.[22] described similar findings, about 60% of infected children developed disease. Ward et al.[11] reported that 30% of participants in a rotavirus feeding study shed virus, but did not display any symptoms.

In one prospective study, the Tecumseh Study,[23] it was reported that during a five year period, the risks of acquiring a rotavirus infection varied from 4.8% to 9.2%. Overall risks were shown to decrease with age. In a follow-up study by Koopman et al.[24] it was shown that community acquisition of rotavirus was as important as acquisition from the household setting.

Cases in adults are relatively infrequent, but have been reported. Attack rates in Truk Islanders in a person-to-person transmission outbreak were 12% of persons over 20 and 62% of 1- to 5-year-olds.[7] Twenty-five percent of adult U.S. transfer students with diarrhea in a school in Mexico City and 12% of controls were found to shed rotavirus. In greater than 50% of the rotavirus-positive cases, other enteropathogens were also present.[13] The Tiriyo Indians in Brazil, a previously unexposed group, suffered an overall attack rate of 88% in an epidemic in 1980.[25] It is not necessary for adults to have contact with ill children to contract the disease.[6,26,27] There are inapparent infections in all age groups.[25]

RESERVOIR

The reservoir of human rotavirus is probably acute-phase humans. It has yet to be shown that animal rotaviruses are pathogenic for man;[2] furthermore, there is no evidence for species cross infection in nature.[3]

MODE OF TRANSMISSION

The most common route of transmission is by the fecal-oral route. The fecal-respiratory route is also suspected to be important.[2] Although common-source outbreaks from contaminated water and food do occur[25,28,29] person-to-person transmission is by far the most frequent.

IMMUNOLOGY

By the age of 2, most individuals have acquired antibody to both serotypes of rotavirus.[1,2] Most persons possessing serum antibody are protected from disease when challenged, but immunity is not absolute,[2] and little is known about protective immunity.[19] Immunity seems to be associated with intestinal antibody secretion more than serum IgA.[30] Infants may have rotavirus infection more than once, usually due to different serotypes.[19,20] Adults are generally, but not always, asymptomatic.[3,20] It is not known why some adults are susceptible. Neonates have been shown to have an infection rate of 30 to 50%[31,32] which is asymptomatic about 90% of the time. This neonatal infection does not confer resistance, but decreases severity of disease during reinfection.[31] Bernstein et al.[33] found that natural infections by rotavirus conferred ≥ 2 years protection from subsequent rotaviral infections. On the other hand, Chiba et al.[34] described that protective immunity was present at seven months, but not at fourteen months in children. Ward and Bernstein[35] reported that natural rotavirus infections conferred protective immunity in 98% of children infected (symptomatic and asymptomatic) to the same serotype of rotavirus. It appears that breast feeding decreases the incidence and severity of rotavirus gastroenteritis in infants[1,32] but this is not universally accepted.[12] There is hope that an effective vaccine can be produced, and active research in this area is under way.[30]

ENVIRONMENTAL PERSISTENCE

Raphael et al.[36] reported that rotavirus can survive up to 10 days in raw fresh water, and up 64 days in municipal treated tap water (free chlorine = 0.05 mg/L). Rotavirus has been shown to survive more than 14 days in estuarine and heavily polluted fresh water.[37] In the marine environment, its rate of inactivation appears to be independent of salinities below 30 ppt.[37] Rotavirus is resistant to acid conditions[6] and is inactivated after 30 min at pH 11.[38] Moe and Shirley[39] reported that rotavirus can survive up to two weeks on inanimate surfaces, dependent upon relative humidity (RH) and temperature. It was found that increased temperatures and RHs' between 33% and 75% negatively impact rotavirus viability significantly. Not surprisingly, rotavirus appears to survive longer at relatively lower temperatures ($< 15°C$).[40,41]

DOSE-RESPONSE RELATIONSHIP

Ward et al.[11] conducted a rotavirus feeding study to determine the infectious dose in adults. Table 1 represents the results of the probit analyses of this study. It was found that the adult human 50% infectious dose was about 10 ffu (focus-forming units) and they estimated that 1 ffu could cause infection in 25% of susceptible adults.

