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Evaluation of Military Field-Water Quality

Volume 5. Infectious Organisms of Military Concern Associated with Consumption: Assessment of Health Risks, and Recommendations for Establishing Related Standards

R. C. Cooper
A. W. Olivieri
R. E. Danielson
P. G. Badger
R. C. Spear
S. Selvin

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Considerable interest exists in establishing realistic standards for water quality as related to the transmission of infectious disease. The development of such standards is a complicated task that consciously, and frequently subconsciously, involves the concept of risk assessment. In the context of our study, risk assessment involves the relationship between the concentration of a pathogen in water and the likelihood of disease occurring in individuals who drink the water. A mathematical model was developed to take into account the variability of the pathogen concentration in water, and hence the dose, as well as the biological variability inherent in the dose-response relationship. An interactive computer program was developed that allows the user to select the organism of interest, the amount of water consumed, the treatment-alternative removal rate, the pathogen concentration, the dose-response model, and the number of susceptible individuals. Based on the users' selections, a computer-generated risk curve is produced.

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This report is the fifth volume of a nine-volume study entitled Evaluation of Military Field-Water Quality. Titles of the other volumes are as follows: Vol. 1, Executive Summary; Vol. 2, Chemical Constituents of Military Concern; Vol. 4, Health Criteria and Recommendations for Standards; Vol. 6, Infectious Organisms of Military Concern Associated with Nonconsumptive Exposure: Assessment of Health Risks, and Recommendations for Establisning Related Standards; Vol. 7, Performance Evaluation of the 600-GPH Reverse Osmosis Water Purification Unit (ROWPU): Reverse Osmosis (RO) Components; Vol. 8, Performance of Mobile Water Purification Unit (MWPU) and Pretreatment Components of the 600-GPH Reverse Osmosis Water Purification Unit (ROWPU) and Consideration of Reverse Osmosis (RO) Bypass, Potable-Water Disinfection, and Water-Quality Analysis Techniques; and Vol. 9, Data for Assessing Health Risks in Potential Theaters of Operation for U.S. Military Forces.

FOREWORD

This report is the fifth volume of a nine-volume study entitled Evaluation of Military Field-Water Quality. Titles of the other volumes are as follows: Vol. 1, Executive Summary; Vol. 2, Chemical Constituents of Military Concern; Vol. 4, Health Criteria and Recommendations for Standards; Vol. 6, Infectious Organisms of Military Concern Associated with Nonconsumptive Exposure: Assessment of Health Risks, and Recommendations for Establishing Related Standards; Vol. 7, Performance Evaluation of the 600-GPH Reverse Osmosis Water Purification Unit (ROWPU): Reverse Osmosis (RO) Components; Vol. 8, Performance of Mobile Water Purification Unit (MWPU) and Pretreatment Components of the 600-GPH Reverse Osmosis Water Purification Unit (ROWPU) and Consideration of Reverse Osmosis (RO) Bypass, Potable-Water Disinfection, and Water-Quality Analysis Techniques; and Vol. 9, Data for Assessing Health Risks in Potential Theaters of Operation for U.S. Military Forces.

As indicated by the titles listed above, the nine volumes of this study contain a comprehensive assessment of the chemical, radiological, and biological constituents of field-water supplies that could pose health risks to military personnel as well as a detailed evaluation of the field-water-treatment capability of the U.S. Armed Forces. The scientific expertise for performing the analyses in this study came from the University of California Lawrence Livermore National Laboratory (LLNL) in Livermore, CA; the University of California campuses located in Berkeley (UCB) and Davis (UCD), CA; the University of Illinois campus in Champaign-Urbana, IL; and the consulting firms of IWG Corporation in San Diego, CA, and V.J. Ciccone & Associates (VJCA), Inc., in Woodbridge, VA. Additionally a Department of Defense (DoD) Multiservice Steering Group (MSG), consisting of both military and civilian representatives from the Armed Forces of the United States (Army, Navy, Air Force, and Marines), as well as representatives from the U.S. Department of Defense, and the U.S. Environmental Protection Agency provided guidance, and critical reviews to the researchers. The reports addressing chemical, radiological, and biological constituents of field-water supplies were also reviewed by scientists at Oak Ridge National Laboratory in Oak Ridge, TN, at the request of the U.S. Army. Furthermore, personnel at several research laboratories, military installations, and agencies of the U.S. Army and the other Armed Forces provided technical assistance and information to the researchers on topics related to field water and the U.S. military community.

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ABSTRACT

Considerable interest exists in establishing realistic standards for water quality as related to the transmission of infectious disease. The development of such standards is a complicated task that consciously, and frequently subconsciously, involves the concept of risk assessment. In the context of our study, risk assessment involves the relationship between the concentration of a pathogen in water and the likelihood of disease occurring in individuals who drink the water. A mathematical model was developed to take into account the variability of the pathogen concentration in water, and hence the dose, as well as the biological variability inherent in the dose-response relationship. An interactive computer program was developed that allows the user to select the organism of interest, the amount of water consumed, the treatment-alternative removal rate, the pathogen concentration, the dose-response model, and the number of susceptible individuals. Based on the users' selections, a computer-generated risk curve is produced.

INTRODUCTION

This volume is divided into two parts, a main body and accompanying appendices. The report is the discussion of health risks and recommendations for establishing standards for infectious organisms in drinking water. It includes the screening methodology used to identify high-priority waterborne pathogens; the mathematical model developed to assess the health risks associated with these pathogens; and recommendations based on use of the model for the development of appropriate drinking-water standards. In cases where certain information on an infectious agent is either lacking, ambiguous, or contradictory, the most conservative data are used in the application of the assessment model. The appendices represent the data base used for assessing the health risks. Emphasis in these appendices is placed on the occurrence and concentration of the pathogen in the environment, dose-response relationships, and indicator organism-pathogen relationships. Readers desiring further details or support of statements made in the report should refer to the appropriate appendix.

The first step in quantifying health risk required the development of a detailed description of the risk and consideration of known factors contributing to this risk. As indicated in Fig. 1, the risk of illness is dependent upon the interaction of human, environmental, and exposure factors. Ideally, the risk could be calculated based on exposing a user population to various concentrations of the pathogens of interest. The incidence of adverse reactions would then be measured and the level of risk calculated. Obviously, such studies cannot be performed. However, an approximation can be made by using data from outbreak reports, epidemiological studies, and animal or human feeding studies.

SCREENING OF WATER-RELATED DISEASES

The general procedure used for identifying diseases, and ultimately the pathogens of concern, is shown in Fig. 2. As presented, the first step involves the identification of, as well as the gathering of data on, the prevalence, morbidity, and mortality of all water-related diseases.

In general, water-related diseases affecting man's health are widespread throughout the world but are most abundant in developing countries. To identify those pathogens that present the greatest risk to military personnel, a list was compiled (Table 1) of the communicable diseases in man[1] that are transmitted via water. Data on the prevalence, mortality, and morbidity of water-related diseases are shown in Tables 2 through 4. Table 2 identifies the most significant water-related diseases endemic to less-developed

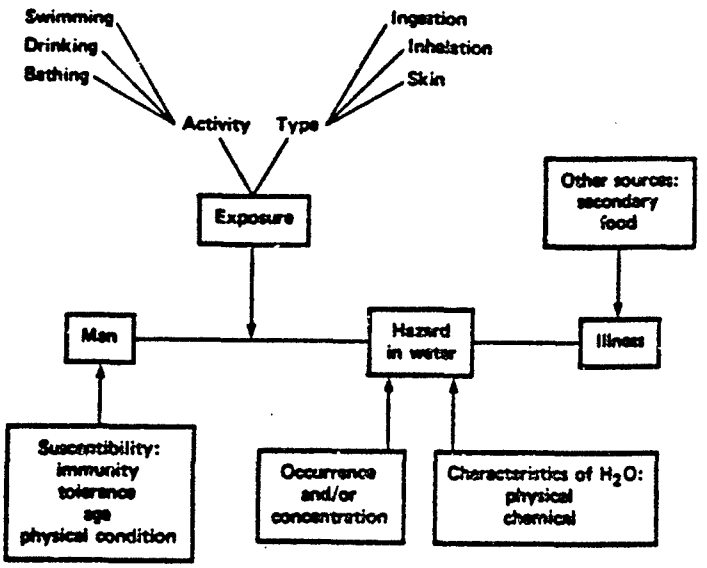

Figure 1. Factors associated with health risks from drinking water.

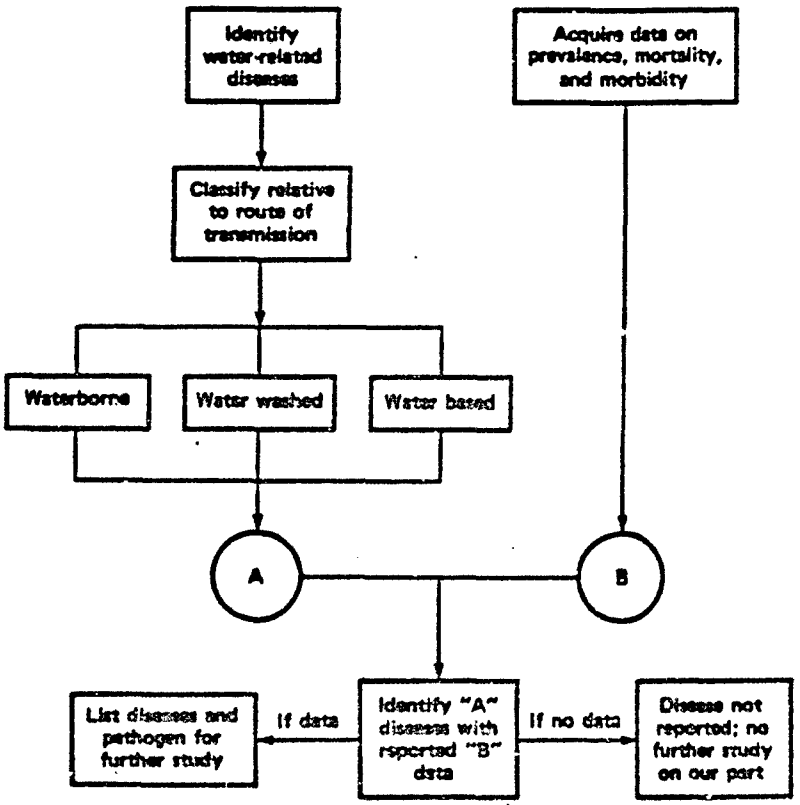

Figure 2. Diagram of generalized screening procedure. A = listing of water-related diseases by transmission route; B = reported data on prevalence, mortality, and morbidity.

Table 1. Water-related diseases (worldwide).

Water-related diseases		
Bacterial	Viral	Parasitic
Aeromonas	Enterovirus diseases	Acanthamebiasis
Cholera	Gastroenteritis	Amebic dysentery
Conjunctivitis	(Norwalk agent, rotavirus)	Ascariasis
Dermatitis	Hepatitis A	Balantidial dysentery
Leptospirosis	Arboviral diseases	Dracontiasis
Melioidosis		Giardiasis
Salmonellosis		Hookworm
Shigellosis		Malaria
Typhoid fever		Meningoencephalitis
Trachoma		Onchocerciasis
Travelers' diarrhea		Schistosomiasis
Tularemia		Sleeping sickness
Yersiniosis		(Trypanosomiasis)
		Trichuriasis

countries based on estimates of prevalence, mortality, and morbidity. Table 3 provides an estimate of the prevalence (as a percentage of the population) of the major water-related pathogens in developing countries, as well as the United States. Table 4 summarizes the etiology of waterborne disease outbreaks in the United States for the period 1946 to 1974. As shown in Table 4, roughly half of the outbreaks (48% from private water systems and 52% from public water systems) were reported as acute gastroenteritis, for which no etiological agent was found. Recently, however, several etiologic agents (including Giardia lamblia, Yersinia enterolitica, enteropathogenic Escherichia coli, Norwalk agent, and rotavirus) have been identified as the causative agents for many of the disease outbreaks associated with drinking water. Therefore, it is likely that some of the earlier cases of gastroenteritis of unknown etiology were caused by these agents.

Water-related diseases were then classified by route of transmission. In this consideration, a distinction was made between various types of disease and the importance of water in their transmission. This approach has been suggested by White and Bradley[12]

Table 2. Prevalence, mortality, and morbidity of major water-related diseases of Africa, Asia, and Latin America.[a]

Infection	Group[b]	Prevalence (10^3 infections/y)	Deaths (10^3/y)	Disease (10^3/y)
Diarrhea	I/II	3–5,000,000[c]	5–10,000	3–5,000,000
Amebiasis	I/II	400,000	30	1,500
Ascariasis	I/II	800,000–1,000,000	20	1,000
Poliomyelitis	I	80,000	10–20	2,000
Typhoid	I/II	1,000	25	500
Schistosomiasis	III	200,000	500–1,000	20,000
Malaria	IV	800,000	1,200	150,000
Onchocerciasis				
Skin	IV	30,000	low	2–5,000
River blindness	IV		20–50	200–500
Filariasis	IV	250,000	low	2–3,000
Dengue	IV	3–4,000	0.1	1–2,000
Hookworm	d	7–900,000	50–60	1,600
Trichuriasis	d	500,000	low	100

[a] Adapted from Ref. 2.

[b] Groups: I = waterborne, II = water-washed, III = water-based, IV = water-related insect vectors.

[c] 3–5,000,000 means 3,000 to 5,000,000,000 infections/y.

[d] Transmitted by contact with contaminated soil.

and is based upon four mechanisms by which disease may be related to water. Table 5 provides a summary of the classification scheme, and Table 8 outlines the causative factors and corresponding preventive strategy for each transmission category.

The final screening task was to compare the reported prevalence, mortality, and morbidity data against the list of diseases and to identify those diseases requiring study. Diseases marked with an asterisk in Table 7 were evaluated within this study. The other diseases are covered in a companion report on water-washed and water-based diseases.[14]

Table 3. Typical prevalence of infections.

Pathogen	Prevalence		Ref.
	Developing country[a]	United States	
	% of population		
Enteric viruses	5	—	—
Salmonella	7	<1	4
Shigella	7	<1	5
Vibrio cholerae	1	—	—
Pathogenic Escherichia coli	—	1–6	6
Entamoeba histolytica	30	0.6–5	7
Ascaris	60	1–12	8
Trichuris	60[b]	—	—
Hookworm	40[b]	2.7	9
Schistosoma mansoni	25	—	—
Taenia saginata	1[c]	—	—
Giardia lamblia	—	3.8–9.2	7

[a] Adapted from Ref. 3.

[b] Transmitted by contact with contaminated soil.

[c] Transmitted by eating raw or inadequately cooked, contaminated meat.

WATERBORNE DISEASES

A waterborne disease is one in which water acts as the passive vehicle for the infecting agent. This category is composed of pathogens originating in fecal material and transmitted via drinking water. Poor water quality, in a biological sense, is the predominant factor governing the incidence of these diseases.

WATER-WASHED DISEASES

Water-washed diseases are of two main types. The first type involves infections of the intestinal tract, generally through the fecal-oral route, and often leads to diarrhea.

Table 4. Water-related disease outbreaks in the United States, 1946 to 1974.[a]

Disease	Public water systems		Private water systems	
	Number	Percent	Number	Percent
Gastroenteritis (unknown etiology)	71	52.2	153	47.6
Infectious hepatitis	22	16.2	44	13.7
Shigellosis	13	9.6	33	10.3
Chemical poisoning	8	5.9	13	4.1
Giardiasis	7	5.1	8	2.5
Typhoid	6	4.4	51	15.9
Salmonellosis	6	4.4	9	2.8
Amebiasis	1	0.7	4	1.3
Poliomyelitis	1	0.7	--	--
Enteropathogenic Escherichia coli	--	--	4	1.3
Tularemia	--	--	2	0.6
Leptospirosis	1	0.7	--	--
Total	136	100	321	100

[a] From Refs. 10 and 11.

The second type involves infections of the skin and eyes. The availability of water for personal hygiene, regardless of quality, appears to play a major role relative to the spread of these diseases.

WATER-BASED DISEASES

Water-based diseases include those infections where a necessary part of the life cycle of the infecting agent occurs in an aquatic animal (e.g., snail) and repeated infections are necessary to build up a debilitating number of parasites in humans. All such infections are caused by parasitic worms; for example, the worm penetrates the skin, as with schistosomiasis, or is ingested, as with guinea worm. Diseases in this category result primarily from contact with the source of the water supply.

Table 5. Classification of water-related diseases.[a]

Transmission category	Transmission pathway
Waterborne	Fecal-oral infections via ingestion of drinking water.
Water-washed	Fecal-oral infections via direct contact with wash water (e.g., swimming, washing, laundering, etc.).
	Skin and eye infections via direct contact with wash water.
Water-based	Helminth (parasitic worm) penetrates skin or is ingested.
Water-related insect vector	Insects breed in or bite near water.

[a] Adapted from Refs. 12 and 13.

Table 6. Cause and prevention of water-related diseases.[a]

Transmission category	Causative factors	Preventive strategy
Waterborne	Poor quality of water	Improve water quality.
Water-washed	Insufficient quantity of water	Increase water quantity. Improve hygiene.
Water-based	Contact with source of water supply	Decrease need for contact. Control snail population.
Water-related insect vector	Proximity to water and related vectors	Improve water management. Decrease need to visit breeding sites.

[a] Adapted from Refs. 12 and 13.

Table 7. Water-related diseases and routes of transmission.

Water-related diseases (worldwide)	Routes of transmission		
	Waterborne	Water-washed	Water-based
Bacterial diseases			
Bacillary dysentery (Shigella spp.)*	X	X	—
Cholera (Vibrio cholerae)*	X	—	—
Diarrhea (Campylobacter)*	X	—	—
Diarrhea (Escherichia coli)*	X	X	—
Leptospirosis (Leptospira spp.)	X	X	—
Salmonellosis (Salmonella spp.)*	X	X	—
Typhoid fever (Salmonella typhi)*	X	X	—
Skin infections (Pseudomonas spp. and Staphylococcus spp.)	—	X	—
Yersiniosis (Yersinia spp.)*	X	—	—
Viral diseases			
Enteroviruses*	X	X	—
Gastroenteritis, Norwalk agent and rotavirus*	X	—	—
Hepatitis A (hepatitis virus)*	X	X	—
Parasitic diseases			
Acanthamebiasis (Acanthamoeba spp.)	X	—	—
Amebic dysentery (Entamoeba histolytica)*	X	—	—
Ascariasis (Ascaris lumbricoides)	X	X	—
Balantidium dysentery (Balantidium coli)	X	X	—
Dracontiasis (Dracunculus medinensis)	—	—	X
Giardiasis (Giardia lamblia)*	X	—	—
Meningoencephalitis (Naegleria spp. and Acanthamoeba spp.)	—	X	—
Schistosomiasis (Schistosoma spp.)	—	—	X

* Indicates that the risk assessment was conducted in this study.

WATER-RELATED INSECT VECTORS

The category, water-related insect vectors, includes those diseases that are spread by insects that either breed in water or bite near water. Malaria and yellow fever are transmitted by mosquitoes that breed in water, whereas trypanosomiasis (Gambian sleeping sickness) is transmitted by the tsetse fly that inhabits areas near water. A person's proximity to water and to related insect vectors is an important factor in the transmission of this disease type. Diseases in this latter category, however, are not included in this report because the military has assigned them to a separate program.

DATA-BASE DEVELOPMENT

LITERATURE COMPILATION

To achieve an adequate literature review, a systematic work plan was constructed as shown in Fig. 3. The emphasis was primarily on recent literature (after 1970).

The criteria shown in Table 8, selected for each disease agent, were derived from a study of basic reference works, recent review articles, the periodical literature, and an overview of related subjects in published collections of journal abstracts. The review involved the following sequence:

- Identification of relevant infectious agents criteria;
- Assembly of bibliographic references;
- Acquisition of pertinent literature;
- Extraction of relevant information;
- Development of a computerized index and data base;
- Evaluation of the data.

As shown in Fig. 3, generation of the data base was cyclic (i.e., continually updated, thus including most, if not all, of the current literature pertinent to the investigation). It is estimated that approximately 3200 relevant abstracts were scanned for selection of those most appropriate. The journals, WRC Information* and Current Contents,† were reviewed for pertinent material. In addition to the manual methods of literature review, the Medline and Aqualine computer data bases were used to retrieve relevant abstracts. Medline corresponds to three printed indices: Index Medicus, Index to Dental Literature, and International Nursing Index, covering over 3000 international journals. Aqualine provides access to information on every aspect of water, wastewater, and the aquatic environment, citing over 400 worldwide periodicals, research reports, books, etc. From the aforementioned lists of abstracts, approximately 1200 articles were retrieved and read, and approximately 700 were abstracted and included in the data base. From the 700 articles, books, reports, proceedings, and other sources, approximately 500 abstracts were

* WRC Information is the weekly journal of the Water Research Center, Medmenham, Marlow, Bucks SL72HD, UK.

† Current Contents is a journal for life sciences and agriculture, biology, and environmental sciences. It is published by the Institute for Scientific Information, Philadelphia, PA 19104.

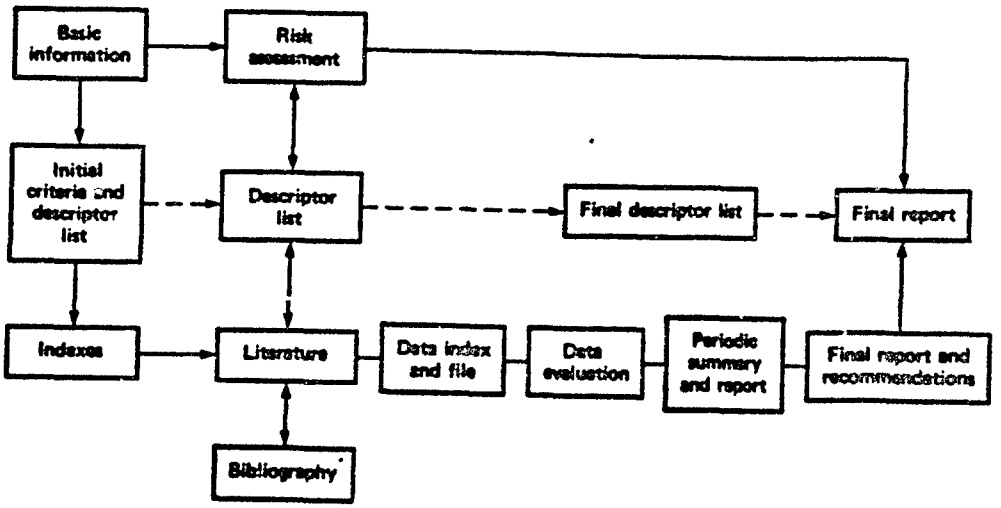

Figure 3. Diagram of general data-base development plan.

chosen for reference and inclusion in this report. We believe that we have identified most, if not all, of the pertinent literature retrievable by feasible methods.

DATA BASE

A significant portion of the literature was devoted to the development of a computerized, command-driven, relational data-base system. The dBase II system, developed by Ashton-Tate,[15] was the data-management software used for this task; the software and manuals are readily available. Over 700 articles are included within this data base. The data-base files, together with the dBase II software, allow easy access and retrieval of the key criteria listed in Table 8. A sample printout from the data base for one complete article is shown in Table 9. The data-base key is shown in Table 10.

RISK-ASSESSMENT METHODOLOGY

Risk assessment, in the context of this project, concerns the relationship between the concentration of a pathogen in water and the likelihood of disease occurring in troops who drink this water. Figure 4 is a diagram of this relationship that stresses the variability of the pathogen concentration in water and, therefore, the dose, as well as the biological variability inherent in the dose-response relationship. In this conceptualization,

Table 8. Infectious agent criteria.

Criteria categories	Category content
Occurrence	Worldwide distribution of disease
Latency	Incubation period
Persistence	Survival time in final infective stage
Infective dose	Dose data
Attack rate	Response data
Multiplication	Multiplication outside human host
Route of transmission	Waterborne, water-washed, etc.
Disinfectant resistance	In disinfected water
Indicator organism-pathogen relationships	Coliform numbers relative to pathogen concentration
Prevalence	Infection rate

we recognize wide variations in the concentrations of pathogens that might be encountered in the field, and we account for this variability to arrive at a realistic estimate of the risk of disease. Moreover, the classical dose-response relation links a particular dose with the fraction of an exposed population responding. The slope of this curve is a descriptor of the biological variability in the population response to this agent. A steep slope corresponds to a low level of variability; conversely, a flat slope corresponds to a high level of variability. When considered together, both sources of variability lead not to a specific level of risk, but to a distribution of risk across the exposed population. Here the term "risk" is used to denote the fraction of a large population that will develop the disease. The important conclusion is that risk is inherently probabilistic, and its assessment must be carried out in that context.

If we were dealing with large numbers of exposed individuals, the issue would be to estimate the risk distribution shown in the top right diagram of Fig. 4. However, the present problem is more complex because the number of troops exposed to a given dose may be small. We chose to deal with this by assuming that each individual within a squad or platoon-sized group was exposed to the same dose and that the total number of troops was comprised of these smaller dose units. Therefore, our attention is focused on the small group and the probability of illness therein.

Before becoming immersed in the detail of these calculations, we consider first the form of the final result because its interpretation is not intuitively obvious. The final

Table 9. Sample data-base printout.

```
RECORD # 00020
REFCODE  : Craun 78 :
SEQNUM   :2:
CITATOR  :pgb:
AUTHOR:LST:Craun G.F.

:
TITLE    :"Waterborne Outbreaks of Giardiasis" in Jakubowski &
Hoff, eds., Waterborne Transmission of Giardiasis: Proceedings of a
Symposium. US EPA

:
CITATION :EPA Office of R&D, Env. Research Center, Cincinnati, Ohio
:
YEAR     :(1978) :
KEY:WORD :Giardiasis, chlorination, filtration, outbreak, coliform
count, Giardia lamblia, giardiasis
:
LOCATION :worLdwide      :
LATENCY  :Y:
ATKRT    :Y:
PESIST   :Y:
MID      :N:
PROPHO   :Y:


RECORD # 00020
CONENV   :fresh   :
DISINTYPE :       :


RECORD # 00020
REFCODE  : Craun 78 :
ABSTRACT : Data are presented on waterborne outbreaks of          :
ABS1     : giardiasis affecting travelers to foreign countries,   :
ABS2     : esp. the USSR, and residents in the US. 23 outbreaks   :
ABS3     : on the US reported since 1965. Usually in mountainous  :
ABS4     : areas of the US: New England, the Pacific Northwest,   :
ABS5     : and the Rocky mountains. Generally involves small      :
ABS6     : municipal systems, or semi-public systems, or          :
ABS7     : untreated water. Most come from consuming              :
ABS8     : untreated or only chlorine treated surface water.      :
ABS9     : Negative results of coliform tests do not provide a    :
ABS10    : guarantee that water is free of Giardia cysts. Attack  :
ABS11    : rate is of visitors to Leninrad who drank tap water. In :
ABS12    : Colorado mountain streams, there are up to 500 fecal   :
ABS13    : coliforms /100ml. This figure may be low. In the       :
ABS14    : Rome, N.Y. outbreak, with 4800 cases, one cyst was     :
ABS15    : isolated from 1 million liters of raw water from the   :
ABS16    : plant intake.                                          :
```

Table 9. (Continued)

```
RECORD # 00020
REFCODE     : Craun78  :
LAT:MAX     : 56       :
LAT:MIN     : 7        :
LAT:AVE     :          :
ATKRTEMAX   : 330      :
ATKRTEMIN   : 230      :
ATKRTEAVE   :          :
PESIST      :2 to 3 months in host.

:
MULTOUTHST  :no  :
MIDMAX      :          :
MIDMIN      :          :
MIDAVE      :          :
RTETRANS    :fecal-oral    :
SIGIMMUN    :?   :
PROPHO      :yes :
PROPHOTYPE  :Filter water in addition to chlorination of surface
water, preceded by sedimentation or coagulation.
:
OPPORTUNE   :    :
OPTENVTEMP  :low    :
OPTENVSAL   :    :


RECORD # 00020
OPTENVPH    :    :
ENVRANGE    :    :
DOSE1       :    :
DOSE2       :    :
DOSE3       :    :
DOSE4       :    :
DOSE5       :    :
RESP1       :        :
RESP2       :        :
RESP3       :        :
RESP4       :        :
RESP5       :        :
INDPATH     :Negative results of coliform tests don't provide
assurance that water is free of Giardia cysts.  Positive results often
correlate with outbreaks.
```

Table 10. Data-base key.

Field	Item
REFCODE	reference code
SEQNUM	sequence number
CITATOR	initials of citator
AUTHOR:LST	list of authors
TITLE	title of article
CITATION	journal, book, report
YEAR	year of publication
KEY:WORD	list of key words
LOCATION	country, state, city of research
LATENCY	information in article, yes or no, Y/N
ATKRT	attack rate information in article, Y/N
PESIST	persistence information in article, Y/N
MID	median infective-dose information, Y/N
PROPHO	prophylactic information, Y/N
CONENV	type of water in which the organism is found
DISINTYPE	type of disinfectant
ABSTRACT	citator abstract
LAT:MAX, LAT:MIN, LAT:AVE	maximum, minimum, average latency data
ATKRTEMAX, MIN, AVE	maximum, minimum, average attack rates
MULTOUTHST	multiplication outside host
MIDMAX, MIDMIN, MIDAVE	median effective-dose data
RTETRANS	route of transmission
SIGIMMUN	immunity data
PROPHOTYPE	prophylactic type
OPPORTUNE	opportunistic organism
OPTENVTEMP	optimum environmental temperature
OPTENVSAL	optimum environmental salinity
OPTENVPH	optimum environmental pH
ENVRANGE	description of environmental conditions of research
DOSE1	disinfectant dose
RESP1	organism response
INPATH	indicator pathogen relationship

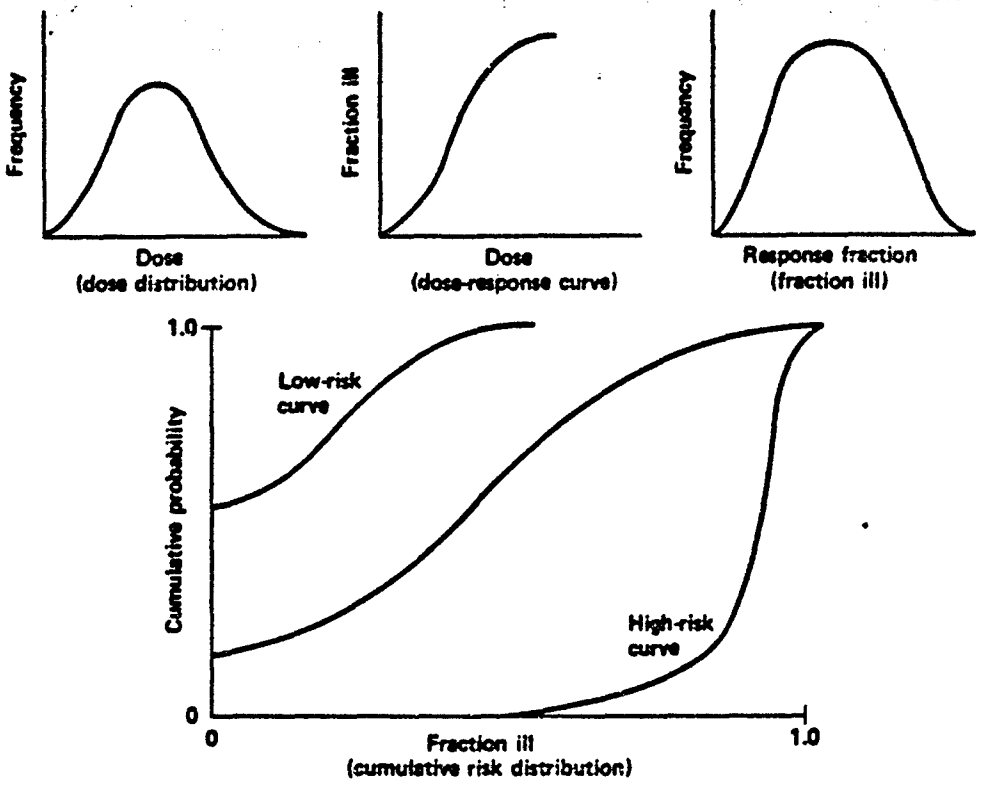

Figure 4. Relationship between dose distribution, dose-response curve, and risk of illness.

result is shown conceptually in the bottom diagram of Fig. 4. Figure 5 shows an example of an actual risk curve. These are cumulative distributions that give the probability that the fraction of troops that become ill is less than or equal to the number on the abscissa. For example, for the high-risk scenario, where there is no treatment of heavily contaminated water, the probability is 0.28 (line A in Fig. 5) that the fraction of troops ill will be less than or equal to 0.4. For that same fraction ill, the corresponding cumulative probabilities for the medium-risk and low-risk scenarios are 0.53 (line B) and 0.85 (line C), respectively. These numbers can be put in perspective by noting that a risk-free situation would be one in which the probability is that the fraction ill is zero or, on the figure, the risk-free situation could be depicted by a single point at (1,0). Hence, the lower the risk, the closer the cumulative distribution will be to this point. These cumulative curves frequently begin at nonzero probabilities, as exemplified by all three curves in Fig. 5. These values give the probability that no illness will occur; this is consistent with the risk-free limiting situation discussed previously.

A final comment on the interpretation of the cumulative-probability curves is suggested by the high-risk curve in Fig. 5. Note the abrupt change in slope at the illness fraction of 0.9. This abruptness is, in part, an artifact of the method of plotting the data;

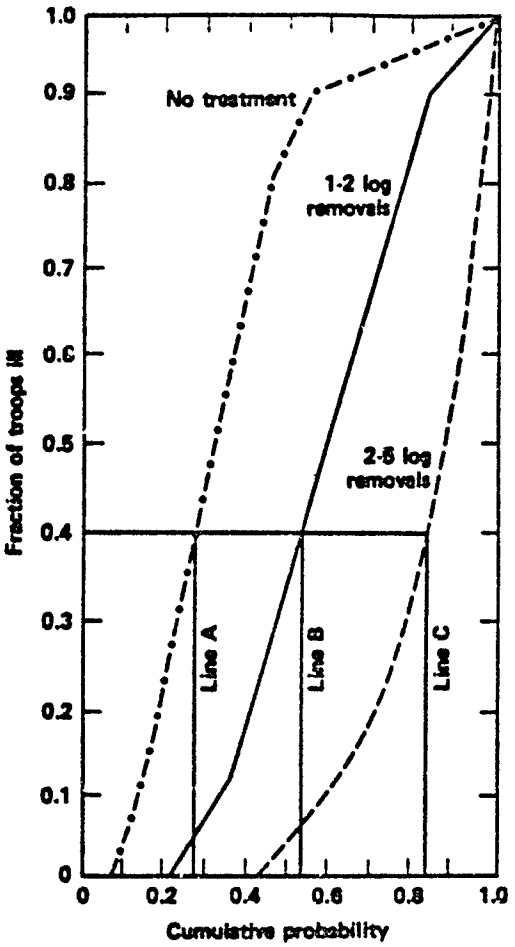

Figure 5. Cumulative-risk curves showing effect of change in treatment efficiency on the risk of becoming ill with typhoid fever. All curves were determined using (1) n = 20 troops and ill = disease symptoms; (2) a logistic dose-response relationship; (3) a dose distribution with a geometric mean (GM) = 172 organisms/L and a geometric standard deviation (GSD) = 99; and (4) treatment efficiency equivalent to either no treatment, 1 to 2 log removals, or 2 to 5 log removals (as noted in figure). Log removal(s) = the logarithm of the factor of reduction in number of organisms per liter (e.g., if initial concentration of organisms = 10^6/L and this concentration of organisms is reduced by treatment to 250/L, then $\log_{10}[10^6/250]$ = 3.6 log removals).

however, the curve does have the general shape shown. In particular, the probabilities given for the illness fractions of 0.9 and 1.0 are exact. The difference between these two probabilities is the probability that the illness fraction will lie in the interval between 0.9

and 1.0, in this case about 0.50. This situation is clearly one of high risk. The point, however, is that the difference in probability at any two points on the cumulative curve gives the probability that the illness fraction lies between the corresponding points on the abscissa.

LINKING EXPOSURE TO RISK

Linking exposure to risk requires first that the distribution of pathogens in the raw water be specified; that is, the parameters describing this distribution are input data to the risk-analysis procedure. The second input is a dose-response function that is assumed to be known without error. Subtle distinctions exist between uncertainty and real environmental variability. A major component of this analysis is dealing with variability in the dose that may be encountered by troops. The possibility of also dealing with uncertainty in the dose-response relationship is a subject for future research.

If the pathogen distribution is assumed to be specified, the next step is to determine the relationship between concentration in the raw water and dose to the squad. Two other factors that we consider are treatment efficiency and the volume of water consumed. The dose-concentration relation is then:

$$D = V (1 - E) C \qquad (1)$$

where

- D = number of organisms consumed,
- V = volume of water consumed,
- E = fraction of organisms removed by treatment, where 1 equals complete removal,
- C = concentration of organisms in raw (untreated) water.

As with the concentration of organisms in the raw water, both treatment efficiency and the volume of water consumed are subject to variability. Because of the multiplicative relation given in Eq. (1), the variability of V and E clearly contributes to that of D. Therefore, the task is to determine the distribution of D from that of V, E, and C. That is, the distributions of volume consumed and of treatment efficiencies are also data inputs required to accomplish this calculation. Figure 6 shows these distributions schematically. For this analysis we assumed the pathogen concentration distribution to be lognormal,[16,17] the treatment-efficiency distribution to be uniform, and the consumption distribution to be normal. Use of the lognormal distribution to represent the

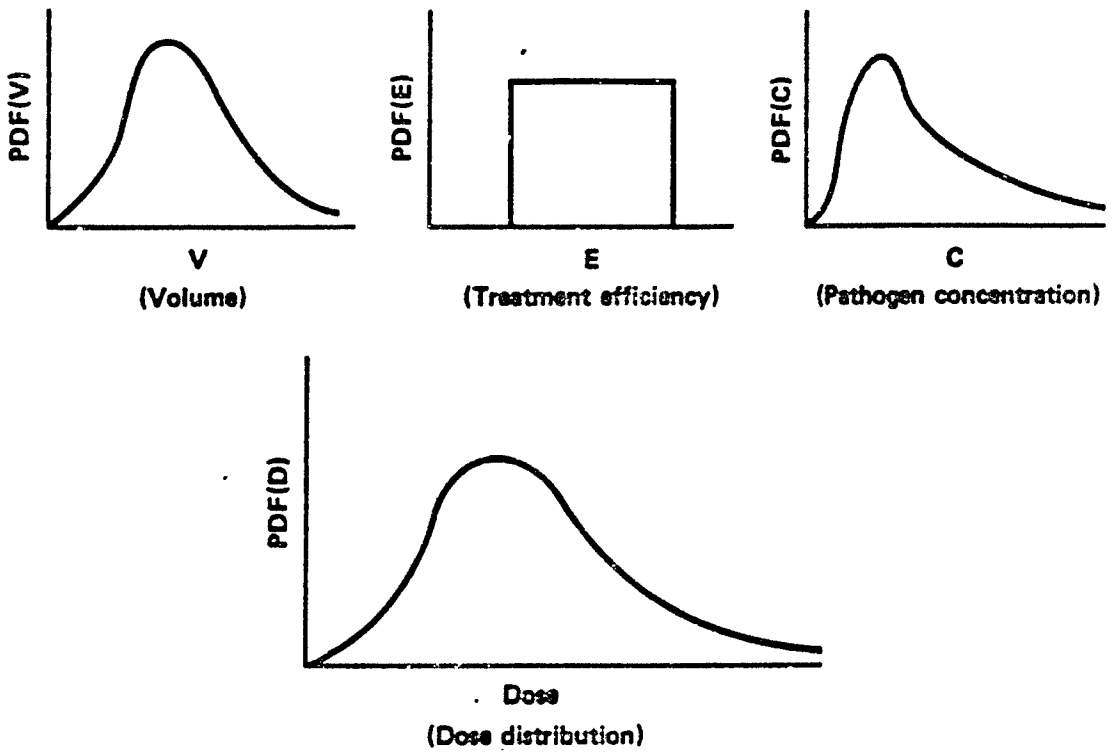

Figure 6. Dose-probability distributions. PDF = probability density function, which indicates the probability that the value of a continuous random variable will be less than or equal to a specific value selected on the x axis (e.g., V, E, C, or D).

concentration of pathogens (C) in water is well established in the literature.[16,17] Data were not available to support use of the uniform distribution to represent treatment system efficiency (E) and the normal distribution to represent the volume of water (V) consumed. However, use of these distributions appears both reasonable and practicable, given the lack of data.

The mixture of distributional forms adopted for V, E, and C means that an analytical approach to the calculation of the probability density function of dose, PDF(D), would not be practical. Therefore, a Monte Carlo approach was taken, which involves random selection of samples from the V, E, and C distributions and calculation of a value for D. Repetition of this process many times results in an estimate of the distribution of D. The larger the number of repetitions, the better the estimate of PDF(D). However, because

the distribution of D is only an intermediate step on the way to the calculation of the risk distribution, $g(\theta)$, where θ = proportion ill at dose D, and because the dose-response function is assumed to be known exactly, it is possible to obtain a value directly for each set of values of V, E, and C. That is, the Monte Carlo process results in an estimate of $g(\theta)$.

Four mathematical models[18,19] were chosen to represent the dose-response data found in the literature. These include

Logistic:

$$\theta = \frac{1}{1 + e^{-(M + N \log D)}}, \quad (2)$$

where

- θ = fraction of an exposed population that becomes ill (i.e., response to a given dose),
- M = shape parameter for the distribution,
- N = shape parameter for the distribution,
- D = dose of organisms.

Beta:

$$\theta = 1 - [1 + (D/\beta)]^{-\alpha}, \quad (3)$$

where

- θ = fraction of an exposed population that becomes ill (i.e., response to a given dose),
- D = dose of organisms,
- β = shape parameter for the distribution,
- α = shape parameter for the distribution.

Exponential:

$$\theta = 1 - e^{-rD}, \quad (4)$$

where

- θ = fraction of an exposed population that becomes ill (i.e., response to a given dose),
- r = fraction of an exposed population that becomes ill per unit dose,
- D = dose of organisms.

Lognormal:

$$\theta = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{Z} e^{-z^2/2} dz \ , \qquad (5)$$

where

$-\infty < z \leq Z$,

θ = fraction of an exposed population that becomes ill (i.e., response to a given dose),

Z = an upper limit of $(\ln D - \mu_{\ell})/ \sigma_{\ell}$,

D = dose of organisms (number),

μ_{ℓ} = ln (GM),

σ_{ℓ} = ln (GSD).

For any particular pathogen, the model is matched to the data by finding the model parameters that give the best fit, usually in a least-squares sense. Definitions for the parameters within these equations are shown in Table 11. We will discuss the equation parameters for the logistic model in the next section; the calculations are shown in Table 12. References 18 and 19 should be reviewed for calculation of the parameters associated with the beta, exponential, and lognormal models.

Recall that the risk, θ, is the fraction of an exposed population that becomes ill. Hence, θ ranges from zero to one, and its distribution is continuous over this interval. The beta-distributional form has these properties and, for theoretical reasons that will be discussed, it is convenient to treat $g(\theta)$ as being beta-distributed. The values of θ obtained from the Monte Carlo simulation are then used to estimate the parameters of the beta distribution describing $g(\theta)$.

The problem now addressed is that of calculating the distribution of the number of illnesses, x, in a group of n men exposed to a risk of disease given by θ. To accomplish this task, we assume that the conditional distribution $f(x \mid \theta)$ is binomially distributed. This is a sensible assumption because the fraction ill would be θ if the group were very large. The binomial assumption is then:

$$f(x \mid \theta) = \binom{n}{x} \theta^{x} (1 - \theta)^{n-x} . \qquad (6)$$

Now, according to the definition of conditional probability,

$f(x \mid \theta) = f(x\theta)/g(\theta)$ for $0 < \theta < 1$; $0 < x < n$; or

Table 11. Parameter definitions.

Symbol		Definition
$\bar{X}$	=	mean
S	=	standard deviation
a	=	lower treatment efficiency
b	=	upper treatment efficiency
GM	=	geometric mean
GSD	=	geometric standard deviation
X_i	=	uniform random number (0,1)
m	=	desired number of different values
A,C	=	coefficients <m
R	=	normal random deviate
X_1, X_2	=	pair of uniform random numbers
E	=	treatment efficiency (uniform distribution)
C	=	pathogen concentration, organisms/liter (lognormal distribution)
V	=	volume of water consumed, liters (normal distribution)
D	=	dose of organisms (number)
θ	=	response (the proportion ill) to a given dose (D)
M,N	=	logistic dose-response equation parameters
α, β	=	beta dose-response equation parameters
r	=	exponential dose-response equation parameters
$\hat{p},\hat{q}$	=	estimates of beta-distribution parameters
h(x)	=	probability of x illnesses or less
x	=	number of ill troops
n	=	number of exposed troops
μ_ℓ	=	ln (geometric mean, GM)
σ_ℓ	=	ln (geometric standard deviation, GSD)
$\bar{X}_\theta$	=	mean of responses (θ)
S^2_θ	=	variance of responses (θ)

Table 12. Example of logistic dose-response calculation.

Consider the following dose-response data for a bacterial pathogen:

Dose (organisms)	x_i = log (dose)	n_i (subjects)	$\hat{p}_i$ (response)
1×10^4	4	10	0.01
5×10^5	5.7	10	0.1
5×10^7	7.7	10	0.5

x_i = log (dose)	$y_i = \ln[\hat{p}_i/(1-\hat{p}_i)]$	$w_i = n_i \hat{p}_i (1-\hat{p}_i)$
4	-4.59	0.099
5.7	-2.20	0.9
7.7	0	2.5

The transformed p values or "logits" (y_i) with weights w give

$$\bar{X} = \frac{\sum w_i X_i}{\sum w_i} = 7.08;$$

$$\bar{Y} = \frac{\sum w_i y_i}{\sum w_i} = -0.69;$$

$$\hat{b} = \frac{\sum w_i (x_i - \bar{X})(y_i - \bar{Y})}{\sum (x_i - X)^2} = 0.35;$$

$$\hat{a} = \bar{Y} - \hat{b}\bar{X} = -3.17.$$

These three estimates yield the estimated relationship between the dose (x_i) and response data (y_i or $\bar{p}$) as follows:

$$\hat{y}_i = -3.17 + 0.35 x_i ,$$

and the logistic equation is:

$$\bar{p} = \frac{1}{1 + e^{-(-3.17 + 0.35\, x_i)}} .$$

Note: $\bar{p}$ is equal to θ within the risk model; $\hat{a}$ is equal to M within the risk model; $\hat{b}$ is equal to N within the risk model.

$f(x\theta) = f(x \mid \theta)\, g(\theta)$, and

$$h(x) = \int_0^1 f(x|\theta)\, g(\theta)\, d\theta = \int_0^1 \left[\binom{n}{x}\theta^x (1-\theta)^{n-x}\right] \frac{\theta^{p-1}(1-\theta)^{q-1}}{\beta(p,q)}\, d\theta \qquad (7)$$

$$= \frac{\binom{n}{x}\beta(p+x, n+q-x)}{\beta(p,q)},$$

where, as discussed above, $g(\theta)$ is assumed to be beta distributed and is given by:

$$g(\theta) = \frac{\theta^{p-1}(1-\theta)^{q-1}}{\beta(p,q)}. \qquad (8)$$

The function $\beta(p,q) = \Gamma(p)\,\Gamma(q) / \Gamma(p+q)$, where $\Gamma(.)$ is the gamma function.

Recall that $g(\theta)$ was generated by a Monte Carlo procedure. These data are used to estimate the parameters of the beta distribution by use of the following equations:

$$\hat{p} = \bar{X}_\theta^2(1-\bar{X}_\theta) - S_\theta^2\,\bar{X}_\theta \,/\, S_\theta^2, \text{ and} \qquad (9)$$

$$\hat{q} = \hat{p}\,(1-\bar{X}_\theta) / \bar{X}_\theta, \qquad (10)$$

where $\bar{X}_\theta$ and S_θ^2 are the sample mean and variance of the risk values θ generated by the Monte Carlo procedure.

The expected number of illnesses is then

$$Ex(x) = (np) / (p+q), \qquad (11)$$

with the corresponding variance

$$Var(x) = \frac{npq\,(p+q+n)}{(p+q)^2\,(p+q+1)}. \qquad (12)$$

A simplification in Eq. (7) occurs when the estimated values of p and q can be rounded to the nearest nonzero integer value. Then, if p becomes $\tilde{p}$ (an integer) and q becomes $\tilde{q}$, the distribution of h(x) is as follows:

$$h(x) = \frac{\binom{\tilde{p}+x-1}{x}\binom{\tilde{q}+n-x-1}{n-x}}{\binom{\tilde{p}+\tilde{q}+n-x-1}{n+x}}. \qquad (13)$$

It is called the negative hypergeometric distribution. Equation (13) is used when the gamma function (Γ) of a number greater than 25 is required. This modification allows for the calculation of h(x), using Eq. (10), when the p and q values are less than 1.

COMPUTER PROGRAM

To calculate the likelihood of illness, h(x), among a group of n individuals, it was necessary to write a computer program using the equations discussed in the previous section. The main program, including its 19 subroutines, is contained in a separate report.[20]

The flowsheet shown in Fig. 7 illustrates the information required as input to the model by the user, the various calculation steps, and the output of the model.

EXAMPLE CALCULATION

The following example is presented to illustrate how the program functions. Input data (Fig. 7, Step 1) for the example are shown in Table 14. As shown, ten random samples will be generated; the mean and standard deviation of the volume of water consumed is set at 10 and 1 L/d, respectively. The treatment-unit efficiency ranges from 99 to 99.999% removal; the geometric mean and geometric standard deviation of the concentration of organisms in the raw water supply are set at 172 and 99 organisms/L, respectively; and the dose-response equation selected is logistic with the parameters for the equation identified in Table 14. (See Table 12 for an example of parameter calculation.) The number of troops at risk is set at 20 persons.

Once the user supplies the above input data, Step 2 is followed to calculate five uniform random numbers as shown in Table 13. Five uniform random numbers are necessary because two random variables (i.e., pathogen concentration and water volume consumed) are calculated using random normal deviates, each of which is computed from two uniform random numbers (see Step 3), and the third variable (i.e., treatment efficiency) only requires a single uniform random number (note: random variables x_1 and x_2 in Table 13 are used for the drinking-water volume; x_3 is used for treatment efficiency; and variables x_4 and x_5 are used for the pathogen concentration).

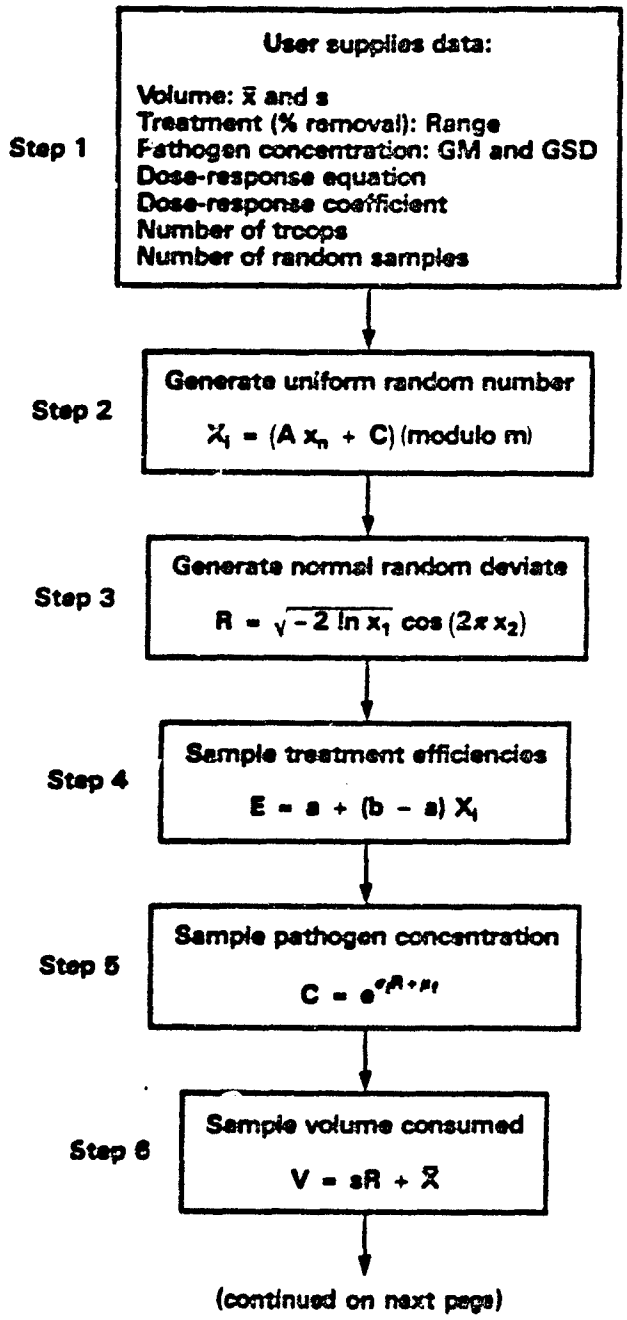

Figure 7. Flowsheet for risk-assessment model. See Table 11 for parameter definitions. (R in step 5 is calculated using random numbers x_4 and x_5 from Table 13 in equation in step 3; R in step 6 is calculated using random numbers x_1 and x_2 from Table 13 in equation in step 3).

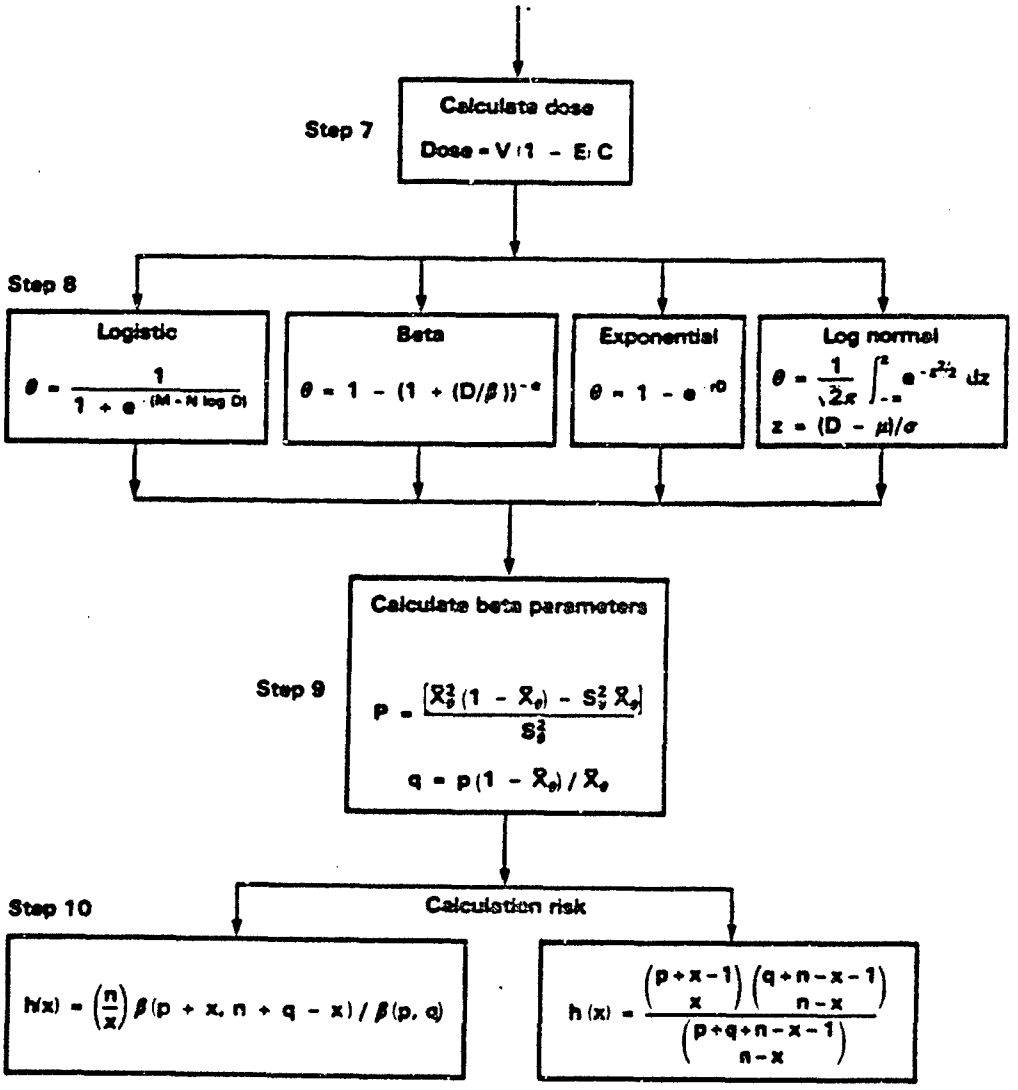

Figure 7. (Continued)

Step 4 is used to calculate the treatment efficiency based on the following equation:

$$E = a + (b - a)(x_i), \tag{14}$$

where E equals treatment efficiency as a decimal fraction, a (minimum) and b (maximum) represent the range of input treatment efficiencies, and x_i is a uniform random number where i = 3, the number of the random sample as shown in Table 13.

For this example, E = 0.991383 as shown in Table 13. The calculation, using Eq. (14) is as follows:

$$E = 0.99 + (0.99999 - 0.99)(0.13899) = 0.991388 .$$

Table 13. Example of model output: Salmonella typhi.

	x_1	x_2	x_3	x_4	x_5
Random x_i =	0.16157	0.43080	0.13899	0.63593	0.11019
V =	8.26830				
E =	0.991388				
C =	4979.54				
Dose is:	354.555				
Dose =	354.555	Log of dose = 2.54968		θ = 0.441801E-01	

$\hat{p}$ = 0.174 $\hat{q}$ = 1.55

Ex(x) = 2.02 Var(x) = 14.4

Distribution of risk:
Number of troops = 20

Number of ill troops	Fraction of ill troops	h(x)[a]	Cumulative h(x)[b]	1- Cumulative probability[c]
0	0.00	0.604	0.605	0.395
2	0.10	0.058	0.765	0.235
4	0.20	0.031	0.838	0.162
6	0.30	0.021	0.885	0.115
8	0.40	0.015	0.918	0.082
10	0.50	0.011	0.943	0.057
12	0.60	0.009	0.962	0.038
14	0.70	0.006	0.977	0.023
16	0.80	0.005	0.988	0.012
18	0.90	0.003	0.996	0.004
20	1.00	0.001	1.000	0

[a] The probability that exactly x out of n (in this case n = 20) troops are ill.

[b] The cumulative probability that x or fewer troops will be ill.

[c] The "risk" that more than x troops will be ill.

Table 14. Example of model input parameters: Salmonella typhi.

Input parameters	Value
Number of random samples	10
Seed for random-number generator	1234
Arithmetic mean of volume (X)	10
Arithmetic standard deviation (S) of volume	1
Minimum treatment efficiency (a)	0.99000
Maximum treatment efficiency (b)	0.99999
Geometric mean (GM) of pathogen concentration (organisms/L)	172
μ_ℓ = 1n (GM)	5.14749
Geometric standard deviation (GSD) for pathogen concentration	99
σ_ℓ = 1n (GSD)	4.59512
Dose-response equation	Logistic
Dose-response parameter M	-7.99340
Dose-response parameter N	1.92930
Number of troops	20

Step 5 is used to calculate concentration of the pathogen based on the following equation:

$$C = e^{\sigma_\ell(R) + \mu_\ell}, \qquad (15)$$

where C equals the concentration of the pathogen in organisms/L, σ_ℓ and μ_ℓ equal the natural log of the geometric mean and geometric standard deviation supplied by the user in Step 1; and R equals a normal random deviate calculated in Step 2. The R for this example, as shown below, is equal to 0.73241 based on the use of x_4 and x_5 from Table 13 (note that the product of 2 π 0.11019 must be in radians).

$$R = -2\ 1n\ (0.63593) \cos\ (2\ \pi\ 0.11019)\ . \qquad (16)$$

For this example, C = 4979 organisms/L as shown in Table 13. The calculation, using Eq. (15), is as follows:

$$C = e^{(1n\ 99)(0.73241) + (1n\ 172)} = 4979\ .$$

Step 6 is used to calculate the volume of water consumed per day based on the following equation:

$$V = SR + \bar{X}, \quad (17)$$

where V equals the volume of water consumed in L/day, S and $\bar{X}$ equal the standard deviation and mean for the normal distribution of water consumption supplied by the user in Step 1, and R is a normal random deviate calculated in Step 3. Following Eq. (16), the R value for calculating the volume consumed is equal to -1.7317 based on the use of x_1 and x_2 in Table 13.

For this example, V = 8.27 L/d as shown in Table 13. The calculation, using Eq. (17) is as follows:

$$V = (1.0)(-1.7317) + 10 = 8.27 \text{ L/d}.$$

Based on the results of the above calculations, the dose of organisms consumed by an individual is calculated in Step 7 as follows:

$$\text{Dose (D)} = V(1 - E)C. \quad (18)$$

For this example, D = 355 organisms as shown in Table 13. The calculation, using Eq. (18) is as follows:

$$D = (8.26)(1 - 0.9914)(4979) = 355.$$

Step 8 is used to calculate the expected response using one of four dose-response equations (numbers 2, 3, 4, and 5) previously discussed. For this example, the logistic equation, Eq. (2), was selected with the values of M and N shown in Table 14. The calculation is as follows:

$$\theta = \frac{1}{1 + e^{-(-7.9934 + 1.92930 \log 354)}} = 0.044.$$

Step 9 is used to calculate the p and q parameters of the beta distribution. These calculations are made using Eqs. (9) and (10). For this example, the $\hat{p}$ and $\hat{q}$ values are 0.174 and 1.55, respectively. In Step 10, the probability of x illnesses or less (out of n at risk), h(x), is calculated following either Eq. (7) or Eq. (13). As previously discussed, Eq. (13) is used when calculation of a $\Gamma(.)$ of a number greater than 25 is required.

For this example, Eq. (7) was used, and the results are shown in Table 13. The expected number of illnesses, Ex(x), and the variance, Var(x), of the distribution are calculated using Eqs. (11) and (12); their values are also shown in Table 13. Plotting the cumulative h(x) values shown in Table 13 results in the risk curve shown in Fig. 8.

The results indicate that a 90% chance exists that 40% or less of the individuals could become ill.

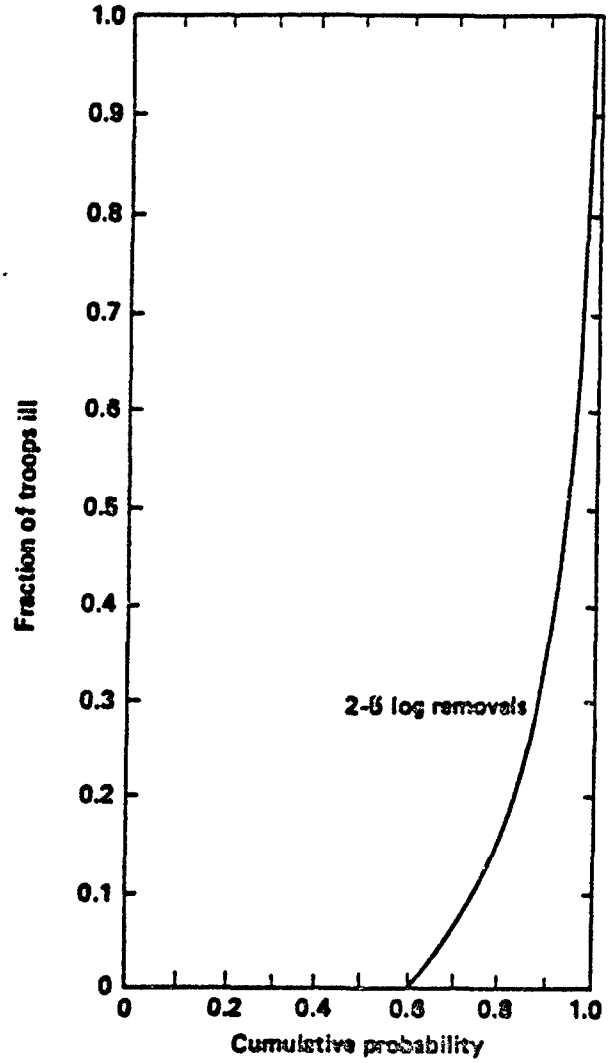

Figure 8. Example of cumulative-risk curve showing the risk of becoming ill with typhoid fever. The curve was determined using (1) n = 20 troops and ill = disease symptoms; (2) a logistic dose-response relationship; (3) a dose distribution with a GM = 172 organisms/L and a GSD = 99; and (4) treatment efficiency equivalent to 2 to 5 log removals, corresponding to limits E = 0.99 to 0.99999.

SENSITIVITY ANALYSIS

Before using the model in the analysis of disease risks for various specific waterborne agents, it is important to be aware of the sensitivity of the model's predictions to variations in the input information. If, for example, the predicted number of illnesses was sensitive to the dose-response function used, it would be important to remember this when interpreting the results of the analysis. In the present case, the end predictions depend on four inputs: the dose-response equation, the consumption distribution, the pathogen-concentration distribution in the raw water, and the treatment-efficiency distribution. Because the fit of the dose-response equation to the data is dependent on the data quality in each case, we defer the topic to the sections of the report dealing with the specific organisms. We discuss the other three variables in the following paragraphs because our sensitivity analyses for them may be similar for all cases considered.

The normal procedure in conducting a sensitivity analysis is to select a normal or baseline case and then perturb the input variables from this case and assess the magnitude of the resulting output change. The greater the change in the output for a given input change, the greater the sensitivity. Generally, a certain ad hoc aspect is apparent in defining commensurate input perturbations and deciding which characteristics of the output are appropriate measures of change in response. Here, we simply define a 10% or greater change in the predicted number of illnesses to be a meaningful output change. The baseline case is defined to be one using the logistic dose-response function, a consumption distribution with a mean of 10 L and a 10% relative standard deviation, and a lognormal pathogen-concentration distribution in raw water. The perturbed cases will use a 15-L mean consumption with the same relative standard deviation, a pathogen-concentration distribution with the same mean but with a standard deviation increased by either 10- or 100-fold, and five treatment alternatives as given in Table 15.

The organism selected for study was Salmonella typhi because of the relatively large amount of data available. The first variable investigated was the consumption volume; the results are shown in Table 16. The predicted number of illnesses for the baseline case was 13.48 and, as can be seen, the perturbed case showed little change in either the mean or the variance.

In the next case, we considered perturbations in the variability of the pathogen concentration in the raw water as indicated in Table 17. Although the case with the largest change in the variable does result in a change in the mean of over 10%, generally, the changes are not dramatic. The cumulative distributions of the fraction ill are shown in Fig. 9. They support this conclusion, although all three cases are high-risk situations.

Table 15. Treatment efficiencies (% removal).[a]

Treatment alternative	Virus	Bacteria	Parasites
No treatment (raw)	0	0	0
ROWPU[b]	99–99.999	99–99.999	99–99.999
Filtration and chlorination	99–99.999	99–99.999	99–99.999
Chlorination (5–10 mg/L FAC)	99–99.999	99–99.999	99–99.999
Filtration (multimedia and 5-μm filter)	0–40	90–99	90–99

[a] See Appendices for further details and support.

[b] ROWPU = reverse osmosis water-purification unit.

Table 16. Volume consumed sensitivity.

Volume (L)	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
10 ± 1	13.48	55.39	—
15 ± 1.5	14.06	53.11	No (4.1)

[a] Ex(x) = mean of distribution for expected number of illnesses.

[b] Var(x) = variance of distribution for expected number of illnesses.

[c] "Yes" or "No" implies whether or not the difference from the first (baseline) case can be considered significant.

Table 17. Pathogen concentration sensitivity.

Pathogen concentration	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
$172 \times 99^{\pm 1}$	13.48	55.39	—
$172 \times 990^{\pm 1}$	14.68	56.19	No (8.9)
$172 \times 9900^{\pm 1}$	15.30	55.38	Yes (13.5)

[a] Ex(x) = mean of distribution for expected number of illnesses.

[b] Var(x) = variance of distribution for expected number of illnesses.

[c] "Yes" or "No" implies whether or not the difference from the first (baseline) case can be considered significant.

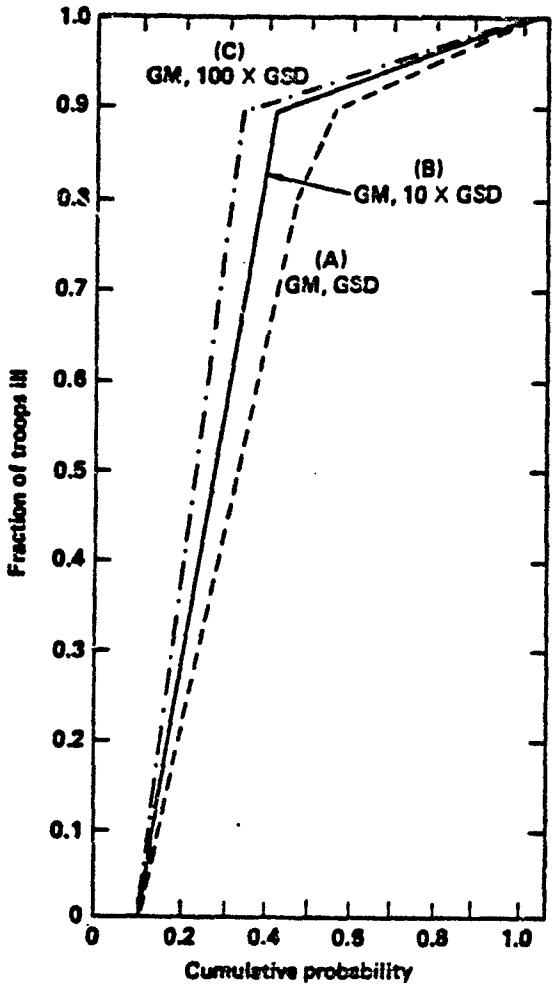

Figure 9. Cumulative-risk curves showing effect of organism-concentration parameters on the risk of becoming ill with typhoid fever. All curves were determined using (1) n = 20 troops and ill = disease symptoms, and (2) a logistic dose-response relationship. However, a dose distribution with a geometric mean (GM) = 172 organisms/L and a GSD = 99 was used to derive curve A; a dose distribution with a GM = 172 organisms/L and a GSD = 990 was used to derive curve B; and a dose distribution with a GM = 172 organisms/L and a GSD = 9900 was used to derive curve C.

This case illustrates one of the pitfalls of sensitivity analysis: conclusions are conditioned on the choice of a baseline case. Here, the baseline case is a high-risk situation in which the variability of the pathogen concentration is not particularly important. On the other hand, one would expect changes in the variance of the pathogen concentration to be important in cases where the predicted number of illnesses was low in the baseline case.

The next variable we considered was treatment efficiency. Table 18 contains the results of the three cases considered. Not surprisingly, treatment efficiency has a large effect on the predicted number of disease cases. The cumulative distributions are shown in Fig. 10 and further illustrate the importance of this variable.

Probably the most important outcome of this analysis is that the model performs much as predicted. For example, treatment efficiency is an important variable; also, the model is sensitive to the concentration distribution but insensitive to the consumption distribution. It must be remembered, however, that the effect of any single variable is conditioned by the values of the other variables that are held constant during that run. For example, treatment efficiency would not have a particularly dramatic effect if the concentration of organisms in the raw water was low at the outset. It does seem safe to conclude, however, that the consumption distribution used in these analyses is unlikely to be an important determinant of risk under any circumstances.

RISK-ASSESSMENT RESULTS

As previously indicated, 12 pathogenic organisms were evaluated relative to the risk of their causing waterborne illness. These organisms are identified in Table 19.

In this section we discuss the effects of modifying the dose-response equation on the sensitivity of the risk model. It is important to note that, when considering host response to water-related pathogens, a distinction must be made between the two most common end points measured: infection and disease. Infection is defined as multiplication of a

Table 18. Treatment alternative sensitivity.

Treatment[a]	Ex(x)[b]	Var(x)[c]	Sensitivity (% difference)[d]
Low (0)	13.48	55.39	—
Medium (90-99)	8.35	55.29	Yes (38)
High (99-99.999)	3.48	23.45	Yes (74.2)

[a] % organism removal.

[b] Ex(x) = mean of distribution.

[c] Var(x) = variance of distribution.

[d] "Yes" or "No" implies whether or not the difference is considered significant, compared to the low-efficiency treatment.

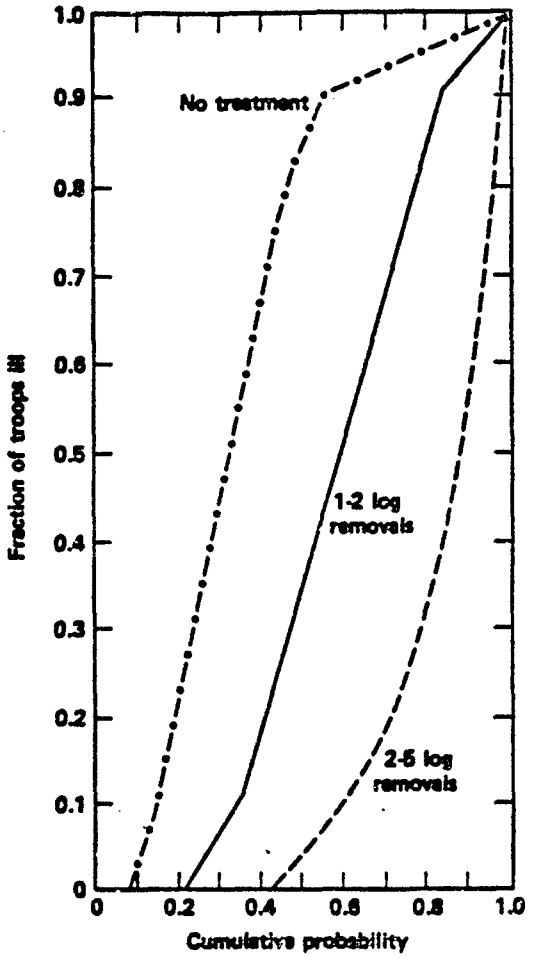

Figure 10. Cumulative-risk curves showing effect of change in treatment efficiency on the risk of becoming ill with typhoid fever. All curves were determined using (1) n = 20 troops and ill = disease symptoms; (2) a logistic dose-response relationship; (3) a dose distribution with a geometric mean (GM) = 172 organisms/L and a geometric standard deviation (GSD) = 99; and (4) treatment efficiency equivalent to either no treatment, 1 to 2 log removals, or 2 to 5 log removals (as noted in figure).

microbial agent within a host, with or without the production of clinical disease. Asymptomatic infection is the desired relationship with a pathogen because immunity may be achieved without the health risk associated with the related disease syndrome (i.e., vaccines). Both end points are used to study dose-response relationships in human populations. In general, much of the information available for bacterial pathogens is associated with the disease end point. The opposite is true for parasites and viruses. The majority of the dose-response data uses infection as the end point. For the purpose of this

Table 19. Waterborne pathogenic organisms.

Bacterial	Viral	Parasitic
Shigella spp.	Enteroviruses	Entamoeba histolytica
Vibrio cholerae	Norwalk agent and rotavirus	Giardia lamblia
Campylobacter	Hepatitis virus	
Escherichia coli		
Salmonella spp.		
Salmonella typhi		
Yersinia spp.		

risk assessment, we have used the available data and appropriate end points. Also, note that the dose-response model that gives the most conservative estimate of risk will be selected for use.

Within this section, low- and high-risk boundary curves are calculated for each organism, assuming 20 exposed individuals, for developed countries (e.g., United States, Europe), and a low-risk boundary is calculated for developing countries. We define the terms "low-risk boundary" and "high-risk boundary" as follows:

Low-risk boundary:

Dose response: Use model that gives highest-risk results

Volume of water consumed: 10 L $\pm$ 10%

Pathogen concentration: Geometric mean and geometric standard deviation

Treatment efficiency: 99 to 99.999% removal

High-risk boundary:

Dose response: Same as low risk

Volume of water consumed: 15 L $\pm$ 10%

Pathogen concentration: Geometric mean, 100 times geometric standard deviation

Treatment efficiency: 0% removal

RISK ASSESSMENT: BACTERIAL ORGANISMS

Seven bacterial agents (Table 19) were selected for assessment, and we discuss them in the order presented.

Shigella spp.

A summary of the dose-response data obtained from the open literature is presented in Table 20. The data for Shigella spp. were analyzed, applying each of the four dose-response equations. The derived parameters associated with each of the four equations are shown in Table 21.