INDICATOR-PATHOGEN RELATIONSHIP

There is no reliable direct correlation between viruses and indicator organisms. It is currently accepted that although the presence of indicator organisms raises the distinct possibility of virus contamination, the absence of indicators does not guarantee the absence of viruses.[42]

Table 1

Dose Response for Rotavirus

Log_{10} DOSE (ffu)[a]	Probability of Infection
$< 10^{-1}$	0.00
10^{0}	0.15
10^{1}	0.73
10^{2}	0.85
10^{3}	0.87
10^{4}	0.73
10^{5}	1.00

[a] focus-forming units.

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Appendix J - Shedding Methodology Supporting Document

APPENDIX J

To lend support to the methodology used in Chapter 2.0 and Chapter 5.0 to determine the rate of pathogen shedding by swimmers, we compared the ratio of concentration of pathogens to concentration of indicator organisms in an infected person's stool determined by two different methods for three organisms. The first method made use of the work of Kehr and Butterfield.[1] The authors reviewed several studies in England, Indonesia and California where enumeration of both coliforms and typhoid bacilli in wastewater and polluted waters was carried out at the time of outbreaks of typhoid fever. They derived a relationship between the morbidity rates from typhoid fever and the ratio of *S. typhi* to total coliform in sewage and polluted waters. A relationship between these organisms was suggested based on correction of the data reviewed for recovery ratios. The relationship can be described by the following equation:

$$y = ar^n,$$

where

a and n are constants (a = 3 and n = 0.46)
y = the number of pathogenic bacteria per 10^6 coliform organisms in wastewater
r = morbidity (relative incidence/100,000 persons)

Based on a morbidity rate of 0.18 per 100,000 persons for typhoid in the United States, the number of *S. typhi* organisms per 10^6 coliforms in wastewater is approximately 1.4 using the above relationship (Table J-1).

Assuming the above relationship holds for *Shigella* spp. and for *Salmonella* spp., a similar calculation can be made using respective morbidities in the United States of 20 and 150 per 100,000 persons (midpoints of the ranges given in Chapter 3.0, Tables 3.5 and 3.6). Table J-2 summarizes the results.

To estimate the ratio of pathogens to coliforms in stool of an infected person, we multiplied the above pathogen to coliform ratios in wastewater by the inverse of the respective incidences for the pathogens. For example, the ratio of *S. typhi* to coliforms is:

$1.4 \cdot (100{,}000/0.18) = 7.8 \times 10^5$ *S. typhi*/10^6 coliforms in stool.

Table J-2 summarizes the results for the above three organisms. Table J-2 also summarizes the ratios determined for these three organisms determined using a second method. This method made use of values for concentration of pathogen and total coliform in stool found in the literature. The same range of 10^5 to 10^9 pathogens per gram stool was estimated for *Shigella* spp. and *Salmonella* spp. (see Chapter 3.0, Tables 3.5 and 3.6). The midpoint of this range, 10^7, was divided by 10^8, the midpoint of the 10^7 to 10^9 range given earlier for total coliforms in stool to yield a ratio of 0.1 pathogens to total coliforms in stool, or 1×10^5 pathogens per million coliforms. Examination of Table J-2 reveals that the ratios determined using the two methods are within an order of magnitude of each other for each of the organisms, which helps support the validity of Method 1 used in Chapter 2.0 and Chapter 5.0 to determine the rate of pathogen shedding.

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TABLE J-1

Estimated Number of Bacterial Pathogens Per Million Coliforms In Wastewater Using Kehr and Butterfield Relationship

Organism	Bacterial Pathogens per Million Coliforms
Salmonella typhi	1.4
Salmonella spp.	11.9
Shigella spp.	30.1

TABLE J-2

Estimated Number of Bacterial Pathogens Per Million Coliforms in Stool

Organism	Kehr and Butterfield Method	Ratio Method
Salmonella typhi	7.8×10^5	1×10^5
Salmonella spp.	5.9×10^4	1×10^5
Shigella spp.	2.0×10^4	1×10^5

Appendix K - Mallows C_p Statistic

Appendix K

Mallows C_P Statistic

$C_P = RSS_P / s^2 - (n - 2p)$

where RSS_P is the residual sum of squares from a linear regression model containing p parameters, p is the number of parameters in the model including the intercept value, and s^2 is the residual mean square from the largest equation postulated containing all the parameters, and is presumed to be a reliable unbiased estimate of the error variance, σ^2.

From "Applied Regression Analysis," 2nd Edition
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New York, 1981
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