Published reports on the occurrence and concentration of Shigella spp. in the environment are limited. Thus, an estimation of the probable concentration of these agents in water was required. We made this calculation by combining prevalence rates with the average number of organisms per gram of feces from an infected person, the sewage production rate of a town of 50,000 persons, and an assumed range of stream-dilution values. The assumptions that we used to calculate the concentration of Shigella in fresh water for both developed and developing countries are shown in Table 22.

With the above data used as input to the risk-assessment model, we ran the model and identified the dose-response equation giving the most conservative estimate of risk. The results of these runs are shown in Table 23; they indicate that the use of different dose-response equations modifies the expectation of the risk distribution from +9 to -16% from that of the logistic equation. The beta dose-response equation appears to be a sensitive component of the analysis, based on the criteria of a 10% difference from the baseline case (i.e., logistic). However, the exponential equation was selected to calculate upper and lower risk-curve boundaries because it results in the highest (i.e., most conservative) estimate of risk. The low-risk boundary is calculated by setting the treatment efficiency variable at 99 to 99.999% organism removal, volume at 10 ± 1 L, and pathogen concentration at the calculated geometric mean and geometric standard deviation. The high-risk boundary is calculated by setting the treatment efficiency at 0.0% removal, volume at 15 ± 1.5 L, and the pathogen concentration at the geometric mean and 100 times the geometric standard deviation.

Plots of the Shigella spp. risk boundaries are shown in Fig. 11. For the low-risk scenario in developed countries, the boundaries indicate that there is a cumulative probability of 0.5 that the fraction of troops ill would be less than or equal to 0.08. For

Table 20. Shigella spp. dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
10	0.1	131	21
2×10^2	0.5	4	21
2×10^3	0.7	10	21
1×10^4	0.83	6	21
2×10^2	0.25	4	21
1×10^4	0.33	6	21
1.8×10^2	0.18	33	18
5×10^3	0.67	49	18
1×10^4	0.76	87	18
1×10^5	0.44	34	18
10^4	0.25	4	18
10^5	0.75	4	18
10^6	0.86	8	18
10^7	0.68	19	18
10^8	0.75	8	18

Table 21. Shigella spp. dose-response equation parameters.

Equation	Parameter 1	Parameter 2
Logistic	M = -7.4577	N = 2.0292
Beta	$\alpha = 0.16$	$\beta = 155$
Exponential	$r = 1.03 \times 10^{-3}$	--
Lognormal	$GM = 8.92 \times 10^3$	GSD = 31.8

Table 22. Possible output of pathogens in feces, sewage, and fresh water.

Pathogen	Prevalence of infection (%)[a]		Average number of organisms per gram of feces[b]	Total excreted daily by town[c]		Concentration per liter in town sewage[d]		Concentration in fresh water[e,f,g]	
	Developed country (A)	Developing country (B)		A	B	A	B	A	B
Shigella spp.	<1	4	10^7	5×10^{11}	2×10^{12}	3×10^3	4×10^4	$100 \times 50^{\pm 1}$	$1000 \times 500^{\pm 1}$
Vibrio cholerae	—	1	10^6	—	5×10^{10}	—	1×10^4	h	$32 \times 15^{\pm 1}$
Campylobacter spp.	<1	<1	10^7	5×10^{11}	5×10^{11}	3.3×10^3	1×10^5	$100 \times 50^{\pm 1}$	$300 \times 150^{\pm 1}$
Salmonella spp.	—	7	10^6	—	3.5×10^{11}	—	7×10^3	—	$221 \times 100^{\pm 1}$
S. typhi	—	7	10^6	—	3.5×10^{11}	—	7×10^3	—	$221 \times 100^{\pm 1}$
Yersinia spp.	<1	4	10^7	5×10^{11}	2×10^{12}	3×10^3	4×10^4	$100 \times 50^{\pm 1}$	1000×500
Enteroviruses	?	5	10^6	?	2.5×10^{11}	?	5×10^4	?	$158 \times 79^{\pm 1}$
Entamoeba histolytica	5	30	1.5×10^5	3.8×10^{10}	2.3×10^{11}	2.5×10^3	4.6×10^4	$13 \times 7^{\pm 1}$	$146 \times 73^{\pm 1}$
Giardia lamblia	10	30	10^6	5×10^{11}	1.5×10^{12}	3.3×10^3	3×10^4	$100 \times 50^{\pm 1}$	$1000 \times 500^{\pm 1}$

NOTE: The table is hypothetical, and the data are not taken from any actual, single town. The concentrations derived would be conservative estimates because it is unlikely that the prevalence rate (infection) would occur in any one community.

[a] The prevalences refer to infection and not morbidity.

[b] Feacham et. al.[3]

[c] To calculate these figures, a mean fecal excretion of 100 g/person/d was assumed for a town of 50,000 people.

[d] It must be recognized that the pathogens listed have different abilities to survive outside the host; however, an estimate of 10% survival or 10% reaching a freshwater source appears reasonable.[2] The concentrations of pathogens per liter of sewage of a town of 50,000 were calculated by assuming 100 L of sewage per capita for developing countries or 300 L of sewage per capita for developed countries, and a mean fecal excretion of 100 g/d.

[e] To calculate these figures, the concentration per liter of sewage was diluted by 10- and 100-fold, and the geometric mean of the range was determined. It was assumed that the standard deviation was half of the mean value.

[f] Geometric mean and geometric standard deviation.

[g] Concentration in organisms per L or $TCID_{50}$ (i.e., dose that infects 50% of the tissue cultures) for virus.

[h] Concentration in receiving water $<10^{-5}$ organisms/L.

Table 23. Shigella spp. dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	15.65	52.36	---
Beta	13.13	42.05	Yes (-16.1)
Exponential	17.07	48.81	No (+9.1)
Lognormal	14.69	51.37	No (-6.1)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the logistic equation.

the high-risk scenario, the probability is 0.5 that the fraction of troops ill would be 0.94 or less. For the low-risk scenario in developing countries, there is a probability of 0.5 that the fraction of troops ill would be less than or equal to 0.92.

Vibrio cholerae Classical

In the case of Vibrio cholerae Classical, three out of four dose-response equations fit the available data (see Table 24). Fitting the logistic equation gives a uniform dose-response line, which is unreasonable. The dose-response equation parameters for the remaining equations are shown in Table 25.

As in the case of Shigella, there is a general lack of aquatic occurrence and concentration data for Vibrio cholerae. Therefore, calculations of the estimated probable concentration of organisms in water were required. The results of the calculations are shown in Table 22. Note that for concentrations in developed countries, the calculated geometric mean was 10^{-5} organisms/L. Because of this low value, we did not calculate the health risk for developed countries.

The risk-assessment model, using the above values, was run to identify the dose-response equation that gives the most conservative estimate of risk in developing countries. The results of the runs, shown in Table 26, indicate that by changing the dose-response equation, the expectation of the risk distribution is modified from +27 to

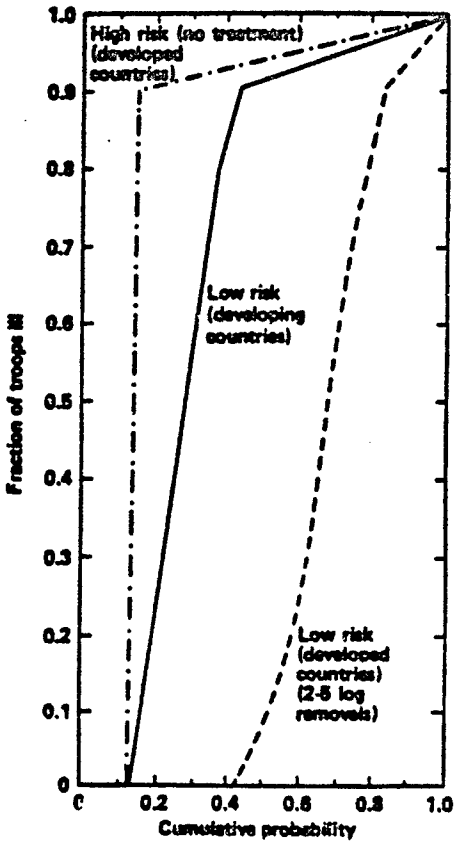

Figure 11. Cumulative-risk curves showing effect of treatment efficiency, organism-concentration parameters, and volume of water consumed on the risk of becoming ill as a consequence of consumption of Shigella spp. in drinking water. Each curve was determined using (1) n = 20 troops and ill = disease symptoms; (2) an exponential dose-response relationship; (3) pathogen concentrations with the geometric means and geometric standard deviations (organism-concentration parameters) as explained in the text; (4) volumes of water consumed as noted in figure; and (5) treatment efficiency equivalent to either no treatment or 2 to 5 log removals (as noted in figure).

+100%. This modification indicates that the dose-response equation is a sensitive component of the analysis. The exponential equation was used to calculate the upper and lower risk-curve boundaries because it resulted in the highest (i.e., most conservative) estimate of risk.

At the low-risk boundary, there is essentially no risk of illness (shown as a dot on the abscissa in Fig. 12). The high-risk curve in Fig. 12 indicates a probability of 0.5 that the fraction of troops ill would be less than or equal to 0.78.

Table 24. Vibrio cholerae-Classical dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
10^6	0.8 - 1.0	20	23
10^6	0.83	12	24
10^8	0.50	4	25
10^9	0.50	2	25
10^{11}	0.50	2	25
10^3-10^4	0.26	19	25
10^4	0.0	2	25
10^6	0.0	4	25
10^7	0.0	4	25
10^{10}	0.0	1	25
10^5	0.67	6	24
10^6	0.96	23	24
10^6	0.89	27	26

Table 25. Vibrio cholerae-Classical dose-response equation parameters.

	Parameter	
Equation	1	2
Logistic	—	—
Beta	$\alpha = 0.097$	$\beta = 13{,}020$
Exponential	$r = 7.45 \times 10^{-6}$	—
Lognormal	$GM = 3.2 \times 10^6$	GSD = 14.5

Vibrio cholerae El Tor

The fit of four dose-response equations to the available dose-response data (see Table 27) was determined. The equation parameters are shown in Table 28.

Table 26. Vibrio cholerae-Classical dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	—	—	—
Beta	5.54	25.89	—
Exponential	11.16	83.77	Yes (101)
Lognormal	7.07	58.29	Yes (27.6)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the beta equation.

Table 27. Vibrio cholerae-El Tor dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
10^5	0.60	10	24
10^6	1.0	10	24
10^5	0.60	5	24
$>10^4$	0.111	274	27
10^6	0.860	37	26

The same estimates for concentration of the agent in water were used for the El Tor biotype as for the Classical biotype. The risk model, using these values, was run to identify the dose-response equation that gives the most conservative estimate of risk in developing countries. The results are shown in Table 29. The results indicate that by changing the dose-response equation, we modify the expectation of the risk distribution from -5 to -13%. This modification indicates that the beta dose-response equation is a sensitive component of the risk calculation, based on the criterion of a 10% difference

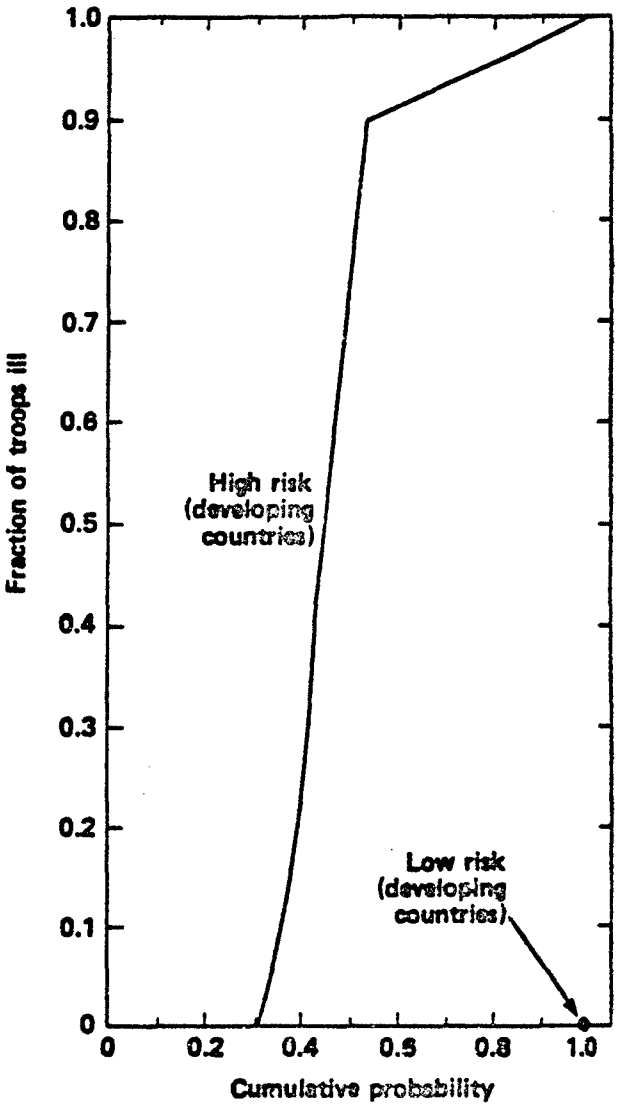

Figure 12. Cumulative-risk curves showing effect of change in treatment efficiency on the risk of becoming ill as a consequence of consumption of Vibrio cholerae Classical in drinking water in developing countries. Each curve was determined using (1) n = 20 troops and ill = disease symptoms; (2) an exponential dose-response relationship; (3) a dose distribution with a GM = 32 organisms/L and a GSD = 15; and (4) treatment efficiency equivalent to either no treatment in the high-risk curve, or 2 to 5 log removals in the low-risk curve.

Table 28. Vibrio cholerae-El Tor dose-response equation parameters.

Equation	Parameter 1	Parameter 2
Logistic	M = - 24.82	N = 5.39
Beta	$\alpha = 1.33$	$\beta = 2.7 \times 10^{5}$
Exponential	$r = 4.99 \times 10^{-6}$	—
Lognormal	$GM = 7.2 \times 10^{4}$	GSD = 5.8

Table 29. Vibrio cholerae-El Tor dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	12.0	84.07	—
Beta	10.43	82.93	Yes (-13.1)
Exponential	10.82	87.17	No (-9.8)
Lognormal	11.39	77.23	No (-5.1)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the logistic equation.

from the logistic model. Because the logistic equation resulted in the highest risk estimate, it was selected as the dose-response model; a plot of the risk boundaries is shown in Fig. 13. The low-risk boundary corresponds to a zero-risk level, shown as a dot on the abscissa.

Review of Fig. 13 indicates that at the low-risk boundary, there would be zero risk, and at the high-risk boundary, the probability is 0.50 that the fraction of troops ill would be less than or equal to 0.9.

Campylobacter

Three of the four dose-response equations could be applied to the available dose-response data for Campylobacter (see Table 30). The logistic equation could not be

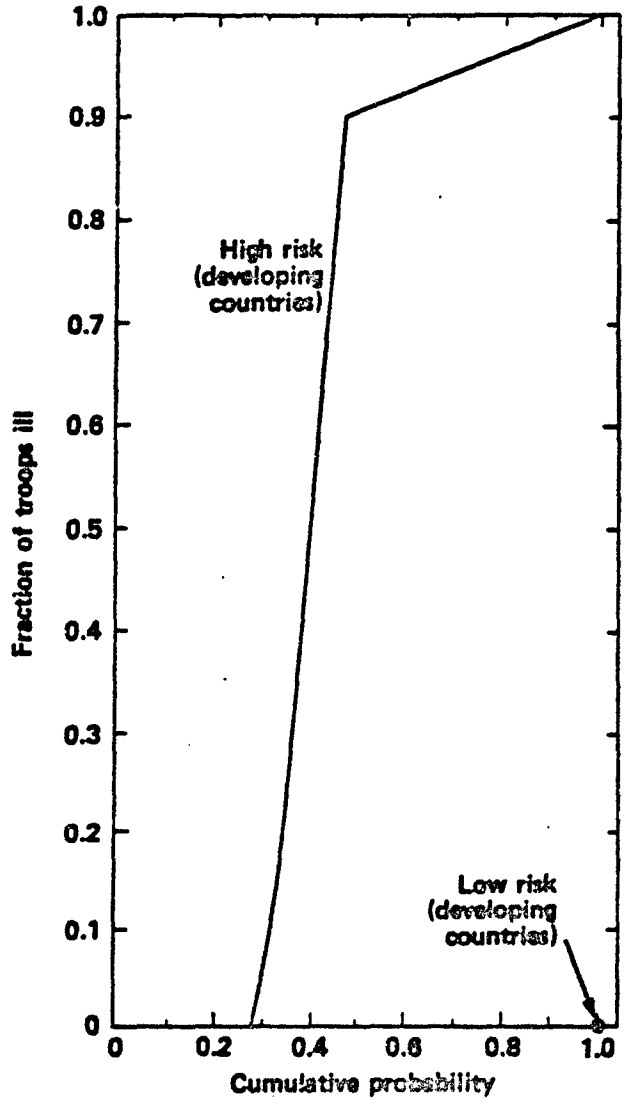

Figure 13. Cumulative-risk curves showing effect of change in treatment efficiency on the risk of becoming ill as a consequence of consumption of Vibrio cholerae El Tor in drinking water in developing countries. Each curve was determined using (1) n = 20 troops and ill = disease symptoms; (2) a logistic dose-response relationship; (3) a dose distribution with a GM = 32 organisms/L and a GSD = 15; and (4) treatment efficiency equivalent to either no treatment in the high-risk curve, or 2 to 5 log removals at the low-risk point, which implies that no troops will be affected.

Table 30. Campylobacter dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
500	1.0	1	28
10^6	1.0	1	29
1	0.0001	1	Estimate

Table 31. Campylobacter dose-response equation parameters.

Equation	Parameter 1	Parameter 2
Logistic	—	—
Beta	$\alpha = 0.39$	$\beta = 55$
Exponential	$r = 7.003 \times 10^{-4}$	—
Lognormal	GM = 30	GSD = 2.4

fit primarily because of the paucity of data. To fit the other three equations, we assumed a low-dose point of one organism with a 0.01% response. The dose-response equation parameters are shown in Table 31.

There is a general lack of occurrence and concentration data in the literature. Therefore, calculation of the probable concentration of the organism in water was required. Table 22 identifies the values used for concentration of this agent in water for developing and developed countries.

Based on the results shown in Tables 22 and 31, the risk-assessment model was run to identify the dose-response equation giving the most conservative estimate of risk. The results of the model runs are given in Table 32. Review of these results indicates that by changing the dose-response equation, we modify the expectation of the risk distribution from 3 to approximately 4%, all less than the sensitivity level of 10%. The lognormal equation was used to calculate the upper- and lower-risk boundaries because it resulted in the highest risk estimate.

Table 32. Campylobacter dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	--	--	--
Beta	16.45	47.0	--
Exponential	16.97	48.62	No (3.1)
Lognormal	17.13	48.98	No (4.1)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the beta equation.

The risk curves for Campylobacter are shown in Fig. 14. The low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.1, and the high-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.95. For a developing country, the low-risk curve indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.93.

Escherichia coli

Three of the four dose-response equations fit the available dose-response data for pathogenic Escherichia coli (see Table 33). The dose-response equation parameters are shown in Table 34.

Table 35 presents selected fecal coliform data for various water sources in developing countries. To calculate environmental concentration levels for E. coli, a worst-case assumption was made that all fecal coliforms are pathogenic. Based on the data in Table 35, a geometric mean of 2.5×10^4 organisms/L and a standard deviation of 35 were calculated for stream and river water quality. For developed countries, a geometric mean of 2000 organisms/L and standard deviation of 2 were used.[33] This limit is used by the State of California for nontidal-contact recreation.

The risk-assessment model was executed to identify the dose-response equation that gives the most conservative estimate of risk. The results of the model runs are shown in Table 36. Review of the results indicates that by changing the dose-response curve, we modify the expectation of the risk distribution from 45 to 50%. The results indicate that

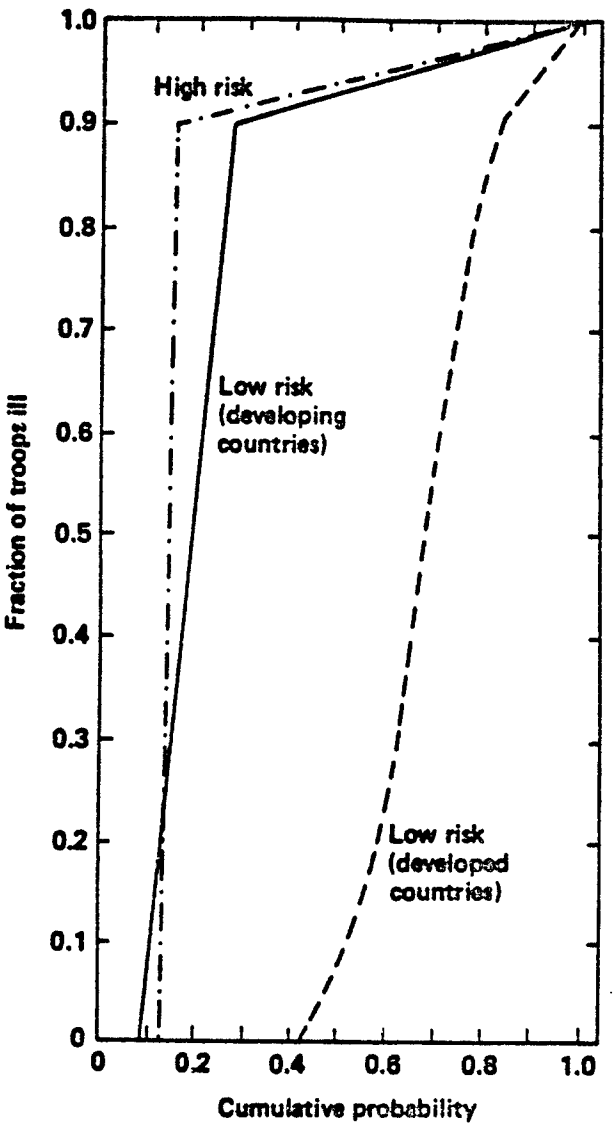

Figure 14. Cumulative-risk curves showing effect of change in treatment efficiency and organism-concentration parameters on the risk of becoming ill as a consequence of consumption of Campylobacter in drinking water. Each curve was determined using (1) n = 20 troops and ill = disease symptoms; (2) a lognormal dose-response relationship. However, the GM of the organism concentrations for developed countries was 100 organisms/L with a GSD = 50; for developing countries, the GM = 300 organisms/L with a GSD = 150. A treatment efficiency equivalent to no treatment was used for the high-risk curve, and a treatment efficiency equivalent to 2 to 5 log removals was used for both low-risk curves.

Table 33. Escherichia coli dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
1.6×10^{10}	0.875	8	30
5×10^{9}	0.75	8	30
1.7×10^{9}	0.625	8	30
1.4×10^{8}	0.750	8	30
9×10^{9}	1.0	12	31
6.5×10^{9}	1.0	11	31
5.3×10^{8}	0.666	12	31
7×10^{6}	0.636	11	31

Table 34. Escherichia coli dose-response equation parameters.

	Parameter	
Equation	1	2
Logistic	M = - 1.2184	N = 0.2406
Beta	—	—
Exponential	$r = 1.217 \times 10^{-8}$	—
Lognormal	$GM = 4.36 \times 10^{7}$	GSD = 36.6

the risk estimates are extremely sensitive to changes in the dose-response equation. The logistic equation was used to calculate the upper- and lower-risk boundaries because it resulted in the highest estimate of risk.

Risk curves are shown in Fig. 15. The low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.29, and the high-risk boundary indicates the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.52. For developing countries, the low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.42.

Table 35. Escherichia coli concentration in water.[a]

Country	Water source	Fecal coliforms (organisms/100 mL)
Gambia	Hand-dug well	<100,000
Indonesia	Canals in Jakarta	3,100–3,100,000
Kenya	Water hole	11–350
	Large river	10–100,000
Lesotho	Unprotected spring	900
	Water hole	860
	Stream	5,000
	Protected spring	200
Nigeria	Pond	1,300–1,900
	Hand-dug well	200–580
Papua New Guinea	Stream	0–10,000
Tanzania	Water hole	61
	Pond	163
	Stream	128
	Open well	343
	Protected well	7
Uganda	River	500–8,000
	Stream	2–1,000
	Unprotected spring	0–2,000
	Protected spring	0–200
	Hand-dug well	8–200
	Bored hole	0 –60

[a] Adapted from Kehr and Butterfield.[32]

Salmonella spp.

The dose-response equations were applied to the available data as shown in Table 37. The dose-response equation parameters are shown in Table 38. The data used to calculate the concentration of Salmonella spp. (excluding S. typhi) in fresh waters of developed countries are presented in Table 39. A geometric mean of 172 organisms/L and

Table 36. Escherichia coli dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	11.21	10.84	--
Beta	--	--	--
Exponential	5.54	61.81	Yes (50.5)
Lognormal	6.13	32.99	Yes (45.3)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the logistic equation.

a GSD of 99 were calculated from the data and used to represent stream and river water quality in developed countries. The calculations and water-quality values for developing countries are presented in Table 22.

The risk-assessment model, using the above results, was run to identify the dose-response equation that gives the most conservative estimate of risk. The results of these runs are shown in Table 40. Review of the results indicates that by changing the dose-response curve, we modify the expectation of the risk distribution from 6.5 to 20.2%. Based on these results, the exponential model was selected for use in calculating the upper- and lower-risk boundaries because it resulted in the highest estimate of risk.

A plot of the risk curves is shown in Fig. 16. The low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.52, and the high-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.94. For developing countries, the low-risk curve indicates that the probability is 0.5 that the fraction of troops ill would be equal to or less than 0.64. The results also indicate that the low risk is essentially the same in a developed country as in a developing country.

The risk calculations for Salmonella spp. appear to be high. The risk values are due to the low dose-response value of 17 organisms and a 12% response value (Table 37). This value is an estimate from an epidemiological study, not from a feeding study. Without this value, the risk assessment would approximate the S. typhi curves shown in Fig. 17.

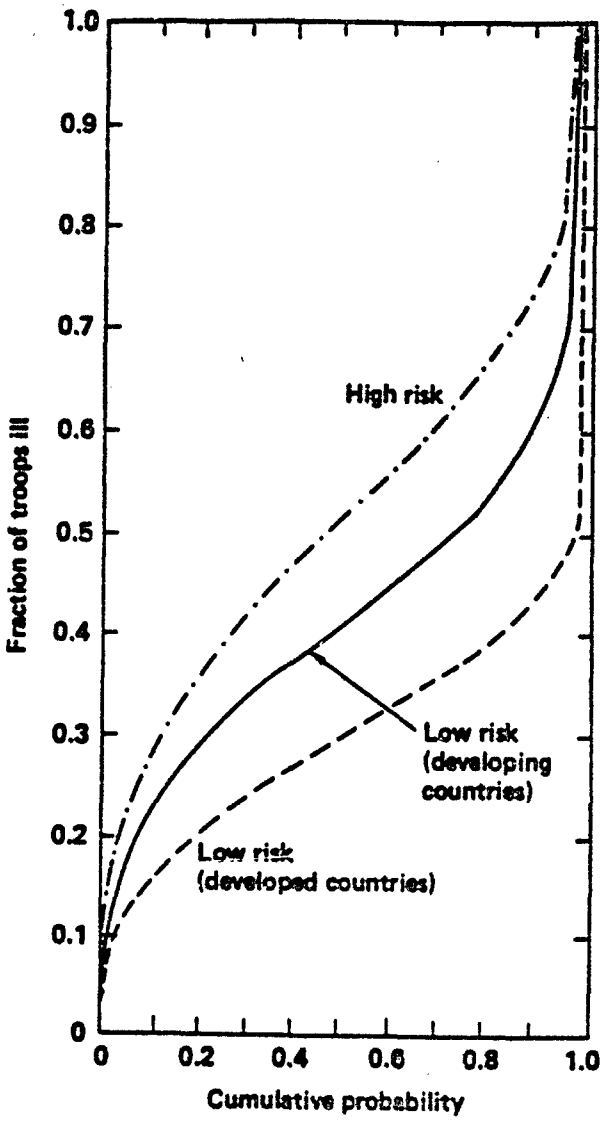

Figure 15. Cumulative-risk curves showing effect of change in treatment efficiencies and organism-concentration parameters on the risk of becoming ill as a consequence of consumption of Escherichia coli in drinking water. Each curve was determined using (1) n = 20 troops and ill = disease symptoms and (2) a logistic dose-response relationship. However, a concentration distribution with a GM = 2,000 organisms/L and a GSD = 2 was used for developed countries, and a concentration distribution with a GM = 20,000 organisms/L and a GSD = 35 was used for developing countries. A treatment efficiency equivalent to no treatment was used for the high-risk curve, and a treatment efficiency equivalent to 2 to 5 log removals was used for both low-risk curves.

Table 37. Salmonella spp. dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
17	0.12	16,000	34
2×10^0	1.0	2	34
1×10^{10}	1.0	1	34

Table 38. Salmonella spp. dose-response equation parameters.

Equation	Parameter 1	Parameter 2
Logistic	M = - 1.9927	N = 0.0002
Beta	α = 0.33	β = 139.9
Exponential	$r = 2.353 \times 10^{-3}$	—
Lognormal	$GM = 7.35 \times 10^4$	GSD = 1152

Salmonella typhi

The dose-response parameters for each of the four dose-response equations as determined for Salmonella typhi are shown in Table 41. The dose-response data upon which these calculations are based are given in Table 42.

An estimate of the concentration of S. typhi in the fresh water of developed countries was based upon the same data that were used to set the environmental concentration of nontyphoid-fever Salmonella in the previous section of this report. Using these latter values provided a conservative estimate of the concentration of S. typhi. For developing countries, the calculations and estimates of water-quality values associated with S. typhi are shown in Table 22.

The risk-assessment model was run, using the above results, to identify the dose-response equation giving the most conservative estimate of risk. The results of these

Table 39. Salmonella spp. concentration in water.

Salmonella spp. (organisms/100 mL)	Environment	Ref.
4.5	Storm water	35
<3.0	Storm water	35
43	Mississippi River	35
1	Mississippi River	36
4	Mississippi River	37
77	Untreated water	37
2	Untreated water	37
18	Untreated water	37
16	Untreated water	37
2	Untreated water	37
18	Untreated water	37
4500	Storm water	38

Table 40. Salmonella spp. dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	--	--	--
Beta	16.09	46.70	--
Exponential	17.14	49.06	No (6.5)
Lognormal	12.84	42.80	Yes (20.2)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the beta equation.

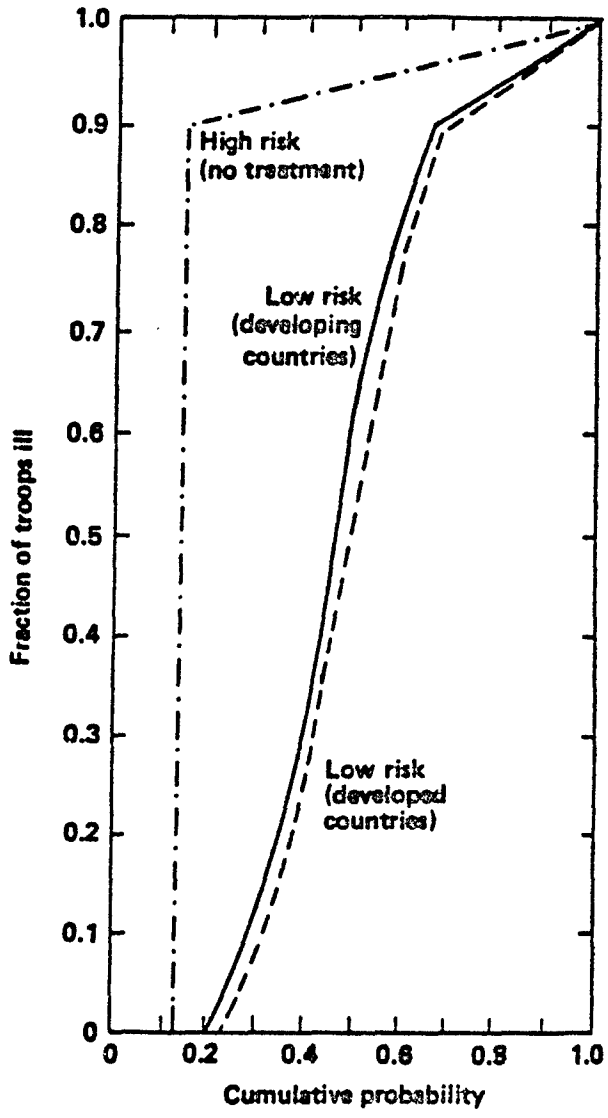

Figure 16. Cumulative-risk curves showing effect of change in treatment efficiencies and organism-concentration parameters on the risk of becoming ill as a consequence of consumption of Salmonella spp. in drinking water. Each curve was determined using (1) n = 20 troops and ill = disease symptoms, and (2) an exponential dose-response relationship. However, a concentration distribution with a GM = 172 organisms/L and a GSD = 99 was used for developed countries, and a concentration distribution with a GM = 221 organisms/L and a GSD = 100 was used for developing countries. A treatment efficiency equivalent to no treatment was used for the high-risk curve, and a treatment efficiency equivalent to 2 to 5 log removals was used for both low-risk curves.

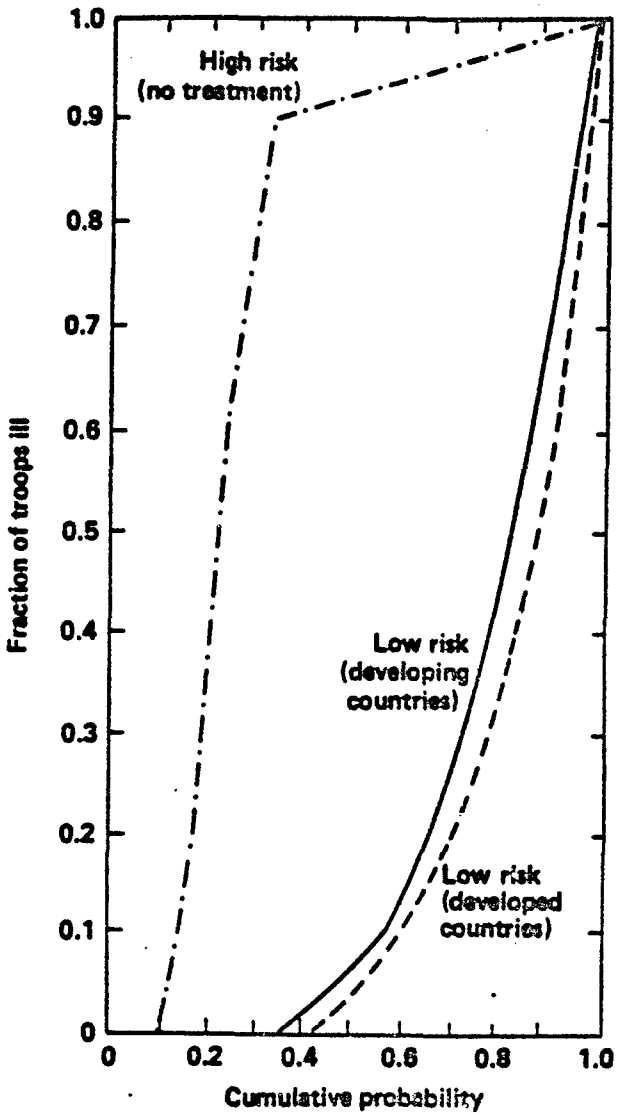

Figure 17. Cumulative-risk curves showing effect of change in treatment efficiencies and organism-concentration parameters on the risk of becoming ill as a consequence of consumption of Salmonella typhi in drinking water. Each curve was determined using (1) n = 20 troops and ill = disease symptoms, and (2) a logistic dose-response relationship. However, a concentration distribution with GM = 172 organisms/L and a GSD = 99 was used for developed countries, and a concentration distribution with a GM = 221 organisms/L and a GSD = 100 was used for developing countries. A treatment efficiency equivalent to no treatment was used for the high-risk curve, and a treatment efficiency equivalent to 2 to 5 log removals was used for both low-risk curves.

Table 41. Salmonella typhi dose-response equation parameters.

Equation	Parameter 1	Parameter 2
Logistic	M = -7.9934	N = 1.9293
Beta	α = 0.21	β = 5531
Exponential	r = 3.79×10^{-5}	—
Lognormal	GM = 3.37×10^{6}	GSD = 71

Table 42. Salmonella typhi dose-response data.

Dose (organisms)	Response (fraction ill)	Number of subjects	Ref.
10^3	0.0001	14	39
10^5	0.275	116	39
10^7	0.5	32	39
10^8	0.89	9	39
10^9	0.95	42	39
10^7	0.53	30	39
10^7	0.55	11	39
10^7	0.33	6	39
10^5	0.27	10,000	39
10^7	0.50	30	39
10^9	1.0	4	39
10^3	0.01	1,300	34
10^3	0.045	11,800	34
10^3	0.04	10,675	34
10^3	0.075	4,293	34
10^3	0.09	378	34
10^3	0.10	1,600,000	40
10^5	0.35	110	41
10^9	0.95	6	42

Table 43. Salmonella typhi dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	15.59	53.20	—
Beta	12.39	52.32	Yes (20.5)
Exponential	15.42	59.96	No (1.1)
Lognormal	10.53	59.39	Yes (32.4)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the logistic equation.

runs are shown in Table 43. Review of the results indicates that by changing the dose-response equation, we modify the expectation of the risk distribution from +1 to +32%. Based on these results, the logistic model was selected for calculating the upper- and lower-risk boundaries because it resulted in the highest estimate of risk.

A plot of the risk curves is shown in Fig. 17. The low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.04, and the high-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.92. For developing countries, the low-risk curve indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.07.

The risk estimates are conservative because the concentration of S. typhi organisms in water was assumed to be the same as Salmonella spp. As previously noted, this estimate is conservative, and the concentration of S. typhi is probably an order of magnitude less than Salmonella spp. Running the model with a reduced concentration of organisms in water resulted in the risk-curve plots shown in Fig. 18. Review of these curves indicates that at the low-risk boundary, the probability is 0.5 that none of the troops would become ill, and at the high-risk boundary, that the probability is 0.82 or less that the troops would become ill. These results appear to be more reasonable, based on the existing incidence of typhoid fever.

Yersinia spp.

Because only one dose-response data point could be found in the literature, only the lognormal distribution was used to represent the dose-response equation. As shown in

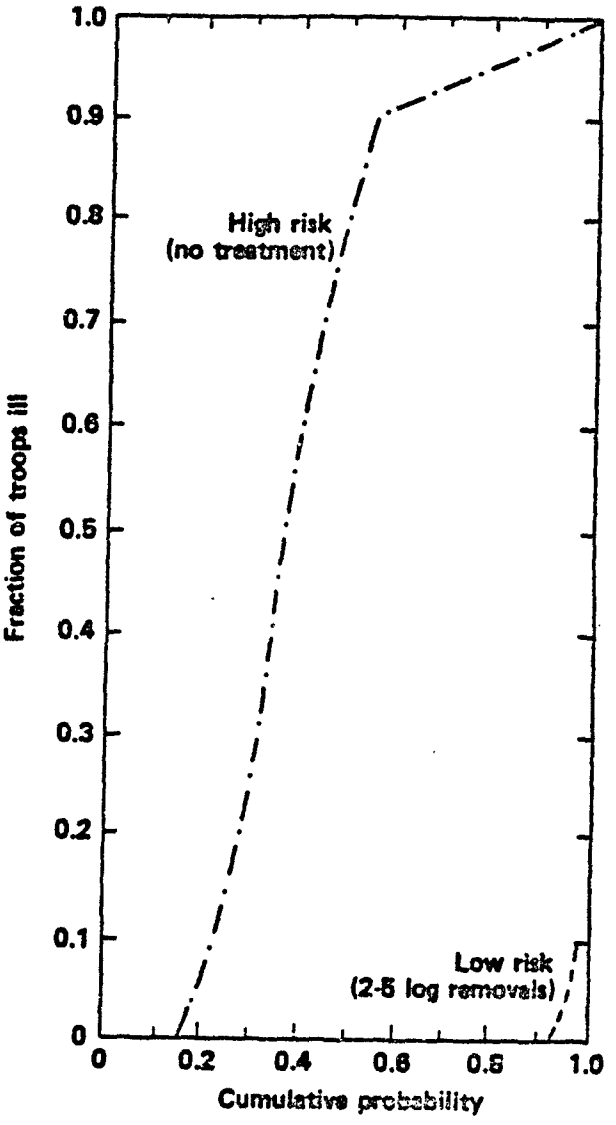

Figure 18. Cumulative-risk curves showing effect of both change in treatment efficiency and initially low organism-concentration parameters for Salmonella typhi on the risk of becoming ill with typhoid fever. Each curve was determined using (1) n = 20 troops and ill = disease symptoms; (2) a logistic dose-response relationship; (3) a concentration distribution with a GM = 17 organisms/L and a GSD = 9; and (4) treatment efficiency equivalent to no treatment for the high-risk curve and 2 to 5 log removals for the low-risk curve.

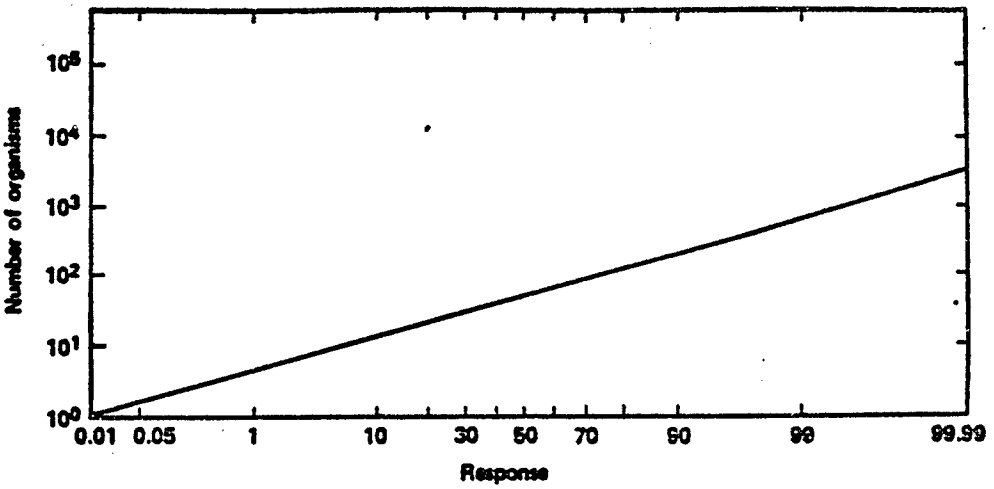

Figure 19. Lognormal dose-response relationship for Yersinia spp.

Table 44. Yersinia spp. risk-assessment results using lognormal equation.

Risk level	Ex(x)[a]	Var(x)[b]
Low (developed country)	11.00	69.70
High	17.13	48.98
Low (developing country)	16.63	48.63

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

Fig. 19, a conservative line was drawn between an assumed low dose-response point (i.e., one organism and 0.01% response) and the dose-response point found in the literature review. This line results in a geometric mean of 70 organisms/L and a geometric standard deviation of 3.

Aside from the lack of dose-response data, there is also a lack of occurrence and concentration data in the literature. Therefore a calculation of the probable concentration of Yersinia spp. in water was made. Table 22 presents the results of this calculation.

The risk-assessment model was run, based on the above values. The results of the model runs are shown in Table 44, and plots of the risk curves are shown in Fig. 20.

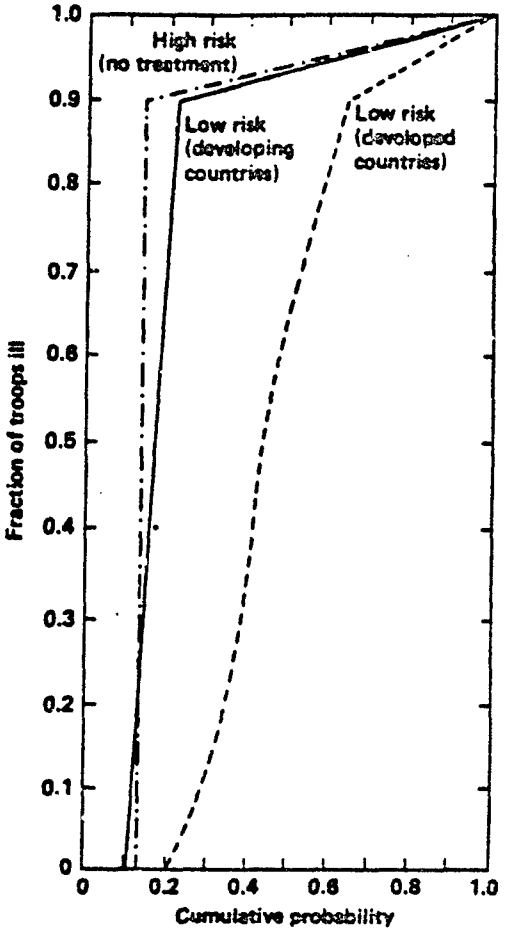

Figure 20. Cumulative-risk curves showing effect of change in treatment efficiency and organism-concentration parameters on the risk of becoming ill as a consequence of consumption of Yersinia spp. in drinking water. Each curve was determined using (1) n = 20 troops and ill = disease symptoms; and (2) a lognormal dose-response relationship. However, a concentration distribution with a GM = 100 organisms/L and a GSD = 50 was used in developed countries; and a concentration distribution with a GM = 1000 organisms/L and a GSD = 500 was used in developing countries. A treatment efficiency equivalent to no treatment was used for the high-risk curve, and a treatment efficiency of 2 to 5 log removals was used for both low-risk curves.

Review of the results indicate that at the low-risk boundary, the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.63 in developed countries and 0.93 in developing countries. At the high-risk boundary, the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.93.

These results, based on the conservative estimate of dose response for Yersinia spp., indicate a relatively high risk of disease even in the low-risk boundary area. In addition, conservative assumptions as a result of the lack of data on the occurrence and concentration of Yersinia spp. in water increases the resulting risk estimate.

RISK ASSESSMENT: VIRAL ORGANISMS

Three separate groups of viruses were identified for assessment. Enteroviruses, which encompass poliovirus, coxsackievirus groups A and B, and echoviruses, were the only viruses for which dose-response and concentration data were available. Therefore risk estimates were made only for enteroviruses.

The dose-response data, as well as data on concentration in water for developed countries, were taken from the work of Mechalas et al.[16] The response for these data is infection rather than disease. This was the case because no additional data other than those reported by Mechalas et al. were found in our literature review. An estimate was made of the concentration of enterovirus in water in developing countries and is shown in Table 22.

Therefore, use of the lognormal dose-response model (see Table 45) of Mechalas et al., as well as their concentration data in fresh water [geometric mean = 113 and standard deviation = 3, tissue-culture infective dose (TCID)], resulted in the low- and high-risk curves for developed countries as shown in Fig. 21. To generate the low-risk curve for developing countries, the dose-response equation of Mechalas et al. was again used, along with the concentration estimates made in Table 22. This risk curve is also shown in Fig. 21.

Analysis of these curves indicates that at the low-risk boundary, the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.18 in developed countries and less than or equal to 0.46 in developing countries. The high-risk curve indicates that the probability is 0.5 that the fraction of troops ill would be less than or equal to 0.9.

Please note that the end point (i.e., response) is infection rather than disease for the viral data. Therefore, when using the risk curves, one must recognize this distinction. If one wishes to calculate the risk curve with disease as an end point, a proportional reduction of the infection-risk curve would be necessary. Adequate data do not presently exist to reach more accurate evaluations of risk to debilitating disease; however, one study has shown that infection with wild poliovirus resulted in disease for 1 in 75 adults

Table 45. Enteroviruses dose-response equation parameters.

	Parameter	
Equation	1	2
Lognormal	GM = 2.5×10^2	GSD = 73

and 1 in 1000 children.[17] These data do not suggest that these are typical ratios. Applying these data to the infection-risk curve would reduce the fraction ill by at least 99%; in other words, approximately 1% of the individuals predicted to develop an infection would also become ill with the disease.

RISK ASSESSMENT: PARASITIC ORGANISMS

Two parasites, Entamoeba histolytica and Giardia lamblia, were selected for risk assessment. The following is a discussion of the assessment for each organism.

Entamoeba histolytica

No dose-response data or appropriate environmental concentration data were identified in the literature review. Some occurrence data were found; however, these data are reported only in qualitative terms (i.e., ± results). To estimate the risk of disease from amebic dysentery, it was assumed that the dose-response data were similar to those of G. lamblia for which such data exist (see Table 46). Because no concentration data were found, estimates were made for both developed and developing countries (see Table 22).

Using the above values, we applied the risk-assessment models and the equation giving the most conservative estimate of risk was determined. The results of the model runs are shown in Table 47. Review of the results indicates that by changing the dose-response equation, we modify the expectation of the risk distribution from +21 to +29%. These results indicate that the dose-response variable is sensitive to the equation used. The exponential model was selected, based on these results, to use in calculating the upper- and lower-risk boundaries because it resulted in the highest estimate of risk.

A plot of the risk curves is shown in Fig. 22. The low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be equal to or less than 0.03, and

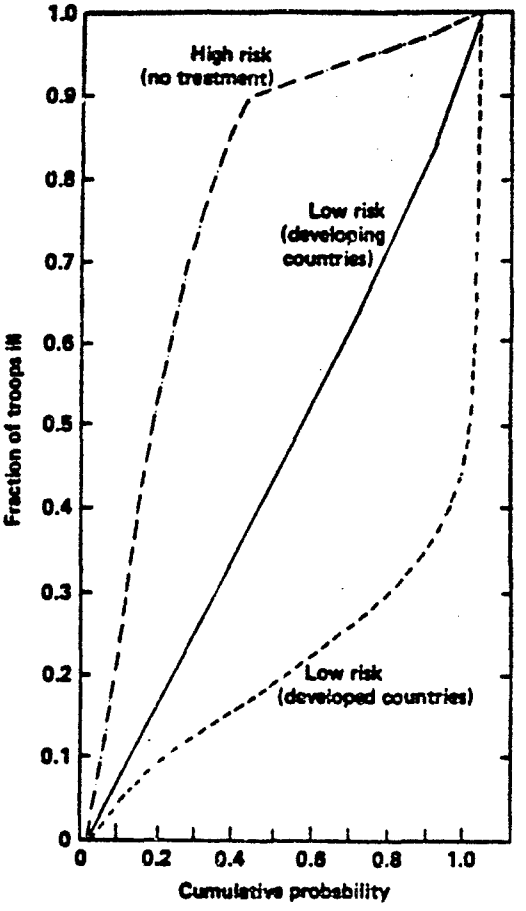

Figure 21. Cumulative-risk curves showing effect of changes in treatment efficiency on the risk of becoming ill as a consequence of consumption of enteroviruses in drinking water. The curves were determined using (1) n = 20 troops and ill = infection symptoms; (2) a lognormal dose-response relationship; (3) a dose distribution with a GM = 113 organisms/L and a GSD = 3; (4) treatment efficiency equivalent to no treatment in the high-risk curve and 2 to 5 log removals in both low-risk curves.

the high-risk boundary indicates that the probability is 0.5 that the fraction ill would be equal to or less than 0.94. In developing countries, the low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be equal to or less than 0.9.

Giardia lamblia

Three of the four dose-response equations were fit to the available data (see Table 46). The dose-response equation parameters are shown in Table 48. An attempt to

Table 46. Giardia lamblia dose-response data.

Dose (cysts)	Response (fraction infected)	Number of subjects	Ref.
1	0	5	43, 44
10	1.0	2	43, 44
25	0.3	20	43, 44
10^2	1.0	2	43, 44
10^4	1.0	3	43, 44
10^5	1.0	3	43, 44
3×10^5	1.0	3	43, 44
10^6	1.0	2	43, 44

Table 47. Entamoeba histolytica dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	--	--	--
Beta	13.3	39.7	--
Exponential	17.1	48.9	Yes (28.5)
Lognormal	16.1	47.0	Yes (21)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the beta equation.

fit the logistic equation to the available dose-response data resulted in a uniform equation (i.e., zero slope). Therefore this equation was not used.

Because no data relative to the occurrence and concentration of Giardia lamblia in water were found in the literature review, calculation of a probable value was necessary. It has been estimated that raw sewage may contain from 96,000 to 2,400,000 cysts/L when 1 to 25% of the population is infected.[17] If we assume a dilution rate of 100:1 for a

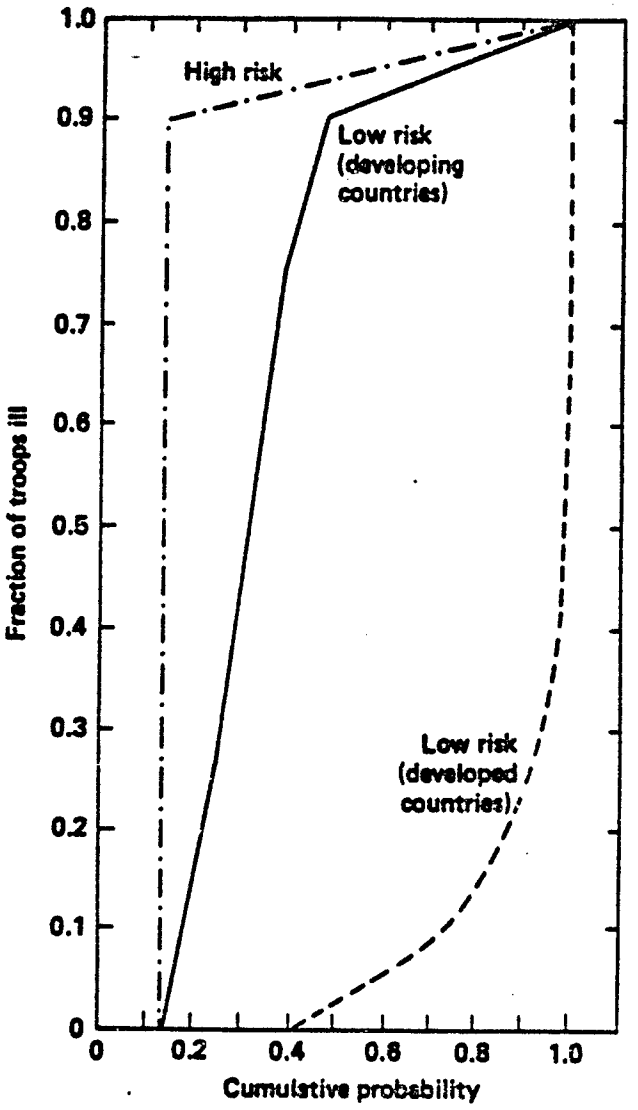

Figure 22. Cumulative-risk curves showing effect of changes in treatment efficiency and organism-concentration parameters on the risk of becoming ill as a consequence of consumption of Entamoeba histolytica in drinking water. Each curve was determined using (1) n = 20 troops and ill = infection, and (2) an exponential dose-response relationship. However, a concentration distribution with a GM = 13 organisms/L and a GSD = 7 was used for developed countries, and a concentration distribution with a GM = 146 and a GSD = 73 was used for the low-risk curve in developing countries. A treatment efficiency equivalent to no treatment was used for the high-risk curve, and a treatment efficiency equivalent to 2 to 5 log removals was used for both low-risk curves.

Table 48. Giardia lamblia dose-response equation parameters.

Equation	Parameter 1	Parameter 2
Logistic	—	—
Beta	$\alpha = 0.18$	$\beta = 11.6$
Exponential	$r = 1.53 \times 10^{-2}$	—
Lognormal	GM = 102	GSD = 17

stream, the result is a calculated geometric mean of approximately 1500 cysts/L (assume standard deviation = 750). A value of 100 was used to run the risk model for both developed and developing countries because it was a more conservative estimate than the values shown in Table 22.

The risk-assessment model, based on the above values, was run to identify the dose-response equation that results in the most conservative estimate of risk. The results of these runs are shown in Table 49. Review of the results indicates that by changing the dose-response equation, we modify the expectation of the risk distribution by roughly 6%, which is not considered sensitive when applying the 10% difference criterion. Because the exponential distribution resulted in the most conservative estimate of risk, it was used to calculate the risk curves for Giardia lamblia.

A plot of the risk curves is shown in Fig. 23. The low-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be equal to or less than 0.92, and the high-risk boundary indicates that the probability is 0.5 that the fraction of troops ill would be equal to or less than 0.94. These results indicate that Giardia lamblia, based on our conservative estimates, presents a significant level of risk.

SUMMARY: RISK-ASSESSMENT RESULTS

The risk-assessment results for water-related pathogens evaluated in this study are summarized in Table 50. Review of the table indicates that the exponential dose-response model provided the most conservative estimate of risk for 5 of 11 pathogens evaluated, and that the logistic model provided the most conservative estimate for 3 of 11 pathogens.

Table 49. Giardia lamblia dose-response equation sensitivity.

Equation	Ex(x)[a]	Var(x)[b]	Sensitivity (% difference)[c]
Logistic	—	—	—
Beta	16.09	45.64	—
Exponential	17.14	49.01	No (6.5)
Lognormal	17.12	48.61	No (6.4)

[a] Ex(x) = mean of distribution.

[b] Var(x) = variance of distribution.

[c] "Yes" or "No" implies whether or not the difference is considered significant, compared to the beta equation.

The most conservative risk estimate for giardiasis was derived using the exponential equation. Because dose-response data for the remaining two agents (Yersinia and enteroviruses) were extremely limited, only the lognormal equation was used in their risk evaluation.

Review of the risk-assessment results for developed countries indicates that the pathogenic organisms, E. coli, Salmonella spp., and Yersinia spp., pose the highest degree of risk of illness (i.e., disease) at the low-risk level (i.e., 2- to 5-log removal treatment efficiency). For the other pathogens at the low-risk level, the fraction of troops ill was generally below 10% at the cumulative probability of 0.5. In general, risk estimates at the high-risk level (i.e., no treatment) indicate that, with the exception of V. cholerae El Tor, all organisms present a high degree of risk of illness.

Review of the risk assessment for developing countries indicates that S. typhi presents the lowest degree of risk, followed by pathogenic E. coli and enteroviruses. In the latter instance, it should be noted that the risk is to clinical disease, whereas the risk associated with enterovirus is to infection. The ratio of infection to clinical disease in the case of enteroviruses is high (i.e., more infection than disease). The remainder of the organisms have a level of risk similar to the high risk level in developed countries. The model was not run for the high-risk level in developing countries because of the findings of the model runs at the low-risk level.

As previously discussed, the risk-assessment model was sensitive to a two-order-of-magnitude change in pathogen concentration variation. Based on a comparison of the

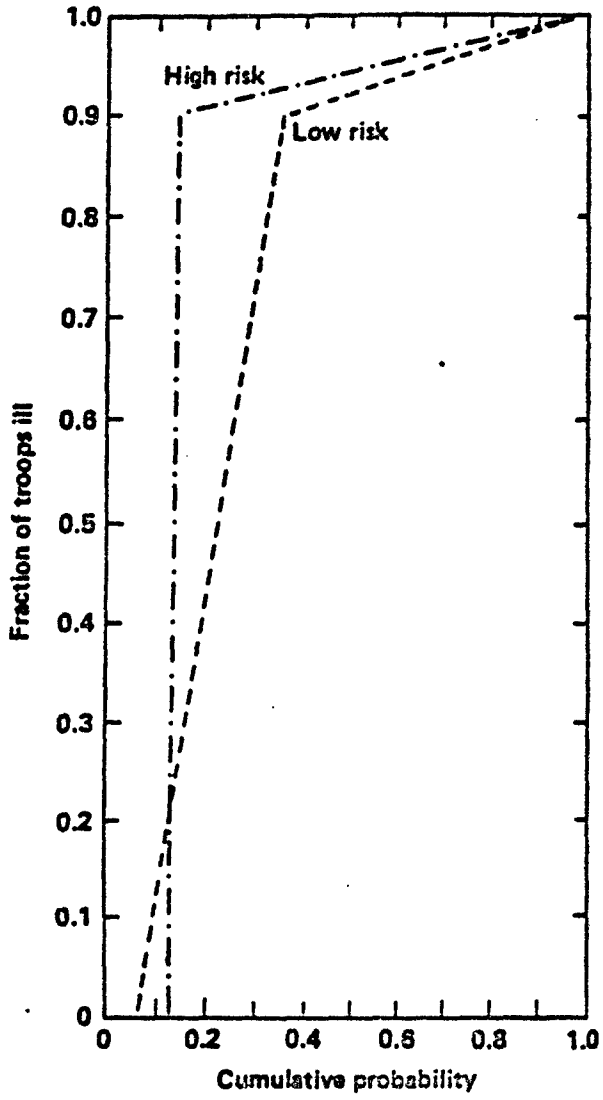

Figure 23. Cumulative-risk curves showing effect of changes in treatment efficiency on the risk of becoming ill as a consequence of consumption of Giardia lamblia in drinking water. The curves were determined using (1) n = 20 troops and ill = infection; (2) an exponential dose-response relationship; (3) a concentration distribution with a GM = 100 organisms/L and a GSD = 50; (4) treatment efficiency equivalent to no treatment in the high-risk curve and 2 to 5 log removals in the low-risk curve.

Table 50. Summary: risk-assessment results.

Pathogen	Dose response[a]	Fraction of troops ill or less at cumulative probability of 0.5: Developed country, low risk[b]	High risk[c]	Developing country, low risk[d]
BACTERIAL:				
Shigella spp.	Exponential	0.08	0.94	0.90
Vibrio cholerae				
Classical	Exponential	0.00	0.78	0.82
V. cholerae El Tor	Logistic	0.00	0.90	0.00
Campylobacter	Lognormal	0.04	0.95	0.93
Escherichia coli	Logistic	0.29	0.50	0.42
Salmonella spp.	Exponential	0.55	0.92	0.62
Salmonella typhi	Logistic	0.00	0.82	0.07
Yersinia spp.	Lognormal[e]	0.63	0.93	0.92
VIRAL:				
Enteroviruses	Lognormal[e]	0.08	0.94	0.46
PARASITIC:				
Entamoeba histolytica	Exponential	0.03	0.94	0.90
Giardia lamblia	Exponential	0.90	0.91	0.90

[a] Disease is the response for bacterial pathogens, and infection is the response for viral and parasitic pathogens.

[b] Low risk: assumes 2- to 5-log removal treatment efficiency.

[c] High risk: assumes no treatment.

[d] Low risk (developing country): assumes 2- to 5-log removal treatment efficiency, as well as a higher raw-water pathogen concentration.

[e] Only model run made.

Table 51. Latency[a] of common waterborne pathogens.

Organism	Latency[b] (d)
Shigella dysenteriae	3 - 6
Campylobacter jejuni	1 - 4
Vibrio cholerae	<1 - 2
Escherichia coli	0.1 - 3
Salmonella typhi	3 - 22
Salmonellosis	0.04 - 4
Yersinia	<1
Enteroviruses	2 - 35
Norwalk agent	0.42 - 2.1
Rotavirus	1 - 4
Entamoeba histolytica	7 - 98
Giardia lamblia	3 - 56

[a] Latency is defined to be the time (in days) from ingestion to the onset of symptoms.
[b] Based on data in the Appendices.

risk-assessment results between developed and developing countries, it appears that the model is also sensitive to changes in the mean concentration of pathogens in water. It was also noted that the volume of water consumed (10 to 15 L/d) had little effect on the risk estimate.

As a final note, latency (i.e., time from ingestion to the onset of symptoms) should be considered when reviewing the risk curves. As shown in Table 51, the latency period for the organisms under consideration is generally 1 to 3 d. This indicates that the expected fraction of individuals predicted to become ill would do so within a 1- to 3-day period after ingestion of the organisms.

RECOMMENDATIONS FOR STANDARDS

The development of water-quality standards is a complicated task that involves the concept of risk. Every human activity involves a certain degree of hazard. Important interrelated questions about standards and risk are (1) How can risk estimates best be used

Table 52. Specified-risk curve: finished (treated) drinking-water pathogen concentrations (organisms/L).

Pathogen	Developed country		Developing country
	Low risk	High risk	Low risk
BACTERIAL:			
Shigella spp.	$7 \times 29^{\pm 1}$	$10^5 \times 3000^{\pm 1}$	$328 \times 239^{\pm 1}$
Vibrio cholerae Classical	1	1	$1 \times 10^{\pm 1}$
V. cholerae El Tor	1	1	$1 \times 10^{\pm 1}$
Campylobacter	$7 \times 29^{\pm 1}$	$10^5 \times 3000^{\pm 1}$	$42 \times 79^{\pm 1}$
Escherichia coli	$14 \times 3^{\pm 1}$	$10^5 \times 3000^{\pm 1}$	$1300 \times 21^{\pm 1}$
Salmonella spp.	$18 \times 54^{\pm 1}$	$10^6 \times 6000^{\pm 1}$	$23 \times 54^{\pm 1}$
Salmonella typhi	$<1 \times 7^{\pm 1}$	$2000 \times 630^{\pm 1}$	$328 \times 239^{\pm 1}$
VIRAL:			
Enteroviruses	$1 \times 4^{\pm 1}$	$6000 \times 220^{\pm 1}$	$14 \times 44^{\pm 1}$
PARASITIC:			
Entamoeba histolytica	$1 \times 6^{\pm 1}$	$1300 \times 490^{\pm 1}$	$12 \times 41^{\pm 1}$
Giardia lamblia	$653 \times 348^{\pm 1}$	$10^7 \times 5^{\pm 1}$	$653 \times 348^{\pm 1}$

Note: Low risk = most conservative dose-response model, 10 L ± 10% water consumed, and 99 to 99.999% organism removal.

Note: High risk = most conservative dose-response model, 15 L ± 10% water consumed, and 0% organism removal.

for setting standards? (2) What is an acceptable level of risk associated with water-related disease? (3) How can this information be used in deciding the appropriate level of resource commitment to achieve the standard?

To address the first question, a concentration of pathogens in finished drinking water (i.e., treated water) can be determined for a selected risk distribution. These values have been determined for both the low- and high-risk curves. The resulting concentrations are shown in Table 52.

Addressing the second question involves consideration of the acceptability of risk or risk evaluation. The latter step is difficult to accomplish because it involves a decision as to the amount of risk that can be tolerated to achieve a defined level of benefit. This

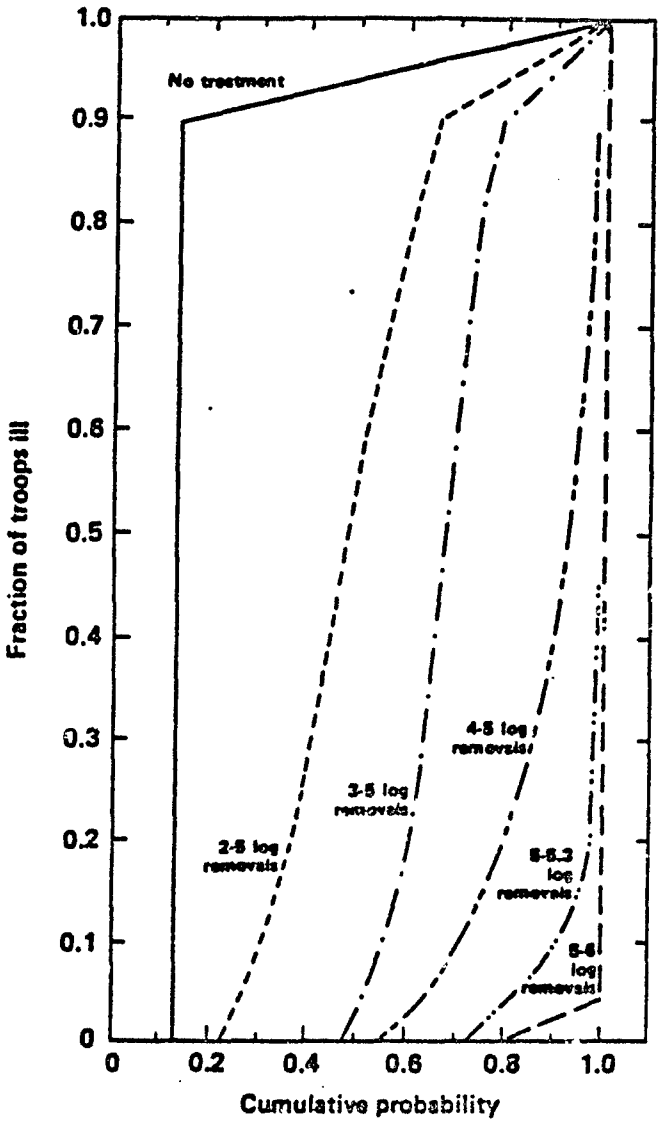

Figure 24. Cumulative-risk curves showing the effect of improving treatment efficiency and knowing precisely the factor of reduction in number of organisms per liter on the risk of becoming ill as a consequence of consumption of Salmonella spp. in drinking water. The curves were determined using (1) n = 20 troops and ill = disease symptoms; (2) an exponential dose-response relationship; (3) a dose distribution with a GM = 172 organisms/L and a GSD = 99; and (4) treatment efficiencies equivalent to no treatment, 2 to 5 log removals, 3 to 5 log removals, 4 to 5 log removals, 5 to 5.3 log removals, and 5 to 6 log removals.

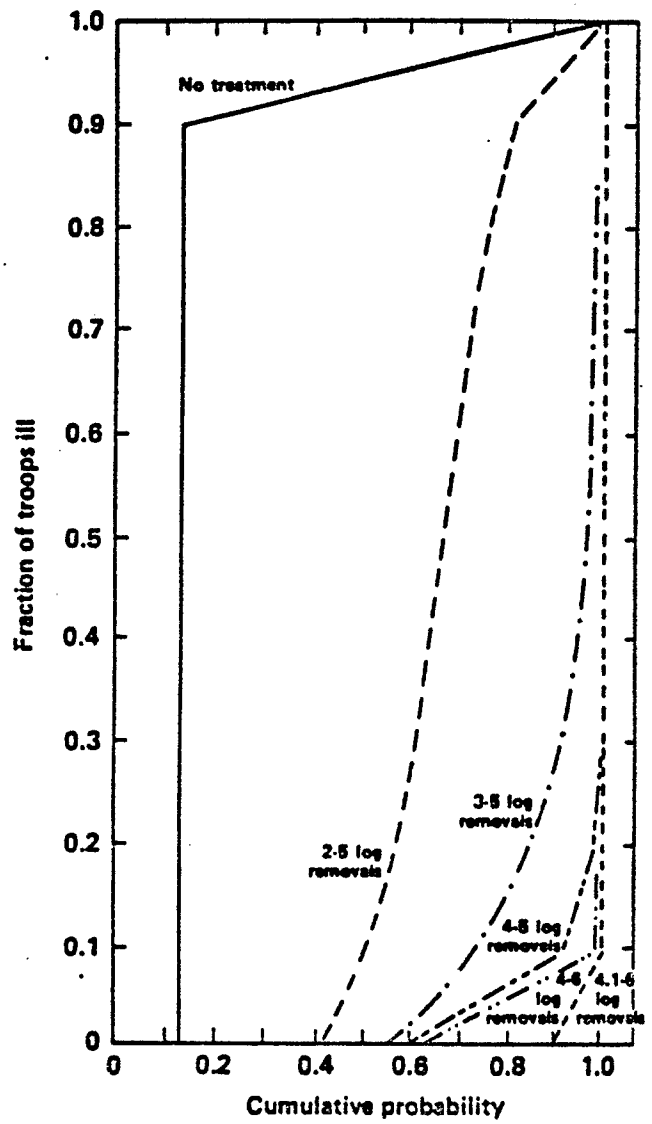

Figure 25. Cumulative-risk curves showing the effect of improving treatment efficiency and knowing precisely the factor of reduction in number of organisms per liter on the risk of becoming ill as a consequence of consumption of Shigella spp. in drinking water. The curves were determined using (1) n = 20 troops and ill = disease symptoms; (2) an exponential dose-response relationship; (3) a dose distribution with a GM = 100 organisms/L and a GSD = 50; and (4) treatment efficiencies equivalent to no treatment, 2 to 5 log removals, 3 to 5 log removals, 4 to 5 log removals, 4 to 6 log removals, and 4.1 to 6 log removals.

Table 53. Alternate-risk curves: finished (treated) drinking-water pathogen concentrations (organisms/L).

Treatment efficiency (% removal)[a]	Concentration	
	Salmonella spp.	Shigella spp.
99–99.999 (2 to 5 log)	$18 \times 54^{\pm 1}$	$7 \times 29^{\pm 1}$
99.9–99.999 (3 to 5 log)	$2 \times 53^{\pm 1}$	$0.7 \times 29^{\pm 1}$
99.99–99.999 (4 to 5 log)	$0.2 \times 56^{\pm 1}$	$0.09 \times 30^{\pm 1}$
99.99–99.9999 (4 to 6 log)	b	$0.07 \times 29^{\pm 1}$
99.993–99.9999 (4.1 to 6 log)	b	$0.05 \times 29^{\pm 1}$
99.999–99.9995 (5 to 5.3 log)	$0.03 \times 68^{\pm 1}$	b
99.999–99.9999 (5 to 6 log)	$0.02 \times 56^{\pm 1}$	b

[a] Range also represents reliability.

[b] Computer run not made.

requires personal and social value judgments, as opposed to the more scientific quantification of risk assessment. From the risk curves developed in this study, the military experts, who are most aware of the judgments required for risk evaluation in the armed services, should make such judgments. Once an acceptable-risk curve is identified, a concentration in the treated water can then be determined. For example, if the low-risk curve for pathogenic E. coli (Fig. 15) is determined to present an acceptable level of risk, then the drinking-water standard necessary to achieve that level of risk is approximately $14 \times 3^{\pm 1}$ organisms/L in the treated water (see Table 52).

To set a standard, a level of risk must be specified. Discussions with Dr. Stephen Schaub[45] indicate that the military would want to be confident (e.g., 0.95 cumulative probability) that less than 5% of the troops would become ill after drinking water with a specified concentration of pathogen organism. To achieve this level of risk, it would be necessary to modify one or more of our assumptions regarding volume consumed, pathogen concentration, and treatment efficiency. Because the model is most sensitive to the treatment-efficiency variable, this variable was selected for modification to illustrate the different levels of risk associated with different levels of treatment efficiency.

The organisms, Salmonella spp. and Shigella spp., were chosen to illustrate the levels of risk, mainly because some data relate the pathogenic organisms to indicator organisms. Shown in Figs. 24 and 25 are the risk curves for these two organisms with varying

treatment removal efficiencies. These data are summarized in Table 53, along with the calculated finished drinking-water concentration values associated with the different risk curves. As shown in Table 53, to achieve a 0.95 cumulative probability of <5% troops ill, a 5- to 6-log reduction (99.999 to 99.9999%) of organisms is necessary for Salmonella spp. and a 4.1- to 6-log reduction (99.993 to 99.9999%) is necessary for Shigella spp. An interesting result of this analysis is that as the reliability of the treatment efficiency increases, the level of risk decreases dramatically. This result is best illustrated in Fig. 24. As the treatment reliability increases from 2- to 5-log removal to a 4- to 5-log removal, the level of risk decreases as shown by the curves moving up to the top of the figure. The results of this analysis again demonstrate the importance of the treatment-efficiency variable, including the question of reliability associated with the treatment system. Because a finished drinking-water concentration has been calculated for the selected organisms at the level of acceptable risk identified by the military, the next step is to relate that concentration to a concentration of indicator organisms.

Ideally, indicators of drinking-water quality are microorganisms whose concentrations in water can be related quantitatively to potential health hazards resulting from drinking the water. Potential indicators can be screened for use against the following criteria.[16] An indicator microorganism:

1. Must be a reliable measure of the potential presence of specific contaminating organisms, both in natural waters and in waters that have been subjected to treatment. To meet this requirement, the indicator organism or organisms must react to the natural aquatic environment and to treatment processes (including disinfection) in relatively the same way as do the contaminating organisms;

2. Must be present in numbers that are relatively much larger than those of the contaminating organism whose potential presence is to be indicated. Otherwise, detection of the contaminating organism itself would serve a more useful purpose;

3. Must be identified readily by relatively simple analytical procedures;

4. Must lend itself to numerical evaluation as well as qualitative identification.

For nearly 80 y,[25] the coliform bacteria and, more recently, fecal coliform have been used as a tool to measure the occurrence and intensity of fecal contamination; for

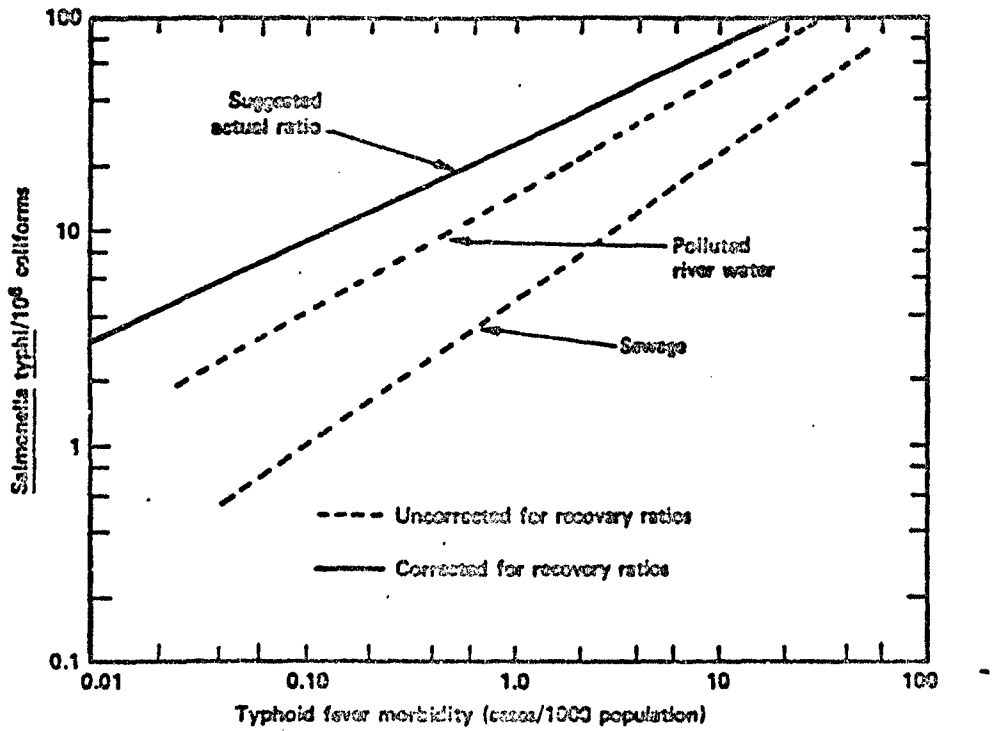

Figure 26. Relationship between the ratio of Salmonella typhi per 10^6 total coliform bacteria and morbidity rates, expressed as cases of typhoid fever per 10^3 individuals. Shown are both the uncorrected ratios of Salmonella typhi to total coliform bacteria determined from monitoring sewage and polluted receiving water (as noted in figure) and the corrected ratios of Salmonella typhi to total coliform bacteria considered to actually occur in either sewage or polluted water.

the most part, they meet the criteria outlined above. Use of the coliform organism still appears reasonable on both theoretical and practical grounds because measuring specific pathogens remains impractical for several reasons:

1. Many different pathogenic organisms (bacteria, viruses, and parasites) can exist in water;

2. Monitoring for each organism would be difficult, time-consuming, and costly;

3. Enumeration methods for some of the more important pathogens are either unavailable or imprecise.

Kehr and Butterfield[32] reviewed several studies in England, Indonesia, and California, where the successful enumeration of both coliforms and typhoid bacilli in sewage and polluted waters was carried out at the time of outbreaks of typhoid fever. As shown in Fig. 26, they derived a relationship between the morbidity rates from typhoid fever in different areas and the ratio of S. typhi to total coliform in sewage and polluted waters. An actual ratio relationship, as shown in Fig. 26, between these organisms was suggested, based on correction of the data reviewed for recovery ratios. This curve can be described by the following equation:

$$y = a r^n, \quad (21)$$

where

a and n are constants = 3 and 0.46, respectively;

y = the number of pathogenic bacteria per 10^6 coliform organisms; and

r = the morbidity (relative incidence/100,000 persons).

Assuming that the relationship between coliforms and enteric pathogens holds for both sewage and the receiving waters, one can then estimate the number of pathogens in the receiving water. Based on a morbidity rate of 0.18 per 100,000 persons for typhoid in the United States, the estimated number of S. typhi organisms is shown in Table 54. Moreover, if we assume that the relationship holds for other Salmonella spp., as well as Shigella spp., and that the morbidity rates for salmonellosis and shigellosis in the United States are 360 and 160 per 100,000,[46] respectively, then the estimates of these organisms in receiving water are as shown in Table 54. Note that the morbidity values for these diseases are inflated by 95% over the reported rates because the reporting of these diseases is roughly 5%.

Based on the ratio of pathogen to indicator organism shown in Table 54 and the concentration of pathogenic organisms in finished drinking water previously calculated (see Table 53), the coliform concentration in finished drinking water can then be calculated. The results of this calculation are shown in Table 55. As shown in Table 55, the coliform density is for a cumulative probability of 0.95 and <5% of the troops ill, which, for the purpose of this study, represents an acceptable level of risk.

Nevertheless, there are no relatively simple field tests for measuring the specific concentration of any of the variety of infectious organisms previously discussed. Until such tests are available for determining either directly or indirectly (based on indicator organisms) the concentration of specific infectious organisms in field water, the military should continue to use the membrane-filter technique for the presumptive determination of the presence of coliform organisms in water. The present field-water quality standard

Table 54. Estimated number of bacterial pathogens per million coliforms.

Organism	Bacterial pathogens per million coliforms
Salmonella typhi	1.4
Salmonella spp.	44.9
Shigella spp.	30.9

Table 55. Finished drinking-water concentration cumulative probability of 0.95 < 5% troops ill.

Pathogen	Pathogen concentration[a]	Treatment efficiency	Coliform density[b]
Salmonella spp.	0.024; 0.0004 to 1.4	99.999 to 99.9999	53; 0.89 to 3110
Shigella spp.	0.049; 0.0017 to 1.4	99.993 to 99.9999	159; 5.5 to 4530

[a] Geometric mean, and 68% confidence interval.

[b] Geometric mean (organisms/100 mL), and 68% confidence interval.

based on this technique (i.e., coliform densities should not exceed one colony-forming unit (CFU) per 100 mL)[47] is considered acceptable as both a short- and long-term standard for pathogenic organisms, including viruses and protozoa. However, further research should be performed with regard to the applicability of a coliform standard to viruses and protozoa as all of these organisms might differ in their survivability and treatability, particularly with respect to disinfection. Nevertheless, no better relationship between an indicator organism and pathogenic organisms in water exists at this time, and the coliform standard is practicable for field application because it eliminates the need to monitor for many different pathogenic organisms that may or may not be present.

To overcome any limitations associated with using a coliform standard for all pathogenic organisms, consideration should be given to transporting water samples collected in the field to a centrally located field laboratory where detailed microbiological analyses could be conducted. Such analyses would permit the concentration of specific infectious organisms to be determined. In a 1985 field exercise involving approximately 5000 troops deployed over a 525-mi^2 area in New Brunswick, Canada, a helicopter was

used successfully to transport field-water samples rapidly to a central field-water testing laboratory, with minimal sample deterioration. Test results were returned to appropriate engineering personnel within a 24-h period.[48] The data from such laboratory analyses could then be used in combination with the risk-assessment methodology discussed earlier to estimate the related health risks to military personnel exposed in the future.

A two-tier analytical approach might also be considered that would capitalize on the use of the membrane-filter technique in the field to determine whether the concentration of pathogenic microorganisms, particularly those of fecal origin, are likely to be of concern and then, if indicated, employ the more sensitive analytical capabilities of a central field laboratory to quantify the concentration of specific infectious organisms in order to estimate health risks in the future. The two-tiered analytical strategy would be useful for prioritizing the locations requiring sample transport to a central laboratory for further analyses.

CONCLUSIONS

In this research effort we have accomplished the following:

1. Developed and documented a rational methodology for evaluating the risk of infectious illness to individuals drinking water of varying quantity and quality.

2. Developed a mathematical model (and computer program) for calculating the risk of illness.

3. Identified a data format appropriate for the model. In this instance, the data are separated into two groups: one describing the likelihood that an individual would encounter a given dose of a pathogen in water, and one describing the capability of the exposed individual to withstand a challenge dose (i.e., dose response). Basically, for given levels of pathogens in water, water volume consumed, treatment efficiency, and a pathogen dose-response relationship, a prediction of the number (or percent) of affected individuals can be made.

4. Compiled an extensive literature review of 11 pathogens of worldwide significance, with particular attention to factors such as occurrence, persistence, dose-response relationships, prevalence, disinfection resistance, and indicator-pathogen relationships; and entered this information into a computer data base. This review is in the Appendices of this report.

5. Conducted a quantitative risk assessment for 11 pathogens.

6. Identified drinking-water standards for low- and high-risk levels in terms of a log mean and a standard deviation. The pathogen that causes the greatest risk should be used in developing an overall numerical standard.

Finally, a rigorous calibration and testing of the model was hampered by the lack of available data. Areas where more information is needed have been indicated. However, the model provides an opportunity to estimate the health risk from many different waterborne pathogens.

UNCERTAINTIES AND RESEARCH RECOMMENDATIONS

In performing this study, it became obvious that the analysis of risk is influenced strongly by the information available on the occurrence and concentration of the pathogen in water, as well as the level (i.e., efficiency) of water treatment. The lack of information on the occurrence and concentration of pathogens in water is disturbing. Better definition of this variable would improve the confidence of the risk estimates.

As discussed in the Appendices, there are many instances in which no techniques exist that would allow for the measurement of pathogens in water. Therefore, it is strongly recommended that additional research be performed to develop reasonable quantitative techniques for the isolation and enumeration of important pathogenic agents in water. These methods could then be applied to determine the concentration of these infectious agents in priority waters in selected geographic areas.

As for the level of treatment, the risk estimates made in this study assumed a maximum treatment-efficiency rate of 2- to 5-log removals. Reducing the uncertainty associated with this variable by reducing the pathogen-removal range and/or increasing the removal rate was shown to improve the confidence of the risk estimate. For example, increasing the treatment-efficiency removal rate from 2 to 5 logs, to 4 to 5 logs, and 5 to 6 logs dramatically lowered the risk distribution as shown in Fig. 24. Documentation of the removal rates of military water-treatment equipment would improve the confidence in the risk estimates.

In addition, several other issues warrant further investigation. These issues include secondary infections, multiple exposure days, the relationship between infection rates and cases of clinical disease, indicator-pathogen relationships, and large numbers of troops at risk (i.e., >20).

In this risk-assessment exercise we have assumed that the infections involved would be associated only with water contact. There may be secondary infections in which the primary infected case transmits the disease via person-to-person contact. The impact of this latter scenario on the risk of disease to exposed troops should be evaluated.

With regard to multiple exposure days, the model, as developed, is based on a single-day exposure. An approach to addressing this issue could be to use the concept of sampling without replacement. That is, on the first day, the entire population is exposed to the dose distribution. A risk curve is calculated, using the model, and an acceptable risk is identified. Subsequent days of exposure are considered independently, and the population is reduced by the number ill for each day. This assumption is conservative

because it does not recognize those whose immunity developed from previous exposures. Because of this assumption, the approach may only be appropriate for time periods up to 7 d. This approach can be represented by the following equation:

$$W = (1 - F)^k N , \quad (19)$$

where:

W = number of individuals remaining well,
F = fraction of individuals ill,
k = days of exposure, and
N = total population.

For example, the military could indicate that F = 10%, that k = 7 d, and that N = 50 individuals. Based on the above equation, W would then equal 26 individuals remaining well or 24 ill individuals. Alternatively, the military could specify the number of troops that must remain well (W) within a specified time period (k). Then F, the acceptable fraction of troops becoming ill, could be calculated. Once F is identified, the risk model can then be used to identify the concentration of pathogens in the finished drinking water.

Frequently, dose-response data on infectious disease are reported in terms of infections rather than of clinical disease; this is particularly true with viral diseases. To reach more accurate evaluations of risk for debilitating disease, it would be very valuable to develop infection/disease ratios in those cases where only infection data exist.

Indicator-pathogen relationships are a major area of concern. Information concerning this relationship is extremely limited. The most important effort in this instance is the 1943 report by Kehr and Butterfield.[32] They attempted to associate coliform number with the concentration of S. typhi as a function of disease morbidity. More adequate information of this kind is urgently needed for the rational interpretation of water-monitoring data.

The last issue relates to the number of individuals (i.e., troops) at risk. The model runs to date have been using n = 20 individuals at risk. An interesting question that warrants investigation is whether the risk distribution would change if the number of individuals at risk were to increase. A run of 100 individuals at risk was made. A comparison of the results that develop with a run of 20 individuals is shown in Fig. 27. Based on this example, it appears that as n increases, the risk distribution changes slightly. This change appears to result from the variance associated with the risk distribution.

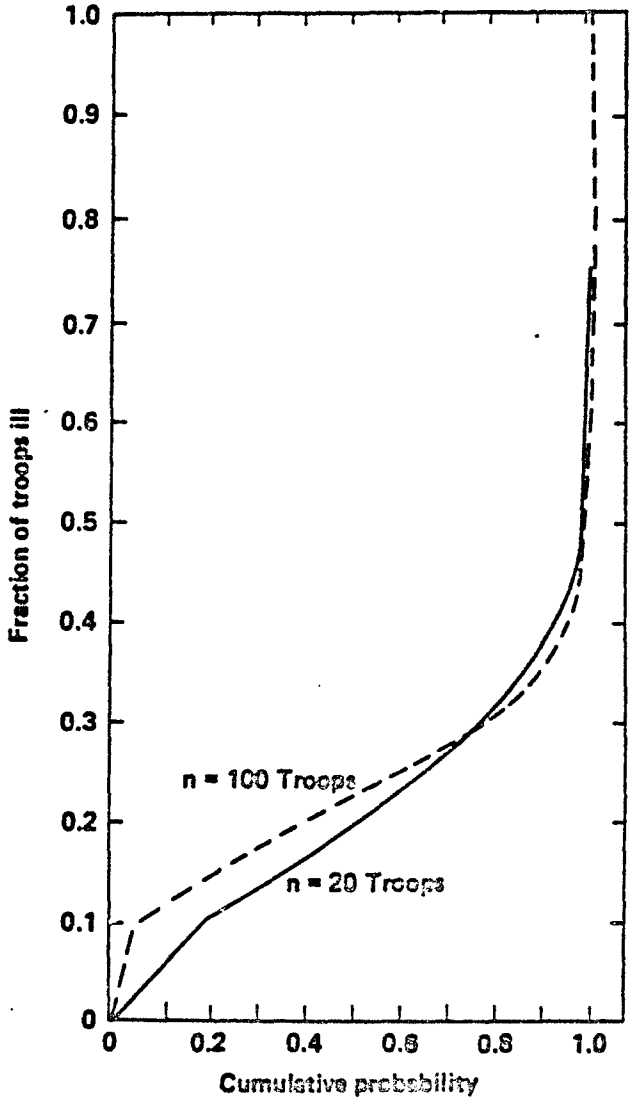

Figure 27. Cumulative-risk curves showing effect of changes in troop number (n) on the risk of becoming ill as a consequence of consumption of enterovirus in drinking water. Each curve was determined using either n = 20 or n = 100 troops.

Review of the risk-distribution variance, Eq. (12), indicates that the variability associated with the observed proportion of ill individuals among a group of size n originates from two sources: (1) the binomial distribution $f(x|\theta)$, and (2) the beta-distribution $g(\theta)$. For large numbers n of individuals, the binomial sampling variability becomes negligible, and it appears that the risk distribution is then approximated by the $g(\theta)$ distribution. Further review of this assumption is necessary. It appears that modification of the model to allow for the calculation of risk for a large population has interesting implications relative to the risk associated with municipal water supplies, and such modification presents the possibility for verification/calibration of the model against reported incidence data. Further work should be conducted in this area.

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APPENDICES

DATA BASE FOR ASSESSING THE HEALTH RISKS ASSOCIATED WITH THE CONSUMPTION OF WATERBORNE INFECTIOUS ORGANISMS

Each of the first three appendices (A, B, and C), which represent the data base used for assessing human health risks, describes the environmental properties of a specific pathogen or group of pathogens, as well as the epidemiology and control of diseases associated with these infectious organisms. These appendices are grouped by biological class of pathogen: bacteria (Appendix A), viruses (Appendix B), and parasites (i.e., protozoa and helminths) (Appendix C). Each appendix is divided into sections that discuss individual topics. The sections are indicated by subscripts to the appendix letter (e.g., A_1, A_2, B_1, B_2, etc.). To keep each sectional topic as an individual unit, the separate sections are each followed by their own references. The tables in each section are identified by a number preceded by the appendix and subscripted section indicator (e.g., Table A_1-1, A_1-2, B_1-1, B_1-2, etc.). Emphasis in each section is placed on (1) the occurrence and concentration of the pathogen in the environment, dose-response relationships, and indicator organism-pathogen relationships; and (2) the presentation of complex (and sometimes contradictory) evidence as clearly and concisely as possible. The source of information for the appendices is the open literature.

Appendix D summarizes the uncertainties encountered in our research and identifies potential areas for further study.

APPENDIX A: BACTERIAL ORGANISMS

SECTION 1. Shigella

ETIOLOGY AND CLINICAL DISEASE

The genus Shigella is made up of Gram-negative, facultatively anaerobic, nonmotile rods. Four species exist and are all pathogenic in humans and other primates. 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). Most other animals are resistant to Shigella infection and disease.[1]

Shigellosis, an acute bacterial disease involving the large intestine, is characterized by diarrhea and is accompanied by fever, nausea, vomiting (sometimes), cramps, and tenesmus. In severe cases, stools may contain blood, mucus, and pus; and both mild and asymptomatic infections occur.[2] Shigellosis differs from salmonellosis in that Shigella organisms rarely invade beyond the mucosal epithelial cells or submucosa lining of the intestine.[3] In severe cases of dysentery, mucosal destruction and ulceration occur but do not extend beyond the intestinal tract.[3] Shigella dysenteriae type 1 produces a heat-labile exo-enterotoxin that affects both the gut and central nervous system.[4,5] The severity of illness and the fatality rate are functions of age, nutrition, and dose of organism. For S. dysenteriae (Shiga bacillus) infection, 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 h to several months.[6-8] Shedding, however, may continue up to several months in asymptomatic carriers. Treatment with ampicillin and tetracycline shortens the clinical disease and shedding stage of Shigella.[9]

OCCURRENCE

Shigellosis occurs worldwide, primarily in children under 10 y old. Outbreaks of this serious disease are common under conditions of crowding and poor sanitation, such as in jails, institutions for children, mental hospitals, crowded camps, and ships. It is endemic in both tropical and temperate climates, and its habitat is the gut of humans and other primates.[2] All waterborne outbreaks have been associated with fresh waters rather than marine waters.

This literature search revealed that, until 1979, Shigella spp. had been the most common bacterial pathogens isolated from waterborne outbreaks in the United States, with rates of 4.1% in 1975 and 5% in 1979.[10-12] In a survey[10] conducted in the U.S. between 1964 and 1973, water was found to be the common source for 14% of all shigellosis outbreaks. Shigella sonnei has been the most common Shigella spp. isolated in waterborne shigellosis.[13-16] Also, the most common U.S. water source of shigellosis has been well water and/or semipublic water systems.[17-19] Mixed infections have been reported to occur in up to 39% of shigellosis patients.[20,21] In developed countries, S. boydii is the most common species, whereas in areas of poor hygiene, S. dysenteriae is predominant.[22] For a summary of attack rates, sources, carrier rates, and secondary attack rates, refer to Table A_1-1.

RESERVOIR

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

MODE OF TRANSMISSION

Transmission is by direct or indirect fecal-oral contact from a patient or carrier. Water, milk, and flyborne transmission may occur as the result of direct fecal contamination.[2]

SUSCEPTIBILITY AND RESISTANCE

Exposed populations are generally susceptible to infection following ingestion of a small number of organisms; 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]

ENVIRONMENTAL PERSISTENCE

The literature search revealed one in-depth experiment describing Shigella survival under environmental conditions. This work by Wang et al.[30] showed that Shigella survived: (1) longer when fecal coliform (FC) numbers were high; (2) poorly when total plate counts were high ($\geq 10^6$ heterotrophic organisms/mL); and (3) longer at lower temperatures

Table A_1-1. Occurrence of Shigella spp.

Shigella spp.	Location	AR[a]	Source	Ref.
S. sonnei	United States	437	Well water	23
	United States	720-920	Well water	14
	Brazil	320	Tap water	21
	United States	185	Tap water	16
	United States	180	River water	16
	Thailand	30	TD[b]	24
S. dysenteriae	Guatemala	60	Water	6
	Bangladesh	120-280	Well water	7
	Bay of Bengal	330	Well water	8
	Thailand	30	TD[b]	24
S. flexneri	Caribbean	350-900	Cruise ship	25
	Thailand	111	TD[b]	24
S. boydii	Thailand	60	TD[b]	24
Shigella spp.	Canada	703	Lake water	26
	United States	681	Lake water	16
	United States	90	U.S.-wide[c]	27

Shigella spp.	Location	CR[d]	SAR[e]	Ref.
S. sonnei	United States	--	90	14
	Great Britain	6	120	28
S. dysenteriae	Guatemala	--	337	6
	Bangladesh	--	204	7
Shigella spp.	United States	--	750	10
	Panama	28	--	29

[a] Attack rate (per 1000).

[b] Travelers' diarrhea.

[c] Based on reported disease outbreaks in the U.S.

[d] Carrier rate (per 1000).

[e] Secondary attack rate (per 1000).

(i.e., 15 to 17°C vs 20°C). Shigella survived 22 d in well water; however, die-off reportedly began within 1 h.[29] Therefore, it can be said that Shigella under ambient conditions (15° to 20°C) will persist longest in fecally polluted fresh water. At lower temperatures (0°C) Shigella has been shown to survive for 47 d in a frozen river in Siberia and 135 d in associated soil. It is thought that the permafrost may maintain a reservoir of Shigella around Siberian settlements.[31]

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 d at 13°C, but only 4 d at 37°C. In seawater, Shigella survival is strain-dependent, persisting from 15 to 70 d.[31] Shigella flexneri strain 6 was found to grow in stored water contaminated with seawater on a cruise ship.[25] Further evidence of survival in seawater was given by Mitchell, showing that Shigella die-off in seawater is not very rapid; about 90%/d.[32]

In fresh water, Shigella displays the same basic survival pattern as most other enteric organisms, except that die-off occurs more steadily, and the organisms completely disappear within 14 d.[33] When compared with other enteric bacterial pathogens, Shigella was second only to Aeromonas in persistence.[34]

DOSE RESPONSE

The dose-response data presented in Table A_1-2 are based on human-volunteer feeding studies with Shigella dysenteriae type 1, strains pandemic M131 and endemic A-1 and S. flexneri 2A# and 2A##. Secondary attack rates are quite high, especially under crowded conditions.[10,35] A waterborne Shigella epidemic in an Iowa school and adjacent buildings resulted in a secondary attack rate of 9%.[14]

LATENCY

Latency data for Shigella spp. are presented in Table A_1-3. Latency of shigellosis typically ranges from 1 to 7 d,[2] and the disease symptomology is usually rapid once the organisms have become established in the gut.

DISINFECTANTS

Most common disinfectants have been shown to be very effective against Shigella. The information in Table A_1-4 has been based mostly on in situ studies on S. dysenteriae.

Table A_1-2. Dose response for Shigella spp.

Dose	AR[a]	Shigella spp.	Ref.
10	100	S. dysenteriae[b]	36
10^2	500	S. dysenteriae[b]	36
1.8×10^2	180	S. flexneri 2A#	22
2×10^2	250	S. dysenteriae[c]	36
2×10^3	700	S. dysenteriae[b]	36
5×10^3	670	S. flexneri 2A#	22
10^4	330	S. dysenteriae[c]	36
10^4	830	S. dysenteriae[b]	36
10^4	760	S. flexneri 2A#	22
10^4	250	S. flexneri 2A##	22
10^5	440	S. flexneri 2A#	22
10^5	750	S. flexneri 2A##	22
10^6	860	S. flexneri 2A##	22
10^7	680	S. flexneri 2A##	22
10^8	750	S. flexneri 2A##	22

[a] Attack rate (per 1000).
[b] Strain - pandemic M131.
[c] Strain - endemic A1.

Table A_1-3. Latency of Shigella dysenteriae.[a]

Dose	Latency (d)	S. dysenteriae strain
10	4.0	Pandemic M131
10^2	3.0	Pandemic M131
2×10^2	6.0	Endemic A1
2×10^3	6.1	Pandemic M131
10^4	5.2	Pandemic M131
10^4	3.0	Endemic A1

[a] Ref. 36.

Table A_1-4. Effect of disinfectants on Shigella spp.

Disinfectant	Dose (ppm)	Contact time (h)	% kill	pH	Temp (°C)	Ref.
Chloramine	1.2	0.33	100.00	7.0	20–25	37
NaOCl	0.05	0.08	100.00	7.0	20–25	33
Javex	—	3.00	100.00*	—	—	26
NaOCl	0.1	0.25	100.00	—	—	25
NaOCl	1.3	—	100.00	—	-	38
NaOCl	3.0	—	100.00	—	-	38
Halazone	—	—	100.00*	—	-	8

* Based on end of outbreak upon administration of disinfectant.

However, in those studies where disinfectants were added in response to an outbreak, the species type was not given.

MONITORING METHODS

Methodology for the detection of Shigella is qualitative and low in sensitivity.[39] This is due in part to the biochemical instability of Shigella characteristics in the water environment, and also from the antagonistic growth effects of coliform bacteria and Proteus vulgaris.[39]

Concentration techniques are those that have been outlined for Salmonella in Section 912A of Ref. 39. Enrichment for Shigella must be done with a selective enrichment medium in order to minimize accumulation of volatile acid by-products from antagonistic bacteria. These media are described in Section 912D.2. of Ref. 39 and feature pH, temperature, and negative-enrichment inhibitory techniques. The selective isolation medium of choice is xylose lysine desoxycholate (XLD) agar, where Shigella colonies are red and non-Shigella colonies are yellow. Further biochemical identification can be carried out on biochemical properties and characteristics of isolated colonies. Serological identification may also be employed using the slide agglutination techniques (Ref. 39, Sec. 912A.4.) with Shigella antisera.

INDICATOR-PATHOGEN RELATIONSHIP

At present, the state of enumeration techniques for Shigella spp. present in water samples are inadequate; thus, a direct comparison with coliform numbers is not available. The only data available are qualitative. McFeters et al.[34] have shown that Shigella survived longer than coliforms in well water at 9 to 13°C. This latter information may raise some doubt about the validity of coliforms as indicators of the presence of Shigella[38] but it should be remembered that it is only one study. Table A_1-5 below contains some information on the presence of Shigella spp. and the number of coliforms.

CONCENTRATION IN THE ENVIRONMENT

As stated previously, there are no adequate techniques for accurate enumeration of Shigella concentration in the aquatic environment; thus, no data are available. The number of Shigella that might be present in a receiving water can be estimated from the number of Shigella found in stool (i.e., approximately 10^6 organisms/g of stool).[30]

Table A_1-5. Indicator-pathogen relationship with Shigella spp.

Shigella spp.[a]	Indicator (coliform/100 mL)	Source	Ref.
S. sonnei	125	Well water	14
S. sonnei	33	Tap water	21
S. sonnei	8	Cl tap water	21
S. dysenteriae	>50	Well water	38
S. dysenteriae	130-900	Well water	8
S. flexneri	49-170	Cruise ship	25
Shigella spp.	36-1260	Lake	26
Shigella spp.	4×10^5	Effluent outfall	40
Shigella spp.	5×10^6	8 km downstream	40
Shigella spp.	17500	River water	16

[a] Reported as present (not quantitative).

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SECTION 2. Campylobacter

ETIOLOGY AND CLINICAL DISEASE

Campylobacter jejuni (C. fetus subsp. jejuni) is a slender, spirally curved rod that is microaerophilic and motile.[1] This organism was originally thought to be a veterinary pathogen only, but now it is recognized as a major cause of enteritis in humans.[2]

C. jejuni enteritis is characterized by acute diarrhea, abdominal pain, malaise, fever, nausea, vomiting, and constitutional complaints. Grees or occult blood in association with white cells is present in the liquid, foul-smelling stools.[3] A typical syndrome of this type rarely involves febrile convulsions and meningitis. The illness is frequently self-limiting within 1 to 4 d, lasting no more than 10 d.[3] Diagnosis of the disease is based on culture of the organisms from stools using special media and incubation conditions.[4] Campylobacter has also been identified as a cause of secondary effects of infection, such as arthritis or carditis.[5]

OCCURRENCE

These microorganisms are found worldwide and occur in all age groups; in developed countries mostly in adults (>15 y old) and in developing countries mostly in children (<5 y old).[2] C. jejuni has been identified in 3 to 10% of diarrhea cases in developed countries.[6] Campylobacter enteritis usually occurs during summer and fall rather than in winter and spring.[1] This may be due in part to the relatively high incidence of Campylobacter enteritis in travelers' diarrhea (Table A_2-1). A survey of diarrheal patients in Bangladesh revealed that mixed infections with other bacterial, viral, or protozoal pathogens occurred more frequently (50%) in patients with C. jejuni. Most cases of Campylobacter have been identified in Northern European countries, probably reflecting special interests and associated expertise. Data on attack rates associated with sources and percent carrier rate and secondary attack rates for Campylobacter spp. are presented in Table A_2-1.

RESERVOIR

A wide variety of domestic animals, including poultry, swine, beef, sheep, cats, dogs, and other pets, as well as wild animals and birds, are known to excrete C. fetus jejuni in their feces. These animals are the major reservoir of infection.[3]

Table A_2-1. Occurrence of Campylobacter spp.

Location	AR[a]	Source	CR[b]	SAR[c]	Ref.
England	480-580	Well water			7
England	71	Diarrheal patients[d]			8
England	140	Diarrheal patients[d]	60	17	9
England	178	Diarrheal patients[d]			10
United States	200	Well water			11
United States	51	Diarrheal patients[d]	370		12
Thailand	30	U.S. tourists[e]			13
United States	--	Nationwide[f]		670	14
United States	--	Nationwide[f]	1.2	660	15
Sweden	90-167	Well water			16
Sweden	69	Diarrheal patients[d]			17
Norway	26	Diarrheal patients[g]			18
Mexico	111	Panamanian tourists[h]			19
Bangladesh	140	Diarrheal patients[d]			6
Worldwide	100	Diarrheal patients[d]			20
Worldwide	30-140	Diarrheal patients[d]			2

[a] Attack rate (per 1000).
[b] Carrier rate (per 1000).
[c] Secondary attack rate (per 1000).
[d] Occurrence of Campylobacter isolation from diarrhea patients.
[e] Incidence of Campylobacter diarrhea in U.S. travelers to Thailand.
[f] Secondary attack rate average in Campylobacter outbreaks in U.S.
[g] Highest rate found in adults 20 to 29 y old; >50% imported cases.
[h] Panamanian travelers to Mexico.

MODE OF TRANSMISSION

Transmission is presumed to be by ingestion of the organisms contained in contaminated water or food and via direct contact with infected humans and animals.[3]

SUSCEPTIBILITY AND RESISTANCE

There is universal susceptibility when a sufficient number of organisms is encountered. The elderly and the very young are the most susceptible. Immune mechanisms for Campylobacter spp. are not well understood.[3]

ENVIRONMENTAL PERSISTENCE

There was only one study found that described the environmental persistence of C. jejuni.[21] Blaser et al. subjected C. jejuni to a variety of environmental conditions. Of those environments tested, hydrochloric acid and stream water are of the most interest.

Campylobacter jejuni persisted in hydrochloric acid for >30 min at pH 2.5 and 37°C (6-log reduction), but was completely eliminated in 5 min at pH 2.3 (>7-log reduction) and within 20 min at pH 2.4. Therefore, it would appear that any rise in stomach pH or achlorhydria would predispose the host to introduction of C. jejuni into the gut. Also, this implies that a large inoculum of C. jejuni is required for infection due to the ability of a normochlorhydric stomach to reduce the number of this organism by four orders of magnitude in 1 min at pH $\leq$ 2.4.

In autoclaved Colorado surface water, C. jejuni survival was shown to be temperature-dependent.[21] Organisms incubated at 25°C survived no more than 4 d, whereas those incubated at 4°C survived for more than 4 wk. Therefore, it is conceivable that in contaminated cold waters, small numbers of C. jejuni are capable of initiating an infection due to rapid wash-through of water to the gut.[9]

DOSE RESPONSE

Only two reports[22,23] on infective dose of C. jejuni in humans were found in the literature review and involved only two individuals (see Tables A_2-2 and A_2-3). Milk was the delivery vehicle in both studies, and because it tends to reduce acid in the stomach, the infective dose was probably lower than would be the case with water as the vehicle.[22] The symptomology for Campylobacter enteritis at these doses persisted for 7 d.

Table A_2-2. Dose response and latency for Campylobacter jejuni.

Dose	Attack rate[a]	Latency (d)	Ref.
500	1000 (1/1)	4	22
10^6	1000 (1/1)	1-3	23

[a] Attack rate (per 1000).

Table A_2-3. Hypochlorite effect on Campylobacter.

Number of organisms tested	Concentration of Cl^- in ppm/negative growth[a] during exposure time (min)				
	1	15	30	240	>240
10^6-10^7	-	5	2.5	1.25	0.312
10^3-10^4	1.25	-	0.625	0.156	0.078

[a] Minutes of exposure to achieve total removal.

LATENCY

The latency associated with a known dose is presented in Table A_2-2. The epidemiological literature, however, indicates incubation periods ranging from 3 h to 10 d, with the average at 3 to 5 d.[2,15,19,20,24]

MONITORING METHODS

The physiological requirements for the growth of C. jejuni in the laboratory have made it difficult to isolate from the environment. A special negative enrichment medium, incubated at 42-43°C in a microaerophilic atmosphere, is employed in order to discourage the growth of competitors.[8] Two methods of isolation are outlined.[4] These procedures are not adequate for the isolation of Campylobacter from water sources, partly because of the filter techniques described for isolation from feces, the media employed, and the low

concentration of Campylobacter in most water sources. Mathewson et al.[19] have described a technique for increased recovery of Campylobacter from water sources with Zeta Plus, Zetapor filters (68-89% recovery efficiency). Bopp et al.[25] have shown that use of a 0.45-μm Millipore filter or Zetapor filter with an enrichment broth gives the best recovery of C. jejuni from surface waters. Work is now being conducted to develop a more efficient selective medium for the isolation of Campylobacter from the environment.

DISINFECTANTS

Only one study on the effects of disinfectants on Campylobacter was found in this search.[26] Many of the disinfectants studied are topical disinfectants that are employed in cleaning surfaces, such as those that may be used in day-care centers. Of the disinfectants listed, the data for removal by hypochlorite are those of importance to water treatment. The effect of hypochlorite on low concentrations of Campylobacter probably best reflects the disinfection of naturally occurring numbers (Table A_2-3).

INDICATOR-PATHOGEN RELATIONSHIP

For most Campylobacter outbreaks, no qualitative data exist on the bacteriological conditions of the suspected source. Mentzing[16] reported 0-50 coliforms/L from a waterborne outbreak in Sweden. More data are required before any relationship can be drawn between the number of coliforms and Campylobacter in water.

CONCENTRATION IN THE ENVIRONMENT

No information was found in the current literature concerning the numbers of Campylobacter occurring naturally in the environment. This lack probably results from a lack of quantitative isolation techniques.

Table A_2-4 represents negative growth after 15 min of contact time of the stated concentration of disinfectant, with an initial Campylobacter dose of 10^6-10^7 organisms.

Table A_2-4. Disinfectant effect on Campylobacter.

Disinfectant	Concentration[a]
Phenol	0.078%
Iodophor	10 ppm
Quaternary NH_3	100 ppm
Ethyl alcohol	70.0%
Formalin	2.5%
Gluteraldehyde	0.0156%
Hypochlorite	5.0 ppm

[a] Negative growth after 15 min contact (initial dose 10^6 to 10^7 organisms)

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SECTION 3. Vibrio cholerae

ETIOLOGY AND CLINICAL DISEASE

Vibrio cholerae is a short, slightly curved, Gram-negative rod responsible for the disease known as cholera. There are several strains of V. cholerae that are pathogenic to man. Vibrio cholerae O-group 1 is made up of Classical and El Tor biotypes and either Inaba, Ogawa, or the rare Hikojima, serotypes.[1] All of these organisms produce an enterotoxin. Another group includes the non-O1 V. cholerae vibrios (nonagglutinable or NAGs), most of which appear to be nontoxigenic but can display a spectrum of enteropathies, including the production of cholera toxin.[1,2] Recently, O'Brien et al.[3] have described environmental and human isolates of V. cholerae that produce a Shigella dysenteriae 1 (Shiga)-like cytotoxin. The Classical and El Tor biotypes are associated with cholera epidemic or pandemic outbreaks. The non-O1 vibrios are associated more with smaller outbreaks and sporadic cases.[4]

Morris et al.[5] recently isolated a strain of V. cholerae O1 from the Gulf of Mexico, which did not produce cholera toxin, but still caused severe gastroenteritis. It was thought that an atypical extracellular toxin may have been the cause.

Cholera is an acute intestinal disease with sudden onset, profuse water in stools, occasional vomiting, rapid dehydration, acidosis, and circulatory collapse. Death may occur within a few hours; the fatality rate in severe, untreated cases is >50%; however, with proper treatment the rate is <1%.[4] 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.[6]

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.[7] 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 10^9 V. cholerae organisms/mL.[7] Cholera can also be foodborne in association with shellfish and marine products.[4]

Cholera symptoms in severe cases may last for very short periods: El Tor, 5 d with nontreatment and 3.2 d with treatment; Classical, 0.7 d with treatment. 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 to deal with cholera diarrhea (i.e., WHO diarrhea treatment solution or Dacca solution).[4]

OCCURRENCE

The distribution and number of V. cholerae are associated with water salinity, and they appear to be autochthonous to estuarine and marine environments.[8-11]

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. More recently, cholera has been reported throughout the Mediterrean area (North Africa, Portugal, and Italy).[4,12] Also, several outbreaks have been reported from the South Pacific in the Gilbert Islands[13] and on Nauru.[14]

In the United States, the first report of cholera in over 60 y occurred in Texas in 1973.[4] Since that time, there have been outbreaks and sporadic occurrences in the U.S., Canada, and Australia.[4,11,15-19] There have been numerous isolated cases of V. cholerae O1 and non-O1 from the three coastal areas of the U.S.; Chesapeake Bay,[15,16] Gulf states,[15,17,18] and California.[11]

Table A_3-1 presents some data on the occurrence of V. cholerae. Secondary-attack rates have been reported in ranges of 42 to 407/1000 individuals exposed, and carrier rates of 7 to 130/1000 with cholerae. In a 1982 review of worldwide cholera distribution, summarized by the United States Centers for Disease Control, there were 33 countries that reported incidences of cholera: 14 in Africa; 17 in Asia; and two in Oceania. Also, there were eight imported cases in Europe and the U.S.[19]

Until 1973, V. cholerae Classical was the predominant biotype in India and Bangladesh.[20,21] However, following an "unexplainable 15-month lapse in cholera cases" in 1973, the El Tor biotype appeared in many outbreaks.[21] Most recently, surveys have shown that the Classical biotype is rapidly replacing the El Tor biotype in epidemics in Bangladesh.[20] No explanation has been found for this cycle of biotype change.

RESERVOIR

In the past, man has been considered to be the single reservoir of Classical and El Tor cholera, but evidence is accumulating that other environmental reservoirs may exist.[4,11,15-18] Sanyal et al.[29] have shown that 0.6% of household animals studied in India were carriers of V. cholerae serotype 1 and that 3.6% were positive for non-O1 vibrios. Cholera vibrios have also been isolated from estuarine, marine, and brackish waters that did not appear to be polluted.[11,15-18]

Table A_3-1. Occurrence of Vibrio cholerae.

Location	Biotype	Water source	AR[a]	SAR[b]	CR[c]	Ref.
Bangladesh	Classical	Brackish	1.3			21
Bangladesh	El Tor	Brackish	2.9			21
Bangladesh	Classical	Fresh	190-250	42-164	7	22
Bangladesh	El Tor	Fresh	80-206	407	5nt,3t[d]	22
Pakistan	—	Fresh	5-20	100-260		23
Pakistan	—	Fresh	1-4	—	—	23
Portugal	El Tor	Spring	2.6			12
Philippines	—	Well	134			24
Bangladesh	El Tor	Surface	111			25
India	—	Well	—	—	100	26
Gilbert Islands	El Tor	Drinking	20-210	—	—	13
Nigeria	non-O1	Well	5	—	—	27
United States	—	—	—	—	130	28

[a] Attack rate (per 1000).

[b] Secondary attack rate (per 1000).

[c] Carrier rate (per 1000).

[d] nt = no treatment; t = treatment.

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, or by food contaminated with filthy water, feces, soiled hands, or flies. Person-to-person spread by direct contact is thought to be of minor importance.[4] Also, seafood (especially shellfish) has been found to be a major carrier of V. cholerae.[4,30] Deb et al.[26] studied intrafamilial transmission of V. cholerae, and found that 10% of family contacts were asymptomatic carriers, and of these, 6% had viable V. cholerae on their fingers. More recently, Hughes et al.[31] described the importance of surface water and V. cholerae transmission. It was found that there was increased risk to those families that utilized water containing V. cholerae for cooking, bathing, or washing. However, increased risk was not found for families drinking surface water containing V. cholerae.[31] These data

are in contrast to the common notion of cholera transmission, which is believed to be by ingestion of contaminated water. However, this probably reflects the role of cross-contamination from skin, clothes, and utensils to foods and the host.

SUSCEPTIBILITY AND RESISTANCE

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 rise in agglutinating, vibriocidal, and antitoxic antibodies against homologous types.[4] Persons in endemic areas acquire antibodies by early adulthood.[4] Vaccines are available, but protection lasts six months at most and does not prevent asymptomatic infection.[4]

ENVIRONMENTAL PERSISTENCE

Vibrio cholerae appear to be autochthonous to estuarine and marine environments.[11,15-18] There have also been numerous isolations from surface, well, and tap water (see Table A_3-2). There have been many studies concerning the survival of V. cholerae, and some of these data are presented in Table A_3-2. 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); (4) best survival and growth appears to be in marine environments during the summer months.

DOSE RESPONSE

Table A_3-3 summarizes the dose-response data for V. cholerae in 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.[22] However, the data in Table A_3-3 show that, in volunteer feeding studies, the rates for Classical and El Tor are very similar. Also, Khan and Shahidullah[22] recently surveyed cholera in Dacca, and found that the severity and attack rates of cholera due to El Tor biotype were equal to those of the Classical biotype.

Table A_3-2. Environmental persistence of Vibrio cholerae.

Location	Source	Salinity (ppt)	Temp (°C)	Survival (d)	Ref.
U.S.	Brackish	25	20-25	Summer months	8
U.S.	Brackish	5	10	4	8
U.S.	Brackish	25	10	25-42[a]	8
U.S.	Brackish	< 3-31.7		Year-round	11
U.S.	Marine	32		In crabs	30
U.S.	Unknown			Several years	18
U.S.	Marine-brackish	≤ 32	Warm	3 mo[b]	17
U.S.	Marine-brackish	6-12		Year-round	16
U.S.	Marine-brackish		9-12	Die-off: 10^2/2 d	30
England	Estuaries	0.5-3.0		Prolonged	32
England	Surface water			3 mo[b]	33
Pakistan	Surface water		Warm	3 mo[b]	23
India	Well water		21	12-51	33
India	Well water		37	1-4	33
Bangladesh	Surface water			7-13	34
Bangladesh	Marine			10	35
Worldwide	Sewage			10	10
Worldwide	Sweaty clothes			7	10
Worldwide	Sewage (USSR)			400	10
(Classical)	Well water			3	10
(Classical)	Surface water			0.75	10
(Classical)	Marine			4	10
(Classical)	Tap water			0.91	10
(Classical)	Sewage			0.5	10
(El Tor)	Well water			5	10
(El Tor)	Surface water			2.2	10
(El Tor)	Marine			2.3	10
(El Tor)	Tap water			2.0	10
(El Tor)	Sewage			2.75	10
Worldwide	Harbor			81	36

Table A_3-2. (Continued)

Location	Source	Salinity (ppt)	Temp (°C)	Survival (d)	Ref.
Worldwide	Marine			64	36
Worldwide	Surface			<32	36
Worldwide	Marine			10-47	36

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

LATENCY

The data collected on latency of V. cholerae indicate that, dependent upon dose, the incubation time for V. cholerae diarrhea ranges from a few hours to 5 d. Usually the incubation time is 2 to 3 d.[4,28,37] See Table A_3-4.

DISINFECTANTS

There have not been many recent studies on the effect of disinfectants on V. cholerae. The data in Table A_3-5 are limited and do not adequately describe the effects of disinfectants on V. cholerae. Referring to Table A_3-2, it appears that V. cholerae does not survive well in fresh water; therefore, unless there has been recent contamination, V. cholerae may not be a major concern. The bacteria survive longer in brackish waters, but these sources are less likely to be utilized for drinking. However, brackish water may present a threat if flooding occurs and it mixes with freshwater sources. Also, if V. cholerae-shedding individuals are present in a community, the threat of cholera contamination of public water sources remains.

MONITORING METHODS

In environmental samples, Vibrio cholerae is found mostly in seawater and estuarine environments and/or alkaline environments (pH 7-9) and in polluted waters of endemic cholera areas.[39] The concentration techniques for V. cholerae are similar to those described for Salmonella in Sec. 912A.1. of Ref. 39.

Table A_3-3. Dose response for Vibrio cholerae.

Biotype or serotype of Vibrio cholerae	Attack rate[a]	Dose	Ref.
O1	800-1000	10^6	2
O1 Classical Inaba	260	10^3-10^4	28
O1 Classical Inaba	0.0	10^4	28
O1 Classical Inaba	0.0	10^6	28
O1 Classical Inaba	830 (10/12)	10^6	5
O1 Classical Inaba	0.0	10^7	28
O1 Classical Inaba	500 (2/4)	10^8	28
O1 Classical Inaba	500 (1/2)	10^9	28
O1 Classical Inaba	0.0	10^{10}	28
O1 Classical Inaba	500 (1/2)	10^{11}	28
O1 Classical Ogawa	670 (4/6)	10^5	5
O1 Classical Ogawa	960 (22/23)	10^6	5
El Tor Inaba	600 (6/10)	10^5	5
El Tor Inaba	1000 (10/10)	10^6	5
El Tor Ogawa	600 (3/5)	10^5	5
El Tor	111 (31/274)	$\geq 10^4$	25
Classical	890 (24/27)	10^6	37
El Tor	860 (32/37)	10^6	37

[a] Attack rate (per 1000); ( ) = number of individuals with cholera per total tested.

Table A_3-4. Latency of Vibrio cholerae.

Dose	Latency (h)	Ref.
10^6-10^8	$\leq$ 24	28
10^3-10^4	48	28

Table A_3-5. Effects of disinfectants on Vibrio cholerae.

Disinfectant	Dose (ppm)	Contact time (h)	Percent kill	Ref.
Hypochlorite	2[a]	—	100.0	10
Chloramine-T	16-160[b]	1-7	0.0	38
$KMnO_4$	—	—	—[c]	24

[a] Free chlorine.

[b] Literature is not clear on whether this is free chlorine or a concentration of chloramine-T.

[c] Addition of $KMnO_4$ to contaminated wells brought an end to an outbreak.

Enrichment for V. cholerae is described in Sec. 912G.2. of Ref. 39. For example, in order to inhibit the growth of competitive, antagonistic organisms, an alkaline (pH 9.0) peptone water medium is suggested.[39] To achieve selective growth for primary isolation, thiosulfate-citrate-bile-salt-sucrose (TCBS) agar is the medium of choice. V. cholerae appear as yellow colonies on this medium. Since there are other interfering organisms that produce yellow colonies, biochemical tests are necessary to positively identify V. cholerae. These tests are outlined in Sec. 912G.4. of Ref. 39.

Serological identification is possible with the slide agglutination technique using appropriate V. cholerae antisera, which can be produced in the laboratory or acquired commercially.[39] In order to distinguish between Classical, El Tor, and NAG vibrio biotypes, techniques are called for that are usually carried out in specialized laboratories.[39]

INDICATOR-PATHOGEN RELATIONSHIP

The literature shows that standard indicator coliforms are probably not good indicators for V. cholerae (see Table A_3-6).[15,16,27] In the case of contaminated freshwater sources, V. cholerae will survive less than coliforms; therefore, in this instance, standard indicators may be useful.[10,27] However, in the estuarine and marine environments, the factors that increase V. cholerae survival are antagonistic to coliforms.[15] Coliform presence in this case probably reflects recent contamination.

Table A_3-6. Indicator-pathogen relationship for Vibrio cholerae.

Vibrio cholerae[a]	TC[b]	Environment	Location	Ref.
(+)	2400	Chesapeake Bay	U.S.	16
(+)	100-2000	Chesapeake Bay	U.S.	16
(+)	0.5	Chesapeake Bay	U.S.	16
10^{3-4}/100 mL	10^{6-8}/100 mL	Sewage influent	Israel	10
0-2/100 mL	10^{4-7}/100 mL	Sewage effluent	Israel	10
(+)	(-)	Chesapeake Bay and Gulf states	U.S.	15
(+)	10^{2-4}/100 mL	Well water	Nigeria	27

[a] Number or presence (+) of V. cholerae.

[b] Total coliforms or their absence (-).

CONCENTRATION IN THE ENVIRONMENT

Only recently, concern for V. cholerae in the environment has stimulated studies of environmental concentration.[10,11,15,40] Vibrio cholerae has now appeared worldwide in the environment, but determining the actual numerical concentrations of this organism has been a problem due to the limitation of isolation and enumeration techniques. Table A_3-7 reflects the lack of quantitative information in the literature.

Table A_3-7. Environmental concentration of Vibrio cholerae.

Vibrio cholerae[a]	Environment	Location	Ref.
(+) samples	Marine	Gulf states	40
(+) samples	Marine	California coast	11
24% (+) samples	Marine	Portugal	41
45% (+) samples	Fresh	Portugal	41
75% (+) samples	River	Bangladesh	31
63% (+) samples	Canals	Bangladesh	31
21% (+) samples	Ditches	Bangladesh	31
33% (+) samples	Tubewell	Bangladesh	31
$10-10^4$/100 mL	Sewage	Jerusalem	10
160-2600/100 mL	Sewage	Bangladesh	10
(+) samples	Estuary	Chesapeake Bay	15
10^9 cfu/mL[b]	Rice-water stools	---	38
5-100 cfu/mL[b]	River	Bangladesh	25
15% (+) samples	Well	India	26
9% (+) samples	Tubewell	India	26
9% (+) samples	Tap water	India	26
3% (+) samples	Pond	India	26

[a] Expressed as number or as percent of samples found with V. cholerae; (+) indicates presence.

[b] cfu/mL = colony-forming units per mL.

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SECTION 4. Pathogenic Escherichia coli

ETIOLOGY AND CLINICAL DISEASE

Escherichia coli is a facultative anaerobic, nonspore-forming, Gram-negative rod and a member of the Enterobacteriaceae.[1] Escherichia coli is a common gut organism in man and mammals; however, some strains have been found to be pathogenic to man. The severity and type of pathogenicity is strain-related, and at present three basic Escherichia enteropathies are recognized: (1) enteropathogenic E. coli (EPEC), (2) enterotoxigenic E. coli (ETEC), and (3) enteroinvasive E. coli (EIEC).[1,2] There are now six different mechanisms of pathogenicity recognized in E. coli diarrhea: 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 V. 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] Symptoms for ETEC may last for 1 to 3 d, whereas EPEC and EIEC symptomology may last for up to 20 d.[9,10]

Specific diagnosis requires isolation of suspect E. coli from infected stools and demonstration of pathogenic activity by animal or cell-culture bioassay, or by immunological methods for certain of the associated enterotoxins. As stated above, there are some common E. coli serotypes associated with enteropathogenic strains; however, it should be pointed out that not all E. coli of these serotypes are pathogenic (i.e., this is not an absolute test for pathogenicity).[2] The most important treatment for E. coli diarrhea is with electrolyte fluid therapy (oral or IV). Antibiotics should be administered only when specifically indicated because antibiotic resistance is found in up to 43% of environmental isolates.[11]

OCCURRENCE

Diarrhea caused by E. coli is found worldwide and the organism is most frequently food- or waterborne. In areas with poor sanitation, endemic diarrhea is frequently due to E. coli.[2] Pathogenic E. coli is the most common cause of "travelers' diarrhea"

(TD) in worldwide travelers.[12-17] Carrier rates have been measured from 13 to 820/1000 population in various areas worldwide (see Table A_4-1). There have been conflicting reports on the incidence of secondary infection rate. Levine et al.[21] showed that none of the uninfected volunteers housed with E. coli-infected volunteers developed diarrhea. However, Cabelli et al.[19] monitored swimmers with diarrhea and found a secondary attack rate of 20/1000 within the study families.

RESERVOIR

The reservoir for E. coli is infected persons, often in asymptomatic cases.[2]

MODE OF TRANSMISSION

Fecal contamination occurs via food, water, or fomites. Persons with diarrhea excrete large numbers of organisms and constitute the greatest hazard. Contaminated hands of uninfected personnel may transmit organisms to other persons. Poor handwashing after patient contact, inadequate personal toilet hygiene of carriers, and poor environmental sanitation contribute to the spread of the disease.[2]

SUSCEPTIBILITY AND RESISTANCE

Infants are the most susceptible to EPEC strains. Immunity to enterotoxin and surface antigens of the bacteria has been demonstrated, but its duration is not known. Local secretory immunity is probably the most important defense mechanism.[2]

ENVIRONMENTAL PERSISTENCE

Escherichia coli survival in the environment has been studied extensively because it is a major component of the coliform-group indicators. Temperature, pH, and organic load are all factors influencing E. coli survival in fresh water. Studies conducted in Canada[27] showed that E. coli survival is prolonged in river water from urban centers due to higher organic loads than those from runoff or in water of pristine quality. Higher temperatures not only contribute to prolonged survival in surface waters, but also to increased bacterial growth rates.[27,28] Under these conditions, in fresh and estuarine waters, 10% of the initial E. coli populations remained after 5 d.[28] On the other hand, Hendricks[29] has shown that in sterile stream water with very low nutrient concentrations

Table A_4-1. Occurrence of pathogenic Escherichia coli.

Location	Source	AR[a]	CR[b]	SAR[c]	MI[d]	Ref.
Worldwide[e]	TD[f]	360-720	150			12
Mexico[g]	TD[f]	700	820			13
Third World[h]	TD[f]	390				18
U.S.[h]	TD[f]	250				17
Thailand[i]	TD[f]	260	260			16
U.S.	Recreation water	42		20		19
U.S.	Recreation water	39				19
U.S.	Well water	380-825				20
U.S.	Person-to-person			0		21
U.S.	DP[j]	250			3.0	11
Bangladesh	DP[j]		560			22
Mexico	DP[j]	300			246	23
Kenya	TD[f]	630				14
Sweden	TD[f]	120				15
Thailand	DP[j]	70			115	24
Thailand	DP[j]	—			16	24
England	DP[j]	18	13			25
Brazil	DP[j]				170	25
Panama	Communities		220			26

[a] Attack rate (per 1000).
[b] Carrier rate (per 1000).
[c] Secondary attack rate (per 1000).
[d] Mixed infection rate (per 1000).
[e] U.S. tourists worldwide.
[f] Travelers' diarrhea.
[g] U.S. tourists in Mexico.
[h] U.S. tourists in Third World Countries.
[i] U.S. tourists in Thailand.
[j] Diarrhea patients.

(<5 μg/mL glucose), E. coli could survive, provided that the temperatures were 5-10°C. In tap water, survival of E. coli is pH-dependent.[30] Sjogren and Gibson[30] have demonstrated that under dilute conditions at pH 7.2, E. coli survival was <24 h, but at pH 5.5, survival was >48 h.

In seawater, E. coli experiences, for the most part, a rapid decline and elimination. Hanes and Fragala[31] clearly showed that as salinity increased in situ, the die-off of E. coli became more rapid. These data are presented in Table A_4-2.

Early studies[32] on E. coli survival in seawater described the normal microbial population of marine bacteria as antagonistic in situ. A 90% die-off for 1.5 d was seen with an overall die-off rate of 10^3 to 10^6 for 6 d when E. coli was grown in the presence of the indigenous bacteriological population. Death rates were greater with elevated temperatures, but survival increased with increased organic loading. Moreover, Enzinger and Cooper[33] have shown that the impact of protozoan bacterial predators can be significant in estuarine water.

DOSE RESPONSE

Table A_4-3 represents the dose-response data for E. coli fed to human volunteers. The effect of buffering the stomach acid barrier on effective dose should be noted. Utilizing the same strain of E. coli with the same dose, the attack rate was six times as high in those with less stomach acid. Not only is the carrier rate less, but the duration of symptomology was half that of the unbuffered stomachs (up to 20 d for unbuffered, 7-10 d for buffered).[9] The organism is listed by strain type; for further details on the strain type, location of isolation, etc., refer to Ref. 9.

LATENCY

The available data for latency of pathogenic E. coli are presented in Table A_4-4. The ranges for latency have been reported to be from a few hours to almost 2 d. Also included in Table A_4-4 is the reported duration of diarrhea in patients infected with pathogenic E. coli.

DISINFECTANTS

There have been several in-depth studies on the effects of disinfectants on E. coli. The data available on the disinfection efficiency of chloramine and hypochlorite are extensive, and inclusion of all of the results is outside the scope of this report. However,

Table A_4-2. Effect of salinity on Escherichia coli.

Water type	Reduction (%)	Time (d)
Seawater	99.99	3
67% Seawater	99.95	5
33% Seawater	99.00	8
BOD[a] dilution water	90.00	8

[a] BOD = biochemical oxygen demand.

review of the data indicates that free chlorine levels of 5-10 ppm will achieve ≥99.999% removal of E. coli.[36-38] The effect of disinfectants on E. coli is dependent upon temperature and pH. Generally, pH values of 6.5 to 7.0 and temperatures of 20 to 25°C are considered to be optimal conditions for disinfection by free chlorine.[36-38]

The study on the effects of chlorine dioxide by Berg et al.[39] reflects the effect of antecedent growth conditions of E. coli on disinfection. Most studies on disinfection utilize stock cultures grown under laboratory conditions; therefore, these data may not reflect the response of the bacteria found in the environment. Berg et al. also found that organisms that were maintained under conditions more closely approximating natural aquatic environments were more resistant than those grown under commonly employed batch-culture conditions. In this study it was found that 13% of the residual chlorine was consumed by the disinfection of bacteria.

MONITORING METHODS

The isolation techniques for E. coli are the same as those for the enumeration of coliforms (Sec. 909A of Ref. 40). Briefly, these methods employ the membrane filter technique with M-FC broth as the growth medium. Colonies of fecal coliforms (primarily E. coli) will appear blue with this medium. In order to confirm the presence of E. coli, biochemical tests, such as the IMViC test, must be performed on each isolate. There are no biochemical tests, per se, available to determine pathogenicity. However, there are commercially available serological tests (i.e., slide agglutination)[2,40] that can be used to identify E. coli serotypes commonly associated with pathogenic strains.

Table A_4-3. Dose response for pathogenic Escherichia coli.

Strain type	Dose	AR[a]	CR[b]	Ref.
B2C (ETEC)	10^8	400(2/5)[c]	1000	9
B2C (ETEC)	10^{10}	600(3/5)	1000	9
B7A (ETEC)	10^8	200(1/5)	800	9
B7A (ETEC)	10^{10}	800(4/5)	1000	9
4608 (EIEC)	10^4	0(0/5)	800	9
4608 (EIEC)	10^6	0(0/5)	0	9
4608 (EIEC)	10^8	625(5/8)	1000	9
1624 (EIEC)	10^4	0(0/5)	0	9
1624 (EIEC)	10^6	111(1/9)	555	9
1624 (EIEC)	10^6	666(2/3)[d]	333	9
1624 (EIEC)	10^8	600(3/5)	1000	9
H10407 (ETEC)	2.7×10^8	560(9/16)		34
055 (ETEC)	1.4×10^8	750(6/8)		10
055 (ETEC)	1.7×10^9	625(5/8)		10
055 (ETEC)	5.0×10^9	750(6/8)		10
055 (ETEC)	1.6×10^{10}	875(7/8)		10
0111 (ETEC)	7.0×10^6	636(7/11)		35
0111 (ETEC)	5.3×10^8	666(8/12)		35
0111 (ETEC)	6.5×10^9	1000(11/11)		35
0111 (ETEC)	9.0×10^9	1000(12/12)		35

[a] Attack rate (per 1000).

[b] Carrier rate (per 1000).

[c] Individuals with diarrhea/total number of persons tested.

[d] Stomach buffered with $NaHCO_3$.

INDICATOR-PATHOGEN RELATIONSHIP

Only recently has it been possible to identify many of the pathogenic strains of E. coli. Unfortunately, there has not been much work on determining the ratio of pathogenic E. coli to nonpathogenic E. coli or coliforms. Table A_4-5 contains some information about the indicator-pathogen relationship to E. coli. Some authors have

Table A_4-4. Latency and duration of pathogenic Escherichia coli.

Strain type	Dose	Latency (d)	Duration (d)	Ref.
B2C (ETEC)	10^8	--	2-3	9
B2C (ETEC)	10^{10}	0.33-1.83	--	9
B7A (ETEC)	10^8	--	2-3	9
B7A (ETEC)	10^{10}	0.33-1.83	--	9
4608 (EIEC)	10^4	0.33-1.00	≤20	9
1624 (EIEC)	10^4	0.33-1.00	≤20	9
1624 (EIEC)	10^8	0.33-1.00	--	9
H10407 (ETEC)	2.7×10^8	0.75	--	34
055 (ETEC)	1.4×10^8	0.40-2.50	1	10
H055 (ETEC)	1.7×10^9	0.16-0.62	1	10
H055 (ETEC)	5.0×10^9	0.2 -0.62	1	10
H055 (ETEC)	1.6×10^{10}	0.20-0.66	1	10
0111 (ETEC)	7.0×10^6	0.50-1.50	--	35
0111 (ETEC)	5.3×10^8	<1.00	--	35
0111 (ETEC)	6.5×10^9	0.50	--	35
0111 (ETEC)	9.0×10^9	0.42	--	35

reported that <1% of the total coliforms are pathogenic E. coli,[28] and that possibly as high as 3-4% of total coliforms may be pathogenic E. coli.[42] Lavoie[43] has suggested that fecal coliform indicators may be more valid in tropical climates, compared with total coliforms, due to the abundance of nonfecal coliforms found in such environments.

CONCENTRATION IN THE ENVIRONMENT

Most enumeration studies count total coliforms or fecal coliforms and usually do not isolate specifically for E. coli, although the FC test is directed toward the isolation of E. coli. The actual percentage of pathogenic E. coli in an environmental population is not known, but as stated previously (see indicator-pathogen section), one author has estimated that <1% of the TC is pathogenic to man.[28] Because the pathogenic traits are plasmid-associated, and passage and maintenance of plasmids are affected by environmental

Table A_4-5. Indicator-pathogen relationship for Escherichia coli.

E. coli[a]	TC[b]	FC[c]	FS[d]	Environment	Location	Ref.
10-900/100 mL			100-8000/100 mL	River	U.S.	44
92%	(+)			Well	West Africa	43
89%		(+)		Well	West Africa	43
1-7%	(+)			Reservoir	Israel	41

[a] E. coli concentration expressed by either number or percent of samples positive.
[b] Total coliforms (TC) present (+).
[c] Fecal coliforms (FC) present (+).
[d] Fecal Streptococci (FS) concentration.

conditions, it is very difficult to estimate this portion of the bacterial population at any given time. The concentration of E. coli in the environment, as revealed by this literature search, is presented in Table A_4-6.

Clarke et al.[45] ranked coliform organisms based on predominance in fresh, mixed, and well water in Canada and found the following:

Fresh water - E. coli > Enterobacter cloacae > A. hydrophila > K. pneumoniae

Mixed water sources - C. freundii > E. coli

Well water - E. cloacae > E. coli

Table A_4-6. Concentration of Escherichia coli in the environment.

E. coli[a]	Source	Location	Ref.
10^4-10^6	Storm sewers	Canada	27
1-10^3	No. Saskatchewan River	Canada	27
2 x 10^9/capita/d	Human feces		34
33-43%	Raw water	Canada	45
10-20%	Drinking water	Canada	45
10^4/mL	Estuary	U.S.	46
10/mL	Marine	U.S.	46
>100/100 mL	Marine	South Africa	47
55%	Well	West Africa	43
70-390/100 mL	Rain runoff	U.S.	48

[a] E. coli concentration expressed by either number or percent of samples positive.

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SECTION 5. Salmonella

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 2000 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 enteric fever, gastroenteritis, 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. (i.e., 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.[2] Ulceration in the ileum can result, producing intestinal hemorrhage or perforation in untreated cases.[2] The fatality rate can reach 10% if symptoms go untreated; however, treatment with antibiotics will lower the 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 (i.e., ampicillin, cotrimoxazole, etc.).[2] 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 y, with some individuals becoming permanent carriers.[2,3] 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] Fecal excretion of Salmonella usually persists for several days or weeks following acute gastroenteritis. Salmonella paratyphi B may persist for 1-20 y.[3]

Antibiotic treatment is symptomatic and can lead to prolonged excretion of Salmonella and/or the development of antibiotic-resistant strains.[4] Treatment of salmonellosis is supportive therapy, i.e., rehydration and electrolyte replacement.[2]

OCCURRENCE

Waterborne outbreaks of Salmonella occur worldwide, and are associated primarily with fresh water. Although the literature is replete with reports on the occurrence of Salmonella typhi and salmonellosis, few of these reports contain information correlating occurrence with attack rates. Table A_5-1 is divided into two parts. In the first part, attack rates for Salmonella spp. are presented in relation to geographic location and water source or type of occurrence. In the second part of Table A_5-1, carrier rate, secondary attack rate, and mixed infection rate for Salmonella spp. are presented in relation to geographic location.

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]

SUSCEPTIBILITY AND RESISTANCE

In the case of typhoid fever, man is the only host, and susceptibility in the population is general; however, susceptibility is increased in individuals with gastric achlorhydria. Immune resistance to typhoid fever follows recovery from clinical disease, from inapparent infection, or active immunization. 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

Table A_5-1. Occurrence of Salmonella spp.

Salmonella spp.	Location	AR[a]	Source/occurrence	Ref.
S. typhi	India	630	TD[b] – English tourists	5
	Mediterranean	130	TD – English tourists	5
	Mideast	51	TD – English tourists	5
	West Africa	65	TD – English tourists	5
	Far East	21	TD – English tourists	5
	U.S.	126–627	Well water	6
	Transatlantic	7–70	Cruise ship	7
	Mexico	490	Soda pop	8
	U.S.	400	Drinking water	9
	U.S.–1976	0.02[c]	Nationwide	10
	U.S.–1961–70	360[d]	Nationwide DP	11
	U.S.–1974	35[d]	Nationwide DP	12
	U.S.	83	Drinking water	13
S. typhimurium	U.S.	100	Drinking water	14
S. paratyphi B	England	540	Stream water	15
S. arechevalata	Trinidad	760	Rainwater runoff	16
S. spp.	Nigeria	9–73	Well water	17
	England	43[e]	DP	18
	U.S.	167	Drinking water	19
	Thailand	30	TD – U.S. tourists	20
	Worldwide	100–150	TD	12
	England	192[e]	DP	21
	U.S.	35	Waterborne outbreak	22
	U.S.–1978	63	Nationwide	23
	England	850	Drinking water	24

Table A_5-1. (Continued)

Salmonella spp.	Location	CR[f]	SAR[g]	MI[h]	Ref.
S. typhi	England		290		18
S. spp.	England	0.0	170		18
S. spp.	England	40	850[i]	170	5

[a] Attack rate per 1000 population.

[b] Travelers diarrhea.

[c] U.S. nationwide attack rate per 1000 total population.

[d] U.S. nationwide attack rate per 1000 total population with diarrhea: diarrhea patients (DP).

[e] Attack rate per 1000 cases of diarrhea.

[f] Carrier rate per 1000.

[g] Secondary attack rate per 1000.

[h] Mixed infection rate per 1000.

[i] Secondary attack rate found in 85% of outbreaks.

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. Severity of the disease is related to the serotype, the number of organisms ingested, and host factors.[2]

ENVIRONMENTAL PERSISTENCE

Persistence of Salmonella in the environment is dependent upon species and environmental conditions. Salmonella follow the same general survival pattern as most enterics in fresh water. The characteristic pattern was described by Beard [25] and later by Andre et al.[26] as (1) rapid decrease of organisms in the first 2 to 3 d; (2) a leveling or lag period of 1 d; (3) regrowth for 2 to 3 d; and (4) death phase lasting more than 10 d. The death phase may or may not result in Salmonella-free water. Salmonella may persist for several (1 to 6) months and be associated with sediments long after they have disappeared from the water column.[17,19,27,28] Salmonella have been isolated from environments with wide ranges of pH (pH 5-8).[8,17,29] Die-off occurs within two weeks at pH 4.5 and just two days at pH 3.5.[8] Seasonality and temperature changes affect Salmonella survival, with a more rapid die-off during the summer months than in the winter.[17,19,29-31] At

20°C, 99% die-off occurs in 10 d, compared with 95% die-off at 10°C after 14 d.[31] Fresh water has been the main source of waterborne outbreaks, although Salmonella have been isolated from estuaries with salinities approaching 17 parts per thousand.[17,32] In general, as salinities increase the presence of Salmonella decreases.[17,32-34] 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.[35] 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 1×10^5 Salmonella organisms in 10 wk with fecal pollution, compared with a die-off of 1×10^6 Salmonella organisms in 2 wk without fecal pollution.[19,28,36]

DOSE RESPONSE

The attack rate of Salmonella in either typhoid fever or salmonellosis is dependent on the dose of the organism. Tables A_5-2 and A_5-3 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.

LATENCY

A summary of latency data based on dose is shown in Tables A_5-4 and A_5-5. Review of the typhoid fever latency data indicates a dose-dependent relationship with a latency of 3 to 22 d. Review of the latency data for salmonellosis indicates a latency period ranging from 6 h to 3 d, with less noticeable dose-dependency.

DISINFECTANTS

The available information on the effects of disinfectants on Salmonella typhi has not been presented in standard format. Some authors report percent kill with fixed concentration of disinfectant with respect to time; others report percent kill at various concentrations within a fixed time frame. The key parameters in all the studies have been dose, pH, temperature, and contact time. A summary of the data is presented in Table A_5-6. Generally, an increase in temperature to 20 to 25°C at a pH of 6.5 to 7.0 increases the effectiveness of chlorine disinfectants.[43]

Table A_5-2. Dose response for Salmonella typhi.

Dose (organisms/mL)	Response (per 1000)	Number of subjects	Ref.
10^3	0.1	14	6
10^3	10.0	1,300	37
10^3	45.0	11,800	37
10^3	40.0	10,675	37
10^3	75.0	4,293	37
10^3	90.0	378	37
10^3	100.0	1.6×10^6	14
10^5	275.0	116	6
10^5	270.0	10^4	6
10^5	350.0	110	22
10^7	500.0	32	6
10^7	530.0	30	6
10^7	330.0	6	6
10^7	500.0	30	6
10^8	890.0	9	6
10^9	950.0	42	6
10^9	950.0	6	36
10^9	1000.0	4	6

Table A_5-3. Dose response for Salmonella spp.

Dose (organisms/mL)	Response (per 1000)	Number of subjects	Ref.
17	120	16,000	14,37
2×10^9	1000	2	37
10^{10}	1000	1	37

Table A_5-4. Latency for typhoid fever.

Dose	Latency (d)	Ref.
$<10^3$	15-22	37
10^3	7-14	2
10^5	9	22,37
10^8	7-8	37
10^9	3-9	36,37

Table A_5-5. Latency for salmonellosis.

Organism	Dose	Latency (d)	Ref.
S. typhimurium	10^3	0.5	14
S. typhimurium	10^4	0.04-4.0	38
Salmonella spp.	10^2-10^3	0.25-3.0	2

MONITORING METHODS

There are no "standardized" methods for the recovery of Salmonella from the environment. Instead, there is a series of techniques that may be adapted to fit a particular set of circumstances.[44] Also, some methods are directed toward the recovery of Salmonella and not enumeration.

Since these organisms are not usually present in large numbers in the environment, the first step must be a concentration procedure. There are four methods recommended in Sec. 912A of Ref. 44: (1) Moore swab technique, (2) diatomaceous earth, (3) large-volume sampler, and (4) membrane-filter technique. The type of method chosen is dependent on the environmental conditions.

Since these organisms have been in the environment, they have existed under relatively low nutrient conditions. Thus, they require time to repair and multiply in order for their presence to be detected. Moreover, organisms taken directly from the environment and placed on synthetic media can suffer from "nutrient shock" and die-off.

Table A_5-6. Effect of disinfectants on Salmonella.[a]

Disinfectant type	Dose (mg/L)	Response (% kill)	Time (h)
NaOCl	6	99.00	—
NaOCl	2	90.00	—
NaOCl	0.85	(+)	
NaOCl	1.4–2.0	100.00	0.008
NaOCl	0.4–0.96	(+)	
NaOCl	0.23	99.9	2.6
NaOCl	0.25	99.99999	
CaOCl	5.00	90.00	0.33
CaOCl	5.00	98.00	0.75
CaOCl	5.00	99.00	1.00
CaOCl	5.00	99.00	2.00
CaOCl	5.00	99.90	4.00
CaOCl	5.00	99.99	18.0
Chloramine	0.23	99.9	2.5
Chloramine	1.2	100.00	0.33

[a] Compiled from data presented in Refs. 7, 25, 30, and 39 to 43.

Therefore, it is necessary to select for enrichment of these organisms and to inhibit other competing organisms that may interfere with the growth of Salmonella. The use of dulcitol selenite broth or tetrathionate broth has been traditionally employed for these enrichment purposes.[44]

To recover Salmonella from the enrichment step and eliminate other species of bacteria, selective growth media can be employed.[44] There are three standard selective media used: (1) brilliant green agar; (2) xylose lysine desoxycholate agar; and (3) bismuth sulfate agar. These media inhibit different groups of organisms competitive with Salmonella; therefore, it is best to use at least two medium types to ensure Salmonella recovery.

To identify the isolated Salmonella organisms, a series of biochemical tests can be carried out as described in Sec. 912A of Ref. 44, or with the use of commercially available, rapid, biochemical identification methods. A more rapid technique for Salmonella identification in water uses an immunofluorescence technique.[44] This

technique requires a fluorescence microscope and Salmonella-specific fluorescent antibody stains. These microorganisms in the water samples must be concentrated by several methods using filtration, followed by incubation in one of the previously mentioned enrichment broths.[44]

Quantitative Salmonella procedures are referred to in Sec. 912C of Ref. 44. Briefly, known volumes of the water sample are concentrated on membrane filters; every filter representing a given volume is placed in an enrichment broth and incubated. This enrichment is then tested for the presence of Salmonella by using the routine biochemical and immunological tests described above. The results of these procedures can be reported as the most probable number (MPN) per given volume of water. Two techniques are described in Ref. 44, one for most Salmonella spp. and the other specifically for S. typhi.

INDICATOR PATHOGEN

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. In Table A_5-7, the relationship of indicator to bacterial pathogen (in this case, coliforms to Salmonella) is illustrated. Unfortunately, the data are generally presented in ratios, and only rarely are the actual Salmonella numbers presented. This is due in part to the lack of accurate quantitative technique for this organism at the time these studies were carried out. Therefore, Salmonella is presented as either a ratio, a number, or percent of samples that were positive for Salmonella, or simply, the presence of Salmonella in that particular environment. 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[34,46,47]; (2) common coliform indicators do not coincide with Salmonella presence in tropical zones[35,42]; (3) at higher temperatures, coliforms tend to die off much more rapidly than Salmonella[31,54]; and (4) coliforms appear to be better indicators in more temperate zones (see Table A_5-7).

Table A_5-7. Indicator-pathogen relationship for Salmonella spp.

Salmonella	TC[a]	FC[b]	FS[c]	Environment	Location	Ref.
Ratio 1:		14,000		Mud	U.S.	27
Ratio 1:		150		River water	U.S.	27
Ratio 1:	32,950	2,737	8,702	River water	U.S.	45
Ratio 1:	11,580	300	191	River water	U.S.	45
# 4/100 mL[d]	0	0		Treated water	France	46
# 7/100 mL	0	0		Treated water	France	46
# 95/100 mL	0	0		Treated water	France	46
# 910/100 mL	0	0		Treated water	France	46
# 6/100 mL	0	0		Treated water	France	46
# 77/100 mL	240	0		Untreated water	France	46
# 2/100 mL	0	0	2	Untreated water	France	46
# 18/100 mL	72	0	6	Untreated water	France	46
# 16/100 mL	0	0	0	Untreated water	France	46
# 2/100 mL	2	0	0	Untreated water	France	46
# 18/100 mL	0	0	0	Untreated water	France	46
Ratio 1:		9		Sediment[e]	U.S.	17
Ratio 1:		13		Sediment[f]	U.S.	17
18%[g]		1-200		Estuaries	U.S.	32
30%[g]		201-2000		Estuaries	U.S.	32
56%[g]		2001-20,000		Estuaries	U.S.	32
62%[g]		>20,000		Estuaries	U.S.	32
(+) samples		230		Irrigation water	U.S.	47
(-) samples		34		Estuaries	U.S.	47
Ratio 1:	255,000			Estuaries	U.S.	47
Ratio 1:			4800	Estuaries	U.S.	47
(+) samples	>10,000			Estuaries	N. Europe	48
(+) samples	<10			Estuaries	N. Europe	48
# 1/100 mL	>1100/100 mL	1100/100 mL		Estuaries	Chesapeake Bay, U.S.	34
# 0/100 mL	>1100/100 mL	15/100 mL		Estuaries	Chesapeake Bay, U.S.	34
10%	79/100 mL to >2400/100 mL			Well water	Nigeria	17

Table A_5-7. (Continued)

Salmonella	TC[a]	FC[b]	FS[c]	Environment	Location	Ref.
(+) samples	30/100 mL			River water	U.S.	31
# isolations				Sewage effluent	England	49
48	72 to 2500 x 10^3					
9	55 to 2300 x 10^3					
60	395 to 6800 x 10^3					
(+) samples	10^6			Well and river	England	15
% (+) samples				Seawater	England	50
13%	0/100 mL to 1000/100 mL					
29%	1000/100 mL to 10,000/100 mL					
40%	> 10,000					
# isolates				Sewage outfall	U.S.	51
1	13 x 10^6	10^6	2 x 10^6			
2	24 x 10^6	5 x 10^6	3 x 10^6			
3	24 x 10^6	8 x 10^6	7 x 10^6			
1	1 x 10^7	11 x 10^6	4 x 10^6			
1	11 x 10^6	5 x 10^6	3 x 10^6			
6	9 x 10^4	1 x 10^4	10^4			
(+) samples	<100/100 mL				So. Africa	42
# 4500	4.5 x 10^5			Urban storm-H_2O	U.S.	52
(+) samples	2.2/100 mL to >1609/100 mL			Cruise ship Storage tank	England	53

[a] Total coliforms.
[b] Fecal coliforms.
[c] Fecal streptococcus.
[d] # = number of organisms.
[e] Estuarine sediment.
[f] Ratio of Salmonella in sediment to FC in the water column.
[g] Percentage of samples positive for Salmonella vs numbers of FC.

CONCENTRATION IN THE ENVIRONMENT

Table A_5-8 summarizes the data obtained by this search for the concentration of Salmonella in the environment. From this table it is clear that the number of Salmonella present in the environment is not very predictable. For example, Sinegre et al.[46] conducted a survey of treated and untreated waters in France. They found that Salmonella was present in higher concentrations in the treated water than in the untreated water. There was no explanation given for this result. Also, the number of Salmonella in storm runoff water is extremely variable, ranging from <3.0/100 mL to 4500/100 mL.[31,40] This may reflect the variable presence of reservoirs of Salmonella.

Table A8-8. Salmonella concentration in the environment.

No. Salmonella per 100 mL	Environment	Ref.
<100	Sewage stabilization pond	55
4.5	Storm water	40
<3.0	Storm water	40
4500	Storm water	31
3-6.2	Municipal wastewater (U.S.)	40
43	Mississippi River water	40
1	Estuary-U.S.	34
4	Treated water	46
7	Treated water	46
95	Treated water	46
910	Treated water	46
6	Treated water	46
77	Untreated water	46
2	Untreated water	46
18	Untreated water	46
16	Untreated water	46
2	Untreated water	46
18	Untreated water	46
150	H_2O sediments	56
1100	Effluent A[a]	21
43	Effluent B[a]	21
23	Below effluent A[b]	21
3	Below effluent B[b]	21
0.9 MPN[c]	Irrigation water	47

[a] Sewage effluent in a river.

[b] 0.5 km below the sewage effluent.

[c] MPN = most probable number.

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SECTION 6. Yersinia

ETIOLOGY AND CLINICAL DISEASE

Yersinia spp. are facultative anaerobic Gram-negative rods that are included in the family Enterobacteriaceae.[1] The pathogens of this family include Y. pestis, Y. pseudotuberculosis, and Y. enterocolitica. Yersinia enterocolitica (more than 30 serotypes) and Y. pseudotuberculosis (6 serotypes) are responsible for yersiniosis as an acute enteric disease manifested by diarrhea.[2] Yersiniosis is characterized by not only diarrhea, but enterocolitis, acute mesenteric lymphadenitis (mimicking appendicitis, especially in older children), low-grade fever, headache, pharyngitis, anorexia, vomiting, erythema nodosum (in adults, particularly women), arthritis, cutaneous ulceration, abscesses, and septicemia. Yersinia enterocolitica infections commonly result in gastroenteritis and have been implicated more with waterborne outbreaks than Y. pseudotuberculosis, which causes severe abdominal pain and has a higher case-fatality rate.[2]

Yersinia enterocolitica and Y. pseudotuberculosis are capable of penetrating the epithelial linings of the intestinal mucosa prior to entering their target reticuloendothelial tissue in the lamina propria and lymph follicles.[3] This tissue invasiveness is directly linked to the presence of a temperature-dependent plasmid. The organism loses its tissue invasiveness with growth at 35°C. It has been proposed that at cooler temperatures (<35°C) Y. enterocolitica sheds or "uncoats" its surface antigens, which permits adherence and tissue invasiveness.[3] These antigens then give way to a phagocytotic protective antigen (VWA). Therefore, it appears that pathogenicity is based on the temperature of antecedent growth conditions, also known as the "hot-cold" virulence cycle.[3] Y. enterocolitica also produces a heat-stable toxin (like E. coli), but it is not elaborated at temperatures >35°C or under anaerobic conditions.[3]

One of the secondary effects of yersiniosis is arthritis. This can be quite severe and debilitating and may last for up to 2 to 3 y. Individuals with HLA (human leukocyte antigen) B27 are at high risk for yersiniosis arthritis.[4,5] In two studies it was found that 25 to 67% of those individuals with Y. enterocolitica gastroenteritis developed arthritis of varying severity.[6,7] In one study,[6] arthritis in gastroenteritis patients was predominantly found in men older than 16 y. Circulating IgA and IgG antibodies to Y. enterocolitica may last for 6 to 8 mo and can be used as a diagnostic measure of arthritis due to yersiniosis. IgM lasts only 2 to 3 mo and can be used to diagnose recent infection.[7]

OCCURRENCE

Yersiniosis occurs worldwide as gastroenteritis and mesenteric lymphadenitis.[2] Northern zones of the Western Hemisphere (Canada, Finland, Norway) seem to be quite sensitive to outbreaks of yersiniosis. Most of the data come from these countries.[7-10] Table A_6-1 contains occurrence and other related data for Yersinia spp. that have been reported for the U.S., Belgium, Canada, and Thailand. This sensitivity may well reflect special interest and associated expertise of these countries in working with Yersinia. As the isolation techniques have improved in recent years, Y. enterocolitica has been recovered from diarrhea patients at equal or greater frequency than has Salmonella and Shigella in Europe and North America.[4]

RESERVOIR

The principal reservoirs for Y. enterocolitica and Y. pseudotuberculosis are animals. Y. pseudotuberculosis is widespread among avian and mammalian hosts; Y. enterocolitica has been recovered from healthy as well as diseased animals. Organisms have been isolated from bodies of water that met potable standard bacteriological criteria.[2]

MODE OF TRANSMISSION

Usually fecal-oral transmission takes place by contact with infected persons or animals, or by eating and drinking fecally contaminated food or water.[2] Milk has also been implicated in two outbreaks.[12,13]

SUSCEPTIBILITY AND RESISTANCE

Susceptibility to this disease is universal in the human population, but the disease is more common and severe in children and the aged. Y. pseudotuberculosis exhibits a predilection for adolescents, while Y. enterocolitica attacks all groups of both sexes, but more commonly children and adolescents.[2]

ENVIRONMENTAL PERSISTENCE

Until recently there has been little published on the survival of Yersinia under environmental conditions.[18,19] Yersinia enterocolitica was isolated from an unchlorinated well source 15 to 40 d following an outbreak.[20] DePaola et al.[18] studied the effects of an

Table A_6-1. Occurrence of Yersinia spp.

Location	AR[a]	Source or occurrence	CR[b]	SAR[c]	Ref.
U.S.	410	Water			11
U.S.	83	Milk			12
U.S.	810	Milk			13
U.S.	—	Water	111	111	14
Belgium	11	DP[d]			15
Canada	28	DP[d]		475	16
Thailand	30	TD[e]	30		17

[a] Attack rate per 1000 population.

[b] Carrier rate per 1000 population.

[c] Secondary attack rate per 1000 population.

[d] Diarrhea patients (Yersinia-positive patients/1000 diarrhea patients).

[e] Incidence among 'travelers' diarrhea patients per 1000.

estuarine environment on Yersinia enterocolitica and coliform survival. They reported that Y. enterocolitica survived for 4 to 8 d, while fecal coliform (FC) persisted longer. As salinity (8-30 parts/1000), temperature (12-30°C), and sunlight increased, survival for Y. enterocolitica decreased. Elais-Maldonado and Hazen[19] showed that, when introduced into a tropical river watershed, Yersinia enterocolitica displayed a rapid decrease within the first 24 h. From the little data available, it seems that Yersinia do not survive well in the environment. A survey by Harvey et al.[21] of the Mammoth Lakes area of California revealed that 34 lakes contained Yersinia. Because many of these lakes were not frequently visited by man, they probably were contaminated by animals. Further research is needed in order to describe accurately the survival of Y. enterocolitica and Y. pseudotuberculosis in the environment.

DOSE RESPONSE

The only dose-response data available for yersiniosis are from a self-feed volunteer study by Szita et al.,[22] presented in Table A_6-2. Unfortunately, the vehicle of dose delivery was not specified. The volunteer was not given antibiotics, and the symptoms persisted for 4 wk.

LATENCY

The dose given in the Szita study[22] is quite high, and the volunteer developed symptoms within 1 d (Table A_6-2). This study is extremely limited. The range of latency of yersiniosis is thought to be 3 to 7 d.[2]

DISINFECTANTS

Only one paper was found that dealt with the disinfection of Yersinia.[23] Chlorine dioxide was the disinfectant chosen, and it was found that 0.25 mg/L ClO_2 was sufficient to inactivate 100% of the added culture. The major factor affecting Yersinia survival was antecedent growth conditions. Yersinia was found to be more resistant when taken from a low-nutrient environment rather than an environment with more of an organic load.

MONITORING METHODS

Yersinia recovery is not addressed directly in Standard Methods[24]; however, since it is a member of the Enterobacteriaceae family, it can be isolated using the same methods (Sec. 912A) as those used for other members of this family (E. coli, Salmonella, Shigella, etc.). Yersinia can be isolated with the following media: MacConkey agar, DCL agar, Salmonella-Shigella agar, or SS-D agar, to name a few.[1] Differentiation of Yersinia spp. from other members of the Enterobacteriaceae may be accomplished by biochemical or serological tests, as described in any microbiological reference source such as Bergey's Manual of Systematic Bacteriology[1] or Manual of Clinical Microbiology.[25]

INDICATOR-PATHOGEN RELATIONSHIP

Generally, the presence of Yersinia has not been associated with indicators.[8] Studies in Norway have shown the presence of Yersinia in water that was acceptable by bacteriological standards. However, because Yersinia can be transmitted via feces, the presence of coliforms can at least be a warning of possible Yersinia contamination. As noted before, DePaola et al.[18] and Elais-Maldonado and Hazen[19] have shown that Yersinia survive less well than total coliforms (TC) and fecal coliforms (FC) in a tropical environment. Therefore, in the case of Yersinia in tropical waters, TC and FC may be good indicators.

Table A_6-2. Dose response and latency for Yersinia enterocolitica.

Dose	Attack rate (per 1000)	Latency (d)	Ref.
3.5×10^9	1000(1/1)	<1	22

Table A_6-3 reflects the lack of data in this area and the need to describe better the association of indicators and Yersinia.

CONCENTRATION IN THE ENVIRONMENT

As with most diarrhea outbreaks, the etiologic agent is usually isolated from feces from those infected, and then environmental isolation is attempted. However, due to the long incubation period of Yersinia, sources are not identified until well after a population has been exposed. Based on the survival data, this time period may be long enough to allow complete die-off of Yersinia organisms unless there is a continuous source of contamination. This literature search did not recover any information concerning actual numbers from the environment (Table A_6-). Estimates of environmental concentration could be calculated from infections. Bottone has estimated 10^6 Yersinia organisms/mL can be excreted from cases with urinary tract infections.[26]

Table A_8-3. Indicator-pathogen relationships for Yersinia spp.

Yersinia	Total coliforms	Source	Ref.
(+)[a]	1 - >16/100 mL	Well water	11
(+)	(+)	Well water	20

[a] Organism present.

Table A_6-4. Concentration of Yersinia in the environment.

Location	Source	Concentration	Ref.
Norway	Unchlorinated water	20% of samples[a]	8
U.S.	Lake water	30% of samples	21
U.S.	Abscesses/urinary infections	10^6/mL	26

[a] 15% of these met Norwegian bacteriological standards.

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APPENDIX B: VIRAL ORGANISMS

SECTION 1. 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 cause a wide variety of disease symptoms,[1-3] as described in Table B_1-1.

Polioviruses are the best studied of any of the enteroviruses; coxsackie A viruses are the least studied.[1] Poliovirus can cause the most serious of the enterovirus symptoms and polio is the major permanently crippling disease of infectious origin ascribed within this group of agents.[7] Mortality among the paralytic cases of polio infection ranges from 2 to 10% and increases markedly with age.[8] Although most infections caused by enteroviruses have no lasting effect, some of the "new enteroviruses" (most recently discovered) can also cause permanent paralysis.[3] 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 d.[1]

OCCURRENCE

The enteroviruses have worldwide distribution, and infections by them are common. There are local variations in virus types and virulence of strains.[7] 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.[6]

Poliomyelitis is characteristically a disease of children and adolescents.[8] 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.

In 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.[7]

Table B_1-2 gives attack rates for various enterovirus outbreaks.

Table B_1-1. The enteroviruses.

Virus	No. serotypes	Disease symptoms
Polio	3	Paralysis, aseptic meningitis, fever, nonparalytic polio
Echovirus	4	Aseptic meningitis, respiratory disease, rash, diarrhea, fever
Coxsackie virus A	5	Herpangina, respiratory disease, aseptic meningitis, fever
Coxsackie virus B	6	Myocarditis, congenital heart anomalies, rash, fever, aseptic meningitis, respiratory disease, pleurodynia
New enteroviruses	7	Aseptic meningitis, encephalitis, respiratory disease, acute hemorrhagic conjunctivitis, fever, paralysis

RESERVOIR

The reservoir of enteroviruses is the infected human. 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.[7]

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.[17] Strong evidence exists that person-to-person transmission is the primary route of contagion.[18]. There is little epidemiological evidence available concerning the waterborne disease potential of the enteroviruses.[18,19] Epidemiological methods are not sensitive enough to detect low-level waterborne transmission.[20] A reported waterborne polio outbreak in Huskerville, NE, involved contaminated tap water.[10] Two outbreaks, one of echovirus 16 and one of coxsackie B5, may have been at least partly from waterborne viruses.[11] Oysters are known to harbor enteroviruses.[21-24] In rare instances, food has been implicated in polio transmission.[8]

Table B_1-2. Attack rates of the enteroviruses.

Year of published report	Virus/disease	Attack rate[a]	Comment	Ref.
1962	Polio, paralytic	34.5	All socioecon. groups, epidemic, Des Moines, IA, 1959	9
1962	Polio, paralytic	3.1	High socioeconomic group	9
1962	Polio, paralytic	24.7	Middle socioeconomic group	9
1962	Polio, paralytic	73	Low socioeconomic group	9
1962	Polio, total ill	68.5	Overall, Des Moines, IA, 1959	9
1962	Polio, total ill	16.0	Upper socioeconomic group	9
1962	Polio, total ill	51.5	Middle socioeconomic group	9
1962	Polio, total ill	170	Lower socioeconomic group	9
1957	Polio	1.5	Lincoln, NE (an epidemic year)	10
1957	Polio	100	Lincoln, NE, waterborne outbreak overall attack rate	10
1957	Polio	45	Lincoln, NE, no. dead/disabled >1 y	10
1962	Echo 16, meningitis	640	N. Carolina, epidemic, possibly waterborne	11
1962	Echo 16, enteroviral illness	760	N. Carolina, overall attack rate	11
1983	Enterovirus 70, hemorrhagic conjunctivitis	0.2-3.62	Dade County, FL, 1981, wide range of economic groups	12
1984	Polio	0.1-6.2	S. Africa, <3 y, total 13.6% case fatality rate	13
Surveys of total infection (asymptomatic and symptomatic)				
1978	Echo, polio, cox.	113	Venezuela, hospital 0-5 y, gastroenteritis	14
1978	Echo, polio, cox.	131	Venezuela, controls	14
1970	Enterovirus (not polio)	14	Stool survey, Aspen, CO	15
1983	Enterovirus	266	Children <6 y, ill, Brazil	16

[a] Number of cases per 1000.

SUSCEPTIBILITY AND RESISTANCE

Susceptibility to infection with poliovirus and the other enteroviruses is general.[8] Small children are the most susceptible age group to polio infection, because most adults have acquired resistance through earlier infection or vaccination.[7] There is long-standing type-specific resistance to polio after infection, whether clinical disease is present or not. Second attacks are rare and result from a different virus type.[8] Vaccine for polio is widely available; there are no vaccines for any of the other enteroviruses. Infection by these also confer type-specific resistance.[7]

ENVIRONMENTAL PERSISTENCE

Enteroviruses are capable of surviving for extended periods under certain environmental conditions. Tables B_1-3, B_1-4, and B_1-5 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.[21] Enteroviruses are more labile in summer than in winter in free-flowing ocean water. They cease to be viable within 7 d at 37°C in seawater, and are more labile in natural waters than in artificially prepared marine and estuarine waters.[2] There is some antiviral activity in natural waters.[26,27] Viruses can survive for more than 175 d in soil particles with a moist environment at neutral pH, and at low temperature.[18]

Enteroviruses have been known to survive several weeks in pit latrines[18] and up to 130 d in sewage.[22] The time required to reduce numbers of enteroviruses by 99.9% in the environment ranges from 2 to 160 d. They can last up to 14 to 16 d in the sea.[5]

DOSE-RESPONSE RELATIONSHIP

Available dose-response information is given in Table B_1-6. In addition to the data included in this table, Westwood and Sattar[29] 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}).

Table B_1-3. Survival of enteroviruses in river water (Tanana River, AK), 0°C.[25]

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 B_1-4. Survival of enteroviruses in ocean water (in days).[2]

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

Table B_1-5. Effects of salinity and incubation temperature on virus survival (in weeks).[2]

Virus	4°C			15°C			25°C		
(ppt NaCl)	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

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.[22,29]

Table B_1-6. Infective doses of enteroviruses.

Virus	Dose	Carrier rate	%	Comments
Polio 1	200 PFU[a]	4/4	100	Koprowski, 1955, as reported in Ref. 28
Polio 1	20 PFU	4/4	100	Koprowski, 1955 (adults, oral route).[28]
Polio 1	2 PFU	2/3	67	Described in Ref. 28
Polio 1	0.2 PFU	0/2	0	Described in Ref. 28
Polio 3	10 $TCID_{50}$[b]	2/3	67	Premature infants,[28] oral route
Polio 3	2.5 $TCID_{50}$	3/9	33	Premature infants,[28] oral route
Polio 3	1 $TCID_{50}$	3/10	30	Premature infants,[28] oral route

[a] PFU = plaque-forming unit.

[b] $TCID_{50}$ = tissue-culture infective $dose_{50}$.

LATENCY

The incubation period for the minor illnesses caused by enteroviruses, including minor polio infections, is about 2 to 3 d. When the nervous system is involved (including paralytic polio), the average latency is 7 to 17 d, with a range of 3 to 35 d.[7,8]

DISINFECTION

Virus disinfection data are summarized in Tables B_1-7 through B_1-10. Chlorination efficiency depends on pH, temperature, presence of organic matter, and the physical state of the virus.[22] Polio, coxsackie, and echo viruses are more resistant to free-available chlorine than enteric bacteria. In general, free-available chlorine is more effective than hypoiodous acid; chlorine dioxide is at least equivalent to free-available chlorine (FAC) (and less affected by pH), and ozone is more effective than FAC by weight. For inactivation of poliovirus, HOCl is 10 times as effective as OCl^-.[44] There is increasing evidence that naturally occurring viruses are not as susceptible to chlorination as experimental strains.[30] Although current water-treatment practices do not always remove all viruses,[1] they do provide reasonable assurance of safe drinking water.[30]

It can be seen from Tables B_1-7 and B_1-8 that different studies may find widely differing inactivation times for the same virus under the same stated conditions. Many of

Table B_1-7. Disinfection of enteroviruses.

Virus	pH	Temp (°C)	Time[a]	Disinfectant type	Reduction (mg/L)	%	Comments/ref.
Polio	10	-	15	Iodine	0.8	85	30
Polio	6	-	2	ClO_2	1.0	90	30
Polio	5.2	-	5	Cl_2	22	100	0.5% organic matter/31
Polio	5.2	-	15	Cl_2	19	100	0.5% organic matter/31
Polio	5.2	-	30	Cl_2	19	100	0.5% organic matter/31
Polio	5.2	-	45	Cl_2	17	100	0.5% organic matter/31
Polio	5.2	-	60	Cl_2	14	100	0.5% organic matter/31
Polio 1	-	-	30	Cl_2	33-43	99.99	Wastewater/32
Polio 1	-	-	30	Cl_2	11-16	99.99	Treatment plant effluent/32
Polio 1	-	-	30	Cl_2	20	99.99	Storm overflow, 10%/32
Polio 1	-	-	30	Cl_2	35	99.99	Storm overflow, 20%/32
Polio	6		28 s	HOCl	0.4	99	Unbuffered water/33
Polio	6		16 s	HOCl	0.8	99	Unbuffered water/33
Polio	10		107 s	HOCl	0.4	99	Unbuffered water/33
Polio	10	-	42 s	HOCl	0.8	99	Unbuffered water/33
Polio	6	-	46 s	HOCl	0.4	99	Reclaimed water/33
Polio	6	-	22 s	HOCl	0.8	99	Reclaimed water/33
Polio	10	-	168 s	HOCl	0.4	99	Reclaimed water/33
Polio	10	-	168 s	HOCl	0.8	99	Reclaimed water/33
Polio	5.0	-	5	ClO_2	1.3-1.6	90	34
Polio	7.2	-	5	ClO_2	1.3-1.6	99	34
Polio	8.7	-	5	ClO_2	1.3-1.6	99.99	34
Most virus	8.5	<20	30	FAC	0.2-0.3		Will destroy most viruses/35
Polio	6	-	78	Chloramine-T	10	99[b]	4
Polio	6	-	34	Chloramine-T	20	99[b]	4
Polio	6	-	14	Chloramine-T	40	99[b]	4
Polio	7	-	11	Chloramine-T	60	99[b]	4
Polio	7	-	261	Chloramine-T	10	99[b]	4
Polio	7	-	81	Chloramine-T	40	99[b]	4
Polio	7	-	60	Chloramine-T	60	99[b]	4
Polio	6	5	78	Chloramine-T	10	99[b]	4
Polio	6	10	34	Chloramine-T	10	99[b]	4

Table B_1-7. (Continued)

Virus	pH	Temp (°C)	Time[a]	Disinfectant type	Reduction (mg/L)	%	Comments/ref.
Polio	6	10	34	Chloramine-T	10	99[b]	4
Polio	6	25	13	Chloramine-T	10	99[b]	4
Polio	6	35	6	Chloramine-T	10	99[b]	4
Polio	6	5	3.5	HOCl	0.5	99[b]	4
Polio	6	10	2.15	HOCl	0.5	99[b]	4
Polio 2	7.65	19-25	10	Cl_2	1.0-1.5	100	Lake water/35
Polio 1	6	0	3.5	Cl_2	0.39	99.6	Demand-free water/35
Polio 1	6	0	1.5	Cl_2	0.80	99.6	Demand-free water/35
Polio 1	7	0	8	Cl_2	0.23	99.6	Demand-free water/35
Polio 1	7	0	4.5	Cl_2	0.53	99.6	Demand-free water/35
Polio 1	8.5	0	16	Cl_2	0.53	99.6	Demand-free water/35
Polio 1	8.5	0	7.5	Cl_2	1.95	99.6	Demand-free water/35
Polio 1	8.5	0	3	Cl_2	5.0	99.6	Demand-free water/35
Polio 1	7	25-28	3	Cl_2	0.21-0.3	99.9	Demand-free water/36
Polio 1	9	25-28	8	Cl_2	0.21-0.3	99.6	Demand-free water/36
Polio 3	7	25-28	2	Cl_2	0.11-0.2	99.6	Demand-free water/36
Polio 3	9	25-28	16	Cl_2	0.11-0.2	99.6	Demand-free water/36
Polio 3	6	27	15	Cl_2	30	99.999	Autoclaved/37
Polio 3	7	27	27	Cl_2	30	99.999	Wastewater/37
Polio 3	10	27	30	Cl_2	30	99.999	Wastewater/37
Polio 1	6	5	2.1	FAC	0.47-0.49	99	38
Polio 2	6	5	1.2	FAC	0.48-0.51	99	38
Polio 1	7.8	5	1.3	FAC	0.46-0.51	99	38
Polio 1	10	5	21	FAC	0.50-0.52	99	38
Polio 2	10	5	64	FAC	0.48-0.50	99	38
Cox. A2	7	3-6	10	FAC	0.58-0.62	99.6	Demand-free water/35
Cox. A	7	3-6	4	FAC	1.9-2.2	99.6	Demand-free water/35
Cox. A	7	3-6	2.5	FAC	3.8-4.2	99.6	Demand-free water/35
Cox. A	9	3-6	24	FAC	1.9-2	99.6	Demand-free water/35
Cox. A	9	3-6	9	FAC	3.7-4.3	99.6	Demand-free water/35
Cox. A	9	3-6	5	FAC	7.4-8.3	99.6	Demand-free water/35

Table B_1-7. (Continued)

Virus	pH	Temp (°C)	Time[a]	Disinfectant type	Reduction (mg/L)	%	Comments/ref.
Cox. A2	7	27-29	4	FAC	0.16-182	99.6	Demand-free water/35
Cox. A2	7	27-29	3	FAC	0.44-0.58	99.6	Demand-free water/35
Cox. A2	9	27-29	10	FAC	0.10-0.18	99.6	Demand-free water/35
Cox. A2	9	27-29	7	FAC	0.27-0.32	99.6	Demand-free water/35
Cox. A2	9	27-29	3	FAC	0.92-1.0	99.6	Demand-free water/35
Cox. B5	7	25-28	1	FAC	0.21-0.3	99.9	36
Cox. B5	9	25-28	8	FAC	0.21-0.3	99.9	36
Cox. B5	7	1-5	16	FAC	0.21-0.3	99.9	36
Cox. B5	8	1-5	30	FAC	0.21-0.3	99.9	36
Cox. A9	6	5	0.3	FAC	0.46-0.49	99	38
Cox. A9	10	5	1.5	FAC	0.48-0.5	99	38
Cox. B5	6	5	3.4	FAC	0.51	99	38
Cox. B5	7.81	5	4.5	FAC	0.49	99	38
Cox. B5	10	5	66	FAC	0.50	99	38
Echo 1	6	5	0.5	FAC	0.48	99	38
Echo 1	7.8	5	1.2	FAC	0.48	99	38
Echo 1	10	5	96	FAC	0.49	99	38
Echo 5	6	5	1.3	FAC	0.38-0.49	99	38
Echo 5	7.8	5	1.8	FAC	0.5	99	38
Echo 5	10	5	27	FAC	0.5	99	38

[a] Time in minutes unless otherwise noted.

[b] The limit of measurement in this study was 99% removal.

the studies reported here were performed in the 1950's and 1960's. Subsequently, much has been learned about the importance of conditions, such as buffer type, temperature, water type, etc., that may affect results. Some of these conditions were not reported in the earlier studies, and the studies have not been repeated to obtain more accurate information on disinfection of enteroviruses.

Some of the data on Polio 1 virus from Table B_1-8 are compared to Coxsackie B5 in Table B_1-9.

Table B_1-8. Hours to 99.7% inactivation, Polio 1, 25°C.[39]

	Cl_2 (ppm)										
pH	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
6	6	3	2	3	2	>1.5	1.5	1			>0.5
7	>7	3	3	3	1.5	1.5	1	1	1	0.5	>0.5
8	12	7	4	4	4	>2	>2	2	1.5	1	1
9	8	>6	6	4	4	>2	2	2	1.5	1.5	1

Table B_1-9. Hours to 99.7% inactivation by 1 ppm combined residual Cl^-, 25°C.

pH	Polio 1	Coxsackie B5
6	3	2
7	3	3
8	>6	4
9	6-8	5
10	6-8	5

MONITORING METHODS

The routine examination of waters for enteric viruses is currently not recommended by Standard Methods for the Examination of Water and Wastewater.[45] There may be circumstances where monitoring for viruses is desired, such as in disease outbreaks, wastewater reclamation, and research. Testing requires specially trained water virologists and proper facilities.

Standard Methods[45] describes the virus-concentration techniques as tentative. They all are still under active research and are subject to modification. Yields are variable. Methods most commonly used are:

1. Concentration by adsorption and by elution from microporous filters, where the sample is pressure-filtered through cellulose nitrate or fiberglass-asbestos-epoxy

Table B_1-10. Reduction of enterovirus by water- and sewage-treatment processes.

Treatment	% Removal	Comment	Ref.
Primary		Decreases no. infectious particles, not isolation frequency	40
Trickling filters	40	Decrease of plaque counts	40
Secondary with chlorination		Cl residual, 0.5 ppm	40
Activated sludge	99		40
30-d activated sludge		None recovered	40
Flocculation and sedimentation	99.9	Attenuated polio	41
Flocculation and sedimentation	99.9	Found in sludge, not inactivated	42
Sand filtration	18.8-37.5		41
Activated carbon	21.4-78.5		41
Total removal by sewage plant	99.9995	Found in sludge, not inactivated	41
Stabilization ponds	89;45	Ramleh, Jerusalem; Israel	43
High-rate filtration	62	Tiberias (sand filtration)	43
Secondary with chlorination	81.5	11 ppm chlorine, 30-min contact time, Haifa, Israel	43
Secondary with chlorination	91.5	11 ppm, 4-h contact time	43
Secondary with chlorination	99.9	0.4 mg/L as residual free chlorine	41
Chlorination	99.9	40 mg/L applied to raw sewage	22

filters to which viruses adhere through electrostatic charge. The viruses are eluted in a small volume of alkaline elution fluid. The sample is usually acidified, and polyvalent cations are added prior to filtration.

2. Concentration by aluminum hydroxide adsorption-precipitation: viruses are adsorbed onto preformed $Al(OH)_3$ precipitates, and the precipitate is collected and either (1) it is used directly, or (2) the viruses are eluted by an alkaline buffer or a proteinaceous solution. This method is limited to a relatively small sample volume.
3. Hydroextraction-dialysis with polyethylene glycol: samples are placed in cellulose dialysis bags, which are placed in contact with hygroscopic polyethylene glycol, and the water and microsolutes are drawn out. This method also is limited to small sample volumes.

Viruses in a concentrated sample are enumerated in either whole animals (usually mice) or most commonly in mammalian (primate) cell culture, often Buffalo Green Monkey Kidney (BGMK) cells or the RD cell line. Usually two host systems should be used. Enteroviruses can be identified by standard serological techniques. Neutralization tests are recommended.[43]

INDICATOR-PATHOGEN RELATIONSHIP

The majority of researchers in this field believe that at present there is no reliable indicator organism for enteroviruses in waters.[19,21,23,46-51] 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.[52]

The search for appropriate viral indicators is an active area of study. Gerba et al. reported in 1978 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.[46] La Belle et al.[47] 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:[47]

$Y = 11.93 + 0.008\,X$, where Y = number of viruses in sediment, and
X = number of fecal coliforms in sediment.

Payment et al.[53] found a correlation between virus isolations and water turbidity at between 10 and 30 nephelometric turbidity units (NTU). Berg and Berman[49] 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.[54] 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[41] proposed bacteriophages as indicators of enteric virus removal by water-treatment practices. Roy et al.[55] mention bovine parvovirus as a possible enteric virus indicator, and Scarpino[56] suggested the phage of Serratia marescens as a poliovirus indicator, and the use of other phages to monitor efficiency of virus removal in water treatment. Knott et al.[57] felt the use of E. coli B bacteriophages provided a satisfactory measure of the quality of waters with respect to viruses.

Clarke et al.[58] 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.

CONCENTRATION IN THE ENVIRONMENT

Viruses have been isolated in a wide variety of waters and in shellfish. Their presence is frequently reported as percent samples that contain the virus but are also reported as plaque-forming units (PFU), tissue-culture 50% infective dose ($TCID_{50}$), virus infective unit (VIU), or infectious particle (IP). A summary of the data found relative to concentration in the environment is shown in Table B_1-11.

Table B_1-11. Concentration of enteroviruses in the environment.

Virus[a]	Water type/area	Concentration	% Positive[b]	Ref.
C,E,P	Raw sewage/Canada	1,000 VIU/L	80	59
C,E,P	Primary effluent/Canada	1,000 VIU/L	72	59
C,E,P	Chlorinated effluent/Canada	27 VIU/L	56	59
C,E,P	Ottawa River, estimate/Canada	27 VIU/L		59
C,E,P	Raw sewage/NY	540-1,600 PFU/L		60
C,E,P	Primary effluent/NY	<200-1,000 PFU/L		60
C,E,P	Second. trickl. filt./NY	<200-680 PFU/L		60
C,E,P	Second. settl. and Cl/NY	<200-320 PFU/L		60
C,E,P	Retained sludge/NY	280 PFU/L		60
C,E,P	Sea water/ Israel		67	54
P1,P3,E	Sewage/Jerusalem	0-7,349 PFU/L		43
P1,P3,E	Sewage/Tel Aviv	91-1,711 PFU/L		43
P2, E	Pond effluent, Israel	23 PFU/L		43
P2,3 +	Raw water/Haifa	31-152 PFU/L		43
Not tested	Raw water/Israel	5-11 PFU/L,184/L		43
Not tested	Raw water, Tiberias	114-4,487 PFU/L		43
Not tested	Raw water/Israel	2,283 PFU/L		43
C,E,P	River water/France		9.1	61
Unclassed	Sewage samples/France		35.2	61
Unclassed	Drinking water/France		8	61
P1,2,3	Sewage/Houston, TX	11.6-73.8 PFU/L		62
P1,2,3	Stream water/Houston, TX	33.5-85 PFU/L		62

Table B_1-11. (Continued)

Virus[a]	Water type/area	Concentration	% Positive[b]	Ref.
E7,P1,2,3	River water/Houston, TX	28.4 PFU/L		62
E7,P1,2,3	Sewage plants/Houston, TX	75-212 PFU/L		62
P1-3,E4,	Wastewater 1972/Israel	155-4,542 PFU/L average		63
13,18,24,	Wastewater 1973/Israel	457-12,697 PFU/L average		63
25, C-A1	Effluent 1972/Israel	77-6,968 PFU/L		63
25, C-A1	Effluent 1973/Israel	258-11,522 PFU/L		63
Varied	Lake, June 1976	6.5 PFU/L includes TC and FC		19
Varied	Lake, July 1976	2 PFU/L includes TC and FC		19
Varied	Open shellfish waters/NY, Sept., Nov., Feb.-June	0		19
P2,E22,11	Open shellfish waters/NY, Jul	2 PFU/L includes TC and FC		19
Varied	Open shellfish waters/NY, Aug.	0.3 PFU/L includes TC and FC		19
Varied	Open shellfish waters/NY, Apr.	0.75 PFU/L includes TC and FC		19
Varied	Closed shellfish waters/NY, Mar., Apr., Aug., Sept., Nov., 1976-77	0		19
E20,23	Closed shellfish waters/NY, July, 1976	1 PFU/L		19
P2	Closed shellfish waters/NY, Feb., 1977	1.1 PFU/L		19
P1	Closed shellfish waters/NY, June, 1977	0.28 PFU/L		19
E7,P1,C-B4	7 pools/Houston	0-17 PFU/L	57	13
C-B3,P1	7 wading pools/Houston	0-650 PFU/L	86	13
P1,2E1	Closed shellfish waters/TX	0-880 PFU/L	63	24
P1,2E1	Open shellfish waters/TX	0-1,670 PFU/L	50	24
P1,2E1	Cysters, from closed water/TX	0-2,240 PFU/L	40	24
P1,2E1	Oysters, from open water/TX	0-590 PFU/L	20	24

Table B_1-11. (Continued)

Virus[a]	Water type/area	Concentration	% Positive[b]	Ref.
Varied	Rhine, Seine, Marne, Moselle Rivers	Up to 300 PFU/l		1
Varied	Drinking water, India	0.025-0.175 PFU/L		1
Varied	Tap water/Nagpur, India	0.017-0.233 PFU/L		1
Varied	Thames River/London, winter	100 PFU/L		1
Varied	Thames River/London, summer	0.1 PFU/L		1
Varied	Missouri River	0.1 PFU/L		7
Varied	Mississippi River	0.4 PFU/L		7
	Mississippi River seawater, Miami sewage outfall	0-3 PFU/300 L		7
P1,2	Galveston Bay, oysters/TX	Up to 26 PFU/100 g		7
Varied	Lagoon, tropical	10^{10} in oysters		65
Varied	Sewage-treatment intake/S.Africa	20,000 TCID-50/L		66
E7,C-B2,	Groundwater/Israel	202 PFU/L	6[c]	63
5,6,P1	Potable water/Israel	435 PFU/L	8.7[c]	63
5,6,P1	Grossly polluted/Israel	421 PFU/L	0[c]	63
5,6,P1	Surface water/Israel	125 PFU/L	12.5[c]	63
5,6,P1	Swimming pools/Israel	1,000 PFU/L	50[c]	63
P,others	Drinking water/France, 1961		8.7	30
Various	Drinking water/1962		8	30
P	Treated drinking/VA, 1976		2.4	30
C-A	Treated drinking/Rumania, 1962-71	3.1		30
C-A,B,E,P	Treated drinking/Russia, 1968-71		14.1	30
E,P	Treated drinking/MA, 1969-71		10.9	30

Table B_1-11. (Continued)

Virus[a]	Water type/area	Concentration	% Positive[b]	Ref.
P	Treated drinking/MA, 1972-3		14.3	30
E	Treated drinking/FL, 1975		10.0	30
P	Treated drinking/VA, 1975		33	30
Various	Reclaimed water, ok TC/TX Gulf[d]	43		46
	Reclaimed water, ok FC/TX Gulf[d]		44	46
Various	Shellfish waters, ok/TX Gulf	35		46
Various	Primary sewage sludge	10-1,000 TCID-50/mL		64
P	Wastewater/Israel	0-89,000 PFU/L		48
P,E,C,Un	Wastewater/Israel	0-82,000 PFU/L		48
Varied	Lake/NY, July, Aug., Nov., Jan., May 1976-77	0		19
Varied	Lake/NY, Sept. 1976	6 PFU/L; includes TC and FC		19
Varied	Lake/NY, Mar. 1977	1.7 PFU/L; includes TC and FC		19
Varied	Creek/NY Aug., Oct.-May 1976-1977	0		19
Varied	R.Trent, Nottingham, Jan.-June 1972	0		41
C-B3	R.Trent, Nottingham, Aug. 1971	8.13 IP/L		41
C-B3	R.Trent, Nottingham, Sept. 1971	2.43 IP/L		41
C-B5	R.Trent, Oct.	0.48 IP/L		41
No type	R.Trent, Nov. 1971	<0.24 IP/L		41
C-B3	R.Trent, July 1972	0.66 IP/L		41
C-B3	R.Trent, Aug.	1.04 IP/L		41
No type	R.Trent, Sept.	<0.024 IP/L		41
Varied	R.Avon/ UK, Nov. 1978	2-5 PFU/L		62

Table B_1-11. (Continued)

Virus[a]	Water type/area	Concentration	% Positive[b]	Ref.
Varied	R.Avon/ UK, Dec. 1978	8-540 PFU/L		62
Varied	R. Sowe/UK, Nov. 1978	145 PFU/L		62
Varied	R. Sowe/UK, Dec. 1978	310-620 PFU/L		62
Varied	Gulf coast/TX	1-43 PFU/L	59	24
Varied	Oysters, Gulf coast/TX	1.5-58 PFU/L	35	24
Varied	Raw sewage/India	to 11,000 PFU/L		67
Varied	Seawater/Galveston	0-26 PFU/ L, median = 2		47
Varied	Sediment/Galveston	0-24 virus/L		47
P	Wastewater/Manitoba	61.8		55
P	Effluent/Manitoba	20.5		55
P	River/Manitoba	3		55
P	Drinking water/Manitoba		8.7	55

[a] Viruses are abbreviated: C = coxsackie, E = echo, and P = polio, followed by type number if available.

[b] Percent of positive virus samples.

[c] Number of positive samples with detectable chlorine residual.

[d] Waters tested were acceptable for total coli counts, Texas Gulf Coast.

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SECTION 2. Norwalk Agent

ETIOLOGY AND CLINICAL DISEASE

The Norwalk agent is a round, 27-nm virus, tentatively classified as a parvovirus-like agent, although there is now evidence that it may be a type of calicivirus.[1] It is responsible for an epidemic gastroenteritis that has been referred to as "winter vomiting disease" or acute nonbacterial gastroenteritis. The illness is characterized by nausea, vomiting, diarrhea, and abdominal cramps. Vomiting is the predominant symptom among children; diarrhea is present more often in adults.[2] Norwalk viral gastroenteritis may last from 2 h to several days, but usually lasts 24 to 48 h. Only about 15% of cases are ill longer than 48 h. Hospitalization is unusual. Norwalk infection may occasionally hasten the death of an elderly or debilitated person,[2] but otherwise is not considered fatal.

Of the 74 outbreaks of acute nonbacterial gastroenteritis investigated by the Centers for Disease Control in 1976-1980, 42% were attributed to the Norwalk virus.[2] The rest resembled Norwalk outbreaks and were probably caused by the 27-nm Norwalk-like viruses. These include the Hawaii, Marin County, Snow Mountain, Montgomery County, Ditchling, W or Wollan, and Parranatta viruses. These viruses are morphologically similar, but can be shown to be antigenically distinct from the Norwalk virus.[1] Some may be serotypes of Norwalk virus.[3]

OCCURRENCE

Norwalk and Norwalk-like viral infections have a worldwide distribution. Outbreaks tend to be explosive in nature and can occur year-round. They can infect persons of all ages.[3] Both sexes are equally susceptible. The median duration of an outbreak is 7 d with a range of 1 d to 3 mo. Attack rates of common-source outbreaks in the U.S. for the period 1976 to 1980 had a median of 60% and a range of 23 to 93%. Person-to-person secondary attack rates had a median of 39% and a range of 31 to 42%.[1] Seventy-one percent of 861 adults tested around the world had antibody, indicating past infection. There seems to be no striking difference between developed and less-developed countries, except that children acquire antibody earlier in the latter.[4]

RESERVOIR

Man is the only known reservoir.[3] There have been no reports of a carrier state. The period of communicability is during the acute stage and possibly for a short time thereafter.[3]

MODE OF TRANSMISSION

Spread of Norwalk and Norwalk-like agent is through the fecal-oral route. Person-to-person transmission is probably the most common. Many outbreaks have been associated with contaminated water supplies,[1] and at least two outbreaks were related to recreational water.[5,6] Foodborne outbreaks have occurred, caused by contaminated, raw or insufficiently cooked shellfish.[7-9] The respiratory route has been suspect because of the high secondary-infection rate of some outbreaks; however, there is no solid evidence to support this suspicion.[1]

SUSCEPTIBILITY AND RESISTANCE

Based upon serological studies, susceptibility to the disease is widespread. Whether infection confers resistance or not is still open to debate, because it is unclear at this time whether or not some of the Norwalk-like viruses are actually serotypes of the Norwalk agent.[1-3,10] The susceptibility data remain open to question.

Clinical immunity to Norwalk virus is complex and fails to fit immunological concepts normally associated with common human viral illnesses.[11] There appear to be two forms of immunity to Norwalk virus; long-term[11,12] and short-term,[12,13] neither of which is absolute. Of 12 volunteers challenged and then rechallenged 27 to 42 months later with Norwalk virus, those who became ill the first time (6 of 12) became ill the second time as well. Those not ill the first time were again not ill the second time. A third challenge of 4 of the previously ill volunteers 4 to 8 wk later resulted in 1 of the 4 becoming ill.[12]

Factors other than serum antibody would seem to be important in Norwalk gastroenteritis immunity. Antibody may play a role in short-term immunity but not long-term immunity.[12] The presence of serum or local jejunal antibody to Norwalk virus makes infection more likely than in a person with little or no antibody. This paradox and the lack of demonstrated long-term immunity make prospects of a vaccine to prevent Norwalk infections unlikely.[2]

ENVIRONMENTAL PERSISTENCE

There is very little information on the environmental tenacity of the Norwalk virus. It is known to remain viable after 3 h of exposure to a pH of 2.7. The Norwalk virus can survive 30 min at 60°C and remains viable after 24 h in 20% ether.[1,14] Viability was determined in the above studies by feeding the organisms to volunteers and noting whether or not disease resulted.

DOSE RESPONSE

The median infective dose for the Norwalk virus has not been determined.[15] Although several volunteer studies have been conducted, dose administered is referred to as a dilution of infected fecal material, as compared with the application of nonfecally contaminated controls. Dolin et al.[16] reported that 10 mL of a filtered rectal swab eluate diluted 1:100 produced Norwalk illness in 2 of 3 volunteers taking the material orally. In a second pass of this material, 10 mL of a 2% stool suspension from one of the 2 above volunteers caused illness in 7 of 9 other volunteers.

It is unknown how much virus is present in feces of persons acutely ill. It is reported that the Norwalk and Norwalk-like viruses are present only at low concentrations in diarrheic feces.[1]

LATENCY

The incubation period for the Norwalk virus and Norwalk-like viruses is usually 24 to 48 h. Volunteer studies with Norwalk agent show a range of 10 to 51 h.[3]

DISINFECTION

There is no information available dealing with the disinfection of the Norwalk agent by chlorine or other disinfectants.[17] Waterborne outbreaks to date have been associated with failures in chlorination systems or absence of residual chlorine; many were due to accidental contamination.[6,13,15,17-19] The disinfection of Norwalk and Norwalk-like agents by chlorination is an area where research is needed.

MONITORING METHODS

There are no methods for detecting Norwalk virus or Norwalk-like viruses in environmental waters at this time.[1,17]

INDICATOR-PATHOGEN RELATIONSHIP

There is no reliable direct correlation between viruses and indicator organisms. It is currently believed that although the presence of indicator organisms may indicate virus contamination, the absence of indicators does not guarantee the absence of viruses.[20]

CONCENTRATION IN THE ENVIRONMENT

Because there is no way to detect the presence of Norwalk or Norwalk-like virus in water, there is also no information on their presence in the environment. The disease has been called "winter vomiting disease" and in temperate climates may predominate during the colder months. However, it can occur at any time during the year.

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SECTION 3. Rotavirus

ETIOLOGY AND CLINICAL DISEASE

Rotaviruses are wheel-shaped, 68-nm viruses constituting a new 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 d,[3] but in rare cases has lasted a month.[4] Death may occur, usually with dehydration and associated electrolyte imbalance as complicating factors.[1] In a study of adults with diarrhea in Nonthaburi, Thailand, only individuals with cholera passed more watery stools in 24 h and were more dehydrated than adults with rotavirus infections.[5] Treatment is nonspecific and consists of supportive therapy including rehydration. Once a patient has recovered, there appear to be no secondary effects.

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-mo age group. A Canadian study found that 62% of infants in a prospective study had at least one rotaviral infection by 2 y of age.[6] Older children, neonates, and adults can also be infected; these infections are usually subclinical[2] but can result in severe illness.[5,7-9] In temperate zones, the incidence of rotavirus infection peaks in winter; as many as 80% of the hospitalized gastroenteritis cases aged 6 to 24 mo 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] Rotavirus accounts for 20 to 40% of all acute diarrheas in developing countries.[10] During the epidemic year 1979 in Washington, DC, 3.7/1000 children under 1 y old and 2.2/1000 children 1 to 2 y old were hospitalized for rotavirus gastroenteritis.[11]

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.[12] Twenty-five percent of adult U.S. transfer students with diarrhea in a school in Mexico City and 12% of controls shed rotavirus. In greater than 50% of the rotavirus-positive cases, other enteropathogens were also present.[7] The Tiriyo Indians in Brazil, a previously unexposed group, suffered an overall attack rate of 88% in an epidemic in 1980.[13] It is not necessary for adults to have contact with ill children to contract the disease.[5,14,15] There are inapparent infections in all age groups.[13]

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,[13,16,17] person-to-person transmission is by far the most frequent.

SUSCEPTIBILITY AND RESISTANCE

By the age of 2 y, most individuals have acquired antibody to both serotypes of rotavirus.[1,2] Most persons possessing serum antibody are protected from disease when challenged,[2] but immunity is not absolute, and little is known about protective immunity.[10] Immunity seems to be associated with intestinal antibody secretion more than serum IgA.[18] Infants may have rotavirus infection more than once, usually due to different serotypes.[10,11] Adults are generally, but not always, asymptomatic.[3,11] It is not known why some adults are susceptible. Neonates have been shown to have an infection rate of 30 to 50%,[19,20] which is asymptomatic about 90% of the time. This neonatal infection does not confer resistance, but decreases severity of disease during reinfection.[19] It appears that breast feeding decreases the incidence and severity of rotavirus gastroenteritis in infants,[1,20] but this is not universally accepted.[6] There is hope that an effective vaccine can be produced, and active research in this area is under way.[18]

ENVIRONMENTAL PERSISTENCE

Rotavirus has been shown to survive more than 14 d in estuarine and heavily polluted fresh water.[21] In the marine environment, its rate of inactivation appears to be independent of salinities below 30 ppt.[21] Rotavirus is resistant to acid conditions[4] and is inactivated after 30 min at pH 11.[22] Not surprisingly, it appears to survive longer at low temperatures.[23,24]

DOSE-RESPONSE RELATIONSHIP

The number of rotavirus particles necessary to initiate infection or to cause disease is unknown.[25] Volunteer feeding studies have not been performed; a possible reason for this is that the unpredictable resistance of adults to clinical disease makes these studies of questionable value.

LATENCY

The incubation period for rotavirus is approximately 48 h.[2] The reported range is 1 to 4 d.[4,12,26,27]

DISINFECTION

The simian rotavirus SA-11 has frequently been used as an animal model for human rotavirus in disinfection studies because it can be propagated in cell culture, whereas the human virus cannot be easily cultured. Results of disinfection and inactivation studies[22,28] are given in Tables B_3-1 and B_3-2.

Rotavirus was inactivated by 0.05 mg/L chlorine dioxide or iodine at pH 10 and 0.5 mg/L chlorine at pH 7.0.[30] Rotavirus displayed 99.9% reduction with a UV-irradiation dose of 24 mW•s/cm^2 in phosphate-buffered saline solution.[31]

MONITORING METHODS

There is no standard method for examining environmental samples. To determine its presence in the research laboratory, rotavirus is concentrated by microporous filter adsorption-elution and detected by indirect immunofluorescence, electron microscopy, or enzyme-linked immunosorbent assay.[25] Human rotavirus cannot be assayed routinely in any convenient host systems.[32]

Table B_3-1. Disinfection of SA-11[a] rotavirus by HOCl[29] (25°C, 0.05 M buffer[b]).

pH	Rotavirus concentration (per mL)	Magnitude of reduction in 1 min	Free chlorine 4-log reduction, minutes required	Total available chlorine (mg/L)	Combined residual (mg/L)
6	1×10^5	4 logs	1	0.42	0.64
8	3×10^5	3.6 logs	1.2	0.41	0.80
10	1×10^4	1.7 logs	2.5	0.40	0.89

[a] SA-11 = simian rotavirus.

[b] Phosphate buffer: pH 6 and 8; borate buffer: pH 10.

Table B_3-2. Inactivation of rotavirus (fluorescent foci[a]).[b]

Treatment	Virus titer, foci[a]/0.5 mL	
	Simian rotavirus SA-11[c]	Sewage isolate
None	107	18
Autoclave, 20 min	0	0
Boil, 20 min	0	0
pH 11, 30 min	0	0
Chlorine,[d] 10 mg/L, 30 min	0	0
Formalin, 1:2000, 4 d	10	0

[a] Unit detected by indirect immunofluorescence technique; represents at least 3.8×10^5 rotavirus particles.[25]

[b] Table adapted from Ref. 22.

[c] SA-11 = simian rotavirus.

[d] Chlorine species undefined.

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.[33]

CONCENTRATION IN THE ENVIRONMENT

The measurement of rotavirus concentration in environmental samples has only recently been possible. Rotaviruses, like other viruses, are subject to wide fluctuations in sewage over short time periods.[25] There may also be seasonal fluctuations reflecting peak infection in temperate climates.[22] Rotavirus in sewage in Houston, TX, ranged from 0 to 3480 fluorescent foci (FF) per 20 L for raw sewage (average 1505 FF) and 150 to 7488 FF/20 L (average 1687 FF) for secondary-treatment effluent. Fluorescent foci, the units detected by the indirect immunofluorescence technique, represent at least 3.8×10^5 rotavirus particles.[25] It was apparent that secondary treatment did not decrease rotavirus levels the way it does enteroviruses.[22] Another study on sewage by the same group in Houston found 1 to 321 FF/L (average = 9.8 FF/L) over the course of a 2-y study and a peak in March through April of the first year and November and December of both years.[25] Rotavirus was also detected in 6 of 24 (25%) samples of domestic sewage in Kiel, West Germany, in June and July, 1980.[34] This paper also stated that 2 L of domestic sewage could not be expected to reach a concentration of 10^7 virions (i.e., infectious particles of a virus) of rotavirus.[34]

A study of rotavirus in Galveston Bay[28] reported 119 to 4980 particles per 100 gal bay water. Treated drinking water showed 83% of finished samples from a heavily polluted source contained rotavirus and/or enterovirus. All samples taken during the rainy season were rotavirus-positive.[35]

In feces of acutely ill humans, rotavirus is usually found in amounts of about 1 billion particles per g. Up to 100 billion particles per g have been recorded.[4]

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SECTION 4. 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 wk, to severely disabling and lasting several months, although the latter occurrence is rare. The recovery period is usually prolonged. The mortality rate is 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.[1]

Hepatitis A can be diagnosed by the detection of virus in the stool or by the presence of IgM antibodies against hepatitis A virus, which are only present in the serum of persons acutely or recently ill. There is currently no specific treatment for hepatitis A. 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 wk of illness.

OCCURRENCE

Hepatitis A has a worldwide distribution. It 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.[2] Nearly 100% of Thais by age 15, Ethiopians by age 13, and Taiwanese by age 20 have antibody to the virus.[3-5] The attack rate for viral hepatitis in the United States in 1980 was reported as 26.5/100,000, of which 48% was hepatitis A.[6] Twenty-two percent of tested U.S. Army personnel stationed in Thailand and 25% of tested personnel stationed in Germany possessed antibodies to HAV.[7,8] The disease typically occurs in persons 15 y old and younger; many of the infections in young people are asymptomatic or mild without jaundice. In general, hepatitis A increases in severity with age and decreases in incidence after age 35. Both sexes have comparable attack rates.[1,9]

RESERVOIR

The normal reservoir of hepatitis A 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. There is no known carrier state. Rarely, chimpanzees, or even less frequently, other nonhuman primates may be reservoirs of the virus.[1,10]

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 hepatitis A incidence.[11-13] Mollusks may concentrate virus from areas with minimally polluted water and be a source of disease.[14,15] Food may also be 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 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.[16]

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 type A hepatitis among Americans and Europeans in developing countries suggests a non-person-to-person vehicle association.[16] Generally, except in definite disease outbreaks, any endemic hepatitis A that is spread via water is less than detectable epidemiologically.[11]

SUSCEPTIBILITY AND RESISTANCE

Susceptibility to hepatitis type A is general. Infants and small children have a low apparent attack rate, probably due to the frequency of mild and anicteric infections.[1] Clinical illness may occur in one out of ten hepatitis infections overall.[17] Homologous immunity after infection is generally lifelong.[1]

ENVIRONMENTAL PERSISTENCE

There is very little information on the persistence of hepatitis A in the environment. It is reported to resist 56° C for 1/2 h.[18] Water collected from a well 9 wk after the onset of a 6-wk outbreak of hepatitis A was stored at room temperature unprotected from light for 40 d before 7 L were ingested by each of five volunteers. Four of these developed hepatitis without overt jaundice.[19] Hepatitis A virus is thought to be retained by oysters for up to 2 mo after contamination.[20]

DOSE RESPONSE

At the time that human volunteer studies were performed on hepatitis, 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[19] 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 g feces per 18.5 L.

Two of three persons receiving orally 3 mL of acute serum developed the disease, as did 13 of 21 volunteers that were fed 1.5 to 5 mL of a 10% feces solution.[19] In recent (1983) studies to determine median infective dose in marmoset monkeys, virus was measured by fecal suspension, but no estimate was made of particle number.[21]

Hepatitis A is considered to be very much like enteric viruses in general behavior. Enteric viruses are excreted in concentrations as high as 10^{10} virus particles/g 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.[22,23] Because hepatitis A is considered to be an enterovirus-like particle, it may well occur in similar concentrations in feces and wastewater.

LATENCY

The incubation period of hepatitis A is related to the dose. The average incubation is 28 to 30 d but ranges from 8 to 60 d in the references cited. The most common range is 15 to 50 d.[1]

DISINFECTION

Treatment in the laboratory at pH 7.0 and room temperature for 30 min with 2 mg/L HOCl (free available) completely inactivated the virus when inoculated in marmoset monkeys, whereas 1.5 mg/L only increased the incubation period of the virus.[21]

Information on the behavior of hepatitis A virus in water-treatment processes is limited because practical technology for direct research on the virus is only now becoming available. Recent studies have indicated that no hepatitis A virus was detected after 30 min of breakpoint chlorination in heavily contaminated water, or 1 mg of total or 0.4 mg of free-chlorine residual/L after purification of the above water by coagulation, settling, and filtration through a diatomaceous-silica filter.[24] Specifications generally accepted for the disinfection of drinking-water supplies are expected to satisfactorily inactivate hepatitis A virus. These specifications require a free-available chlorine residual of 1 to 2 mg/L for 1 to 2 h at a pH of less than 8 and turbidity of less than one unit.[24] Some other viruses have been shown to survive this treatment,[11] and the efficacy of treatment on hepatitis A virus is not universally accepted.[25]

MONITORING METHODS

There is currently no standard method for monitoring hepatitis A in the environment. Hepatitis A is currently difficult to propagate, requiring complex tissue-culture methods and specially trained personnel.[26] This is an active area of ongoing research.

INDICATOR-PATHOGEN RELATIONSHIP

There is no reliable direct correlation between viruses and indicator organisms such as coliform bacteria, fecal streptococci, acid-fast bacteria, or coliphages. However, some of these organisms, particularly coliphages, can be useful indicators of the virucidal properties of water-treatment processes.[24] 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.[27]

CONCENTRATION IN THE ENVIRONMENT

There is virtually no information available on the numbers in which hepatitis A virus occurs in the water environment.[24] This is an area where more research is greatly needed. In temperate climates, hepatitis incidence peaks in the late fall and winter. As with many other waterborne diseases, in areas with poor sanitation, the incidence may increase following heavy rainfall or the onset of the rainy season.

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APPENDIX C: PARASITIC ORGANISMS

SECTION 1. Entamoeba histolytica

ETIOLOGY AND CLINICAL DISEASE

Entamoeba histolytica is a potentially invasive protozoan parasite. Although amebiasis (infection with E. histolytica) is usually asymptomatic, it can lead to both intestinal and extraintestinal disease. The clinical response is exceedingly variable and is most frequently intestinal, ranging from intermittent mild diarrhea with blood and mucus in the stools, alternating with constipation to fulminating dysentery with chills, fever, and severe diarrhea.[1] The chronic disease response leads to marked weight loss, wasting, and malnutrition.[2] Pathologic lesions may occur anywhere in the colon but are mainly found in the cecum, ascending colon, and rectum. Intestinal mucosa can become ulcerated and may slough off. Granulomatous lesions called ameboma may develop, which can be mistaken for carcinoma. Liver abscess is the most common extraintestinal presentation. Invasion may develop via amoebae carried from the submucosa by the portal veins. Abscesses may also occur in the diaphragm, lung, and pericardium from penetration by liver abscesses. Rarely, abscesses may form in any part of the body, such as brain, bladder, uterus, or skin.[3]

The infective form of Entamoeba histolytica is the resistant cyst. Persons are capable of spreading disease as long as they are shedding cysts, which may be for years.[1]

Secondary bacterial infections in intestine and liver abscess can and often do occur. The majority of infections, possibly up to 85%, are asymptomatic.[4-6] It is possible that virtually all strains may be avirulent in the intestine, and some sort of stimulus is needed for pathogenicity,[5] but this conversion stimulus is unknown. Subclinical invasion of the intestinal mucosa may be frequent.[6]

In Mexico at the present time, abscess is found at the rate of 2% in all adult patients and in 3 to 4% of autopsies. Before treatment was available in that country, the death rate for liver-abscess sufferers was 80%.[7] Amebiasis was the seventh most frequent cause of death in Guatemalan hospitals in 1974 and sixth most frequent in Mexico in 1970.[6] The etiology of this disease often goes unrecognized until autopsy or surgery,[4] because diagnosis can be difficult.[8]

OCCURRENCE

Entamoeba histolytica is found worldwide.[1,4,7] In general, tropical and subtropical areas have a higher attack rate than temperate countries. This appears to be due more to sanitation than to climate.[8] As a rule, more males are infected than females; this is particularly the case with abscess.[4,9] However, one study in Gambia demonstrated higher attack rates in women than in men.[10] The ratio of men to women with amebiasis in Mexico is 3:1.[7]

Morbidity and mortality increase with age.[6] Seventeen percent of mothers were infected in Sukuta, Gambia, but no babies 0 to 18 mo of age shed cysts.[11] Young children generally have a lower attack rate than that reported as the worldwide occurrence rate.[6,12-16] There may be a racial difference in attack rate[1]; this is confounded by living conditions. Natives of subtropical and tropical areas seem to tolerate the disease to a greater extent than nonnatives, but the carrier rate is greater in the natives.[9] Attack rates reported around the world are shown in Table C_1-1.

RESERVOIR

The reservoir of E. histolytica is the infected human. Probably the most important source is the asymptomatic carrier, although chronically ill persons are also infective.[8] About 50% of those infected pass cysts continually and, as stated above, they may be asymptomatic.[9] It is possible that some animals may form a reservoir, as cysts have been recovered from apes, monkeys, dogs, pigs, cattle, cats, and rats.[9] Even with this possibility, zoonotic transmission is probably not significant.[3,9] In a study in India, the close association between domestic animals and humans did not appear to be associated with the prevalence rate of amebic dysentery.[39]

MODE OF TRANSMISSION

Endemic spread of amebiasis is by the fecal-oral route; this can stem from hand-to-mouth fecal transfer and fecally contaminated raw vegetables, possibly by contaminated hands of food handlers and perhaps by water.[1] Flies can spread amebiasis; cysts can live in fly droppings for 48 h.[9] Epidemics generally stem from water contaminated with cysts from feces of infected persons.[1] Areas where a high level of cleanliness is difficult to maintain, such as mental hospitals, may have chronically high infection rates.[36] Sexual transmission by oral-rectal contact, particularly among male homosexuals, has been reported.[1,40]

Table C_1-1. Attack rates of Entamoeba histolytica.

Year of published report	Attack rate (no./1000)	Location	Description	Ref.
A: Attack rate of persons with symptoms				
1935	600	Chicago, IL	WO[a] firemen (exposed group)	17
1935	150	Chicago, IL	WO controls	17
1935	370-570	Chicago, IL	WO those with symptoms	17
1982	22-140	Mexico	Acute diarrhea and dysentery	7
1983	85	Seychelles	Outpatients, parasite symptoms	18
1983	19	Brazil	Children with diarrhea, <6 y old	12
1978	24	Manila	Poor children, ill	13
1978	0	Manila	Poor children, controls	13
1978	50	Portugal	Travelers with diarrhea	19
1983	5	Indonesia	Poor children with diarrhea, <3 y old	14
1983	40	Indonesia	Poor children controls, <3 y old	14
1983	10	Indonesia	Medium income, diarrhea	14
1983	50	Indonesia	Medium income, controls	14
1978	0	Houston, TX	Children with diarrhea	15
1978	0	Mexico	Children with diarrhea	15
1983	5.9	Bethesda, MD	Military personnel, 1946-57	20
1948	70-100	Tokyo	WO employees before	21
1948	222	Tokyo	WO employees after	21
1948	629	Tokyo	WO occupants of building	21
1955	524	Indiana	WO workers	22
1955	37	Indiana	WO family contacts	22
1956	507	Indiana	WO family stool sample	23
B: Infection, symptomatic plus asymptomatic				
1971	24	Bangkok	Middle class, stool survey	24
1964	170	Gambia	Stool survey; mothers, infants	11
1964	0	Gambia	Stool survey; infants <18 mo	11
1976	8.3	Mexico	Travelers, prospective study	25

Table C_1-1. (Continued)

Year of published report	Attack rate (no./1000)	Location	Description	Ref.
1976	99	Southern U.S.	Blacks and whites, total	26
1976	6	Southern U.S.	Whites	26
1976	60	Southern U.S.	Blacks and whites, total	26
1968	483	Brazil	Xavante Indians, stool survey	27
1970	2	Aspen, CO	Stool survey	28
1980	300–800	Amazon	Acculturating tribes	29
1980	140–280	Amazon	Newly encountered tribe	29
1963	260	Surinam	Reservoir area, villages	30
1963	70	Surinam	Reservoir area, city	30
1963	59	Los Angeles, CA	Commune	31
1983	70	Los Angeles, CA	Commune, returnees from India	31
1982	44	Venezuela	Poor children, 0–12 y; stool survey	32
1982	77	Venezuela	Poor children, 0–12 y; serological survey	32
1981	40	Venezuela	Stool survey	33
1977	255	Gambia	Whole country, city of Banjul	10
1977	155	Gambia	City of Banjul	10
1983	340	Bangladesh	Stool survey, 30–44 y old	34
1962	15–330	U.S.	American Indians; range	35
1962	149	U.S.	American Indians; mean	35
1974	170	U.S.	Mental institution patients, employees	36
1977	120	Texas	Extended family, stool survey	37
1977	457	Texas	Extended family, serological survey	37
1972	190	Europe	Attack rate	16
1972	120	The Americas	Attack rate	16
1972	170	Africa	Attack rate	16
1972	160	Asia	Attack rate	16
1972	15	U.S.	College students	16
1972	90	U.S.	Small town	16
1972	110	U.S.	Indian children	16

Table C_1-1. (Continued)

Year of published report	Attack rate (no./1000)	Location	Description	Ref.
1972	90	U.S.	Agricultural workers	16
1972	140	U.S.	Municipal sewage workers	16
1982	0	Mexico	U.S. students, visiting 4 wk	38
1972	137	India	Stool survey	39
1982	100	Worldwide	Overall attack rate	6
1935	116	U.S.	Mean of 18 stool surveys	17
1974	200-800	Worldwide	Amount asymptomatic	9
1983	30	U.S.	Countrywide estimate	2
1983	267	San Francisco, CA	Homosexual men	40
1983	7	San Francisco, CA	Stool survey	40

[a] WO = waterborne outbreak.

SUSCEPTIBILITY AND RESISTANCE

Susceptibility to amebiasis is general, but most persons infected with Entamoeba histolytica do not develop outright disease.[1] There is little indication of post-infection immunity in humans; reinfection is common.[1,6,8,9] The fact that severity and death from the disease increase with age is an indication that effective immune resistance is not acquired from previous infection.[6] Humoral antibodies are assumed to appear only after invasion, which can be subclinical. It is doubtful whether either the humoral or cellular immune reactions induced by infection confer protection,[6] except for the possible protective role of humoral antibodies in liver-abscess cases.[4]

Amebiasis is known to be exacerbated by immunosuppression. The disease is enhanced by hormonal alterations; pregnancy increases invasiveness. Malnutrition and particularly its concomitant weakening of resistance also enhance the development of amebiasis.[6] Infants, whose immune systems are not fully developed, are especially susceptible to fulminating forms of amebiasis when infected.[6]

ENVIRONMENTAL PERSISTENCE

The form of Entamoeba histolytica found in the environment is the cyst, a highly resistant structure. Cysts have been reported to survive for 153 d in distilled water at 12 to 22°C.[16] They can survive nearly 3 mo at 0°C, 1 mo at 10°C, 10 d at 20°C, and 3 d at 30°C in fresh water. Kept moist and in the shade at room temperature, they have lasted 9 to 21 d and 14 mo in cell cultures at 4°C.[9]

The cysts die rapidly in heavily polluted water but can survive 1 to 5 wk in water of low contamination.[9] The concentration of cysts decreases at a rate of 30% for each 10°C increase in temperature in sewage.[16] Cysts are resistant to drying, freezing, and acidic conditions,[41] but are killed by temperatures above 50°C, complete desiccation, sunlight, hyperchlorination, or extended exposure times in chlorinated water.[3]

The more fragile trophozoites can be passed out of the body during diarrheal phases of the disease, but they are short-lived.[8] There are conflicting reports of their ability to withstand the acid of the stomach,[8] making their ability to transmit infection unclear but doubtful. Optimum growth occurs at 35 to 37°C, at pH 7.0, and under reduced oxygen tension. Trophozoites can survive up to 5 h at 37°C and 96 h at 5°C under laboratory conditions.[2]

DOSE-RESPONSE RELATIONSHIP

The dose-response relationship for Entamoeba histolytica has not been determined. Feeding studies using humans were performed in 1913 on Philippine prisoners by Walker and Sellards;[42] however, cysts were not enumerated, and the data obtained are not useful. Animal studies have also not been effective in determining infective dose.

It appears that the massive and frequent doses acquired in endemic areas are of epidemiological importance; repeated large doses may be needed to infect people under constant exposure. Infection also is known to decrease with increased food intake, increased intestinal motility, and a decrease in the number of organisms.[2]

LATENCY

Acute intestinal amebiasis has an incubation period from 1 to 14 wk in general.[2] The onset can be insidious, and noticeable disease may take months to years to become apparent.[3,8]

DISINFECTION

Entamoeba histolytica cysts are quite resistant to chlorination. Sand filtration appears to be superior to chlorination in removal of E. histolytica cysts from raw water.[9,43] Trickling biofilters decrease the numbers of E. histolytica cysts by 88 to 99% in sewage.[44] A 1-h treatment with 5 mg/L free-available chlorine (FAC) in raw water is recommended for total destruction of cysts. A Cl_2 residual of 1 mg/L should be kept to be assured of the drinking water's safety.[9] The pH, temperature, turbidity, and contact time all affect the disinfection efficiency of chlorinating E. histolytica. Even when these factors are optimal, the level of free chlorine required for amebicidal activity is 3 mg/L, which is six times the recommended level of 0.5 mg/L for municipal drinking water.[45] At 30°C, pH 7, and 10-min contact time, 2 mg/L FAC residual was needed for 99% inactivation of cysts as measured by excystation methods. Under the same conditions, 2.5 mg/L was needed for 99.9% inactivation. Bromine was found to be a superior cysticide, compared with chlorine or iodine, over a pH range of 4 to 10. Iodine was the best disinfectant in the presence of ammonia.[20] Brady et al.[43] showed in 1943 that the recommended dose at that time (3.77 mg calcium hypochlorite/L) was insufficient to kill cysts in raw water. Thirty-nine percent of cysts survived this level after 20 min, and 83% survived 15 min. Up to 50% of cysts survived 56.6 mg calcium hypochlorite/L for 15 min or less, but none survived this chlorine level after 20 min. (It should be noted that addition of calcium hypochlorite to water may raise the pH of water to above optimum for disinfection; this may have affected the work of Brady et al.)

The cysts are known to survive 0.04% $HgCl_2$, 0.5% formalin, 1.0% phenol, and 2% potassium permanganate.[9]

MONITORING METHODS

Standard Methods for the Examination of Water and Wastewater[46] recommends two techniques to detect E. histolytica cysts in water and sewage samples:

1. Sample concentration: Use a membrane filter with a 7- to 10-μm pore size if turbidity is not too great. A sample size of 4 L or more is suggested. A direct microscopic examination of the above filter contents is made in a Sedgewick-Rafter counting cell under low-power magnification for identification and enumeration of cysts or trophozoites.

2. E. histolytica may be cultured on modified liver infusion medium. A 3- to 6-d incubation at 37°C should be followed by microscopic examination for trophozoites. A most-probable-number (MPN) approximation can be made by concentrating and culturing replicate sample portions.

INDICATOR-PATHOGEN RELATIONSHIP

As in the case of Giardia lamblia, there is frequently no apparent correlation between coliform numbers or other indicators and the presence of protozoan cysts.[47] Cysts of E. histolytica were found in 8% of water samples taken from open waste drains in Ibadan, Nigeria. The total coliforms measured in these drains were as high as 1.8×10^7/100 mL; the concentration of fecal coliforms measured as high as 10^2 to 1.8×10^4/100 mL, with an average concentration for all measurements of 2.7 to 4×10^3/100 mL.[48]

CONCENTRATION IN THE ENVIRONMENT

Entamoeba histolytica can be found in low densities in wastewater. Five cysts per liter were detected in raw sewage in Haifa, Israel, and 1 to 2/L in treated effluent.[16] In the Nile Delta region of Egypt, tap water was found to contain cysts in 63.6% of 7 samples and 55.1% of 59 samples of water stored in the earthenware storage jugs ("zirs") commonly used there.[41]

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SECTION 2. 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 G. lamblia 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 d to 3 mo or more.[3] The average duration of symptoms is reportedly 30 to 45 d,[2,4] but may be as short as 10 to 15 d.[5,6] Carriers can shed Giardia for years,[7] but usually self-cure occurs within 2 to 3 mo. In one study, only 2 of 56 (3.5%) infected persons were ill less than 10 d.[8] In another study, Barbout et al.[6] reported 9 of 59 (15.2%) clinical cases relapsed within 3 mo of treatment.

OCCURRENCE

Giardia lamblia is found worldwide. Infection is more frequent in children than adults, particularly among the group aged 6 to 10 y.[2] There is no apparent seasonal fluctuation of attack rate. See Table C_2-1 for reported attack rates.

Areas of the world known to be associated with increased risk of infection include Southeast and South Asia, West and Central Africa, Mexico, Korea, and Western South America.[33] Areas of relatively increased risk in the United States are usually mountainous and include New England, the Pacific Northwest, and the Rocky Mountains.

RESERVOIR

The major reservoir of Giardia is the infected human. The rate of asymptomatic infection can be high; in the Berlin, NH outbreak, 76% of cases were reported to be asymptomatic.[5] In an outbreak in Montana, the asymptomatic attack rate was 13% overall,[31] and a stool survey of persons without symptoms in Finland showed a 12%

Table C_2-1. Attack rates of Giardia lamblia.

Area	Attack rate (per 1000)	Comment	Ref.
Surinam	30–220	Stool survey	9
Dominican Republic	7	Stool survey	10
Amazon	200–260	Tribes; acculturated	11
Amazon	40–50	Unacculturated	11
Mexico	30–60	Students; U.S.	12
Mexico	110–180	Latin	12
Iran	140	Stool survey	13
Finland	80	Asymptomatic stool survey	3
Gambia	124	Stool survey	14
Gambia	10	Infants, 1 y old	14
Gambia	170	Children, 5 y old	14
Gambia	90	Mothers of children 1 y old	14
India	210	Children, 5–9 y old	15
Brazil	67	Indians, stool survey	16
Brazil	331	Children with diarrhea, < 6 y old	17
Manila	30	Poor children	18
Rome	24	Children, stool survey	19
Bangladesh	420–820	Infants, survey, 1.5 y	20
Bangladesh	820	Mothers, survey	20
Israel	300	Children, 3 mo–3 y	21
Worldwide	74	Worldwide, 3 mo–3 y	21
Tokyo	460	Waterborne outbreak, employee	22
Tokyo	762	Waterborne outbreak, residents	22
U.S.	23–240	Stool survey, Indians	23
U.S.	44	Stool survey	24
U.S., south	31	Stool survey	25
U.S.	15.3	Stool survey of children, 11 y old	26
U.S.	109	Outbreak	27
U.S.	53	Controls	27
U.S.	90	Mental institution, patients	28
U.S.	70	Mental institution, employees	28
U.S.	45	Extended Chicano family	29

Table C_2-1. (Continued)

Area	Attack rate (per 1000)	Comment	Ref.
Colorado	50	Stool survey, Aspen, CO	30
Montana	330	Outbreak, residents	31
Montana	130	Outbreak, asymptomatic	31
New Hampshire	460	Outbreak, town	5
New Hampshire	85	Outbreak, controls	5
Utah	98	Outbreak	32
Washington, DC	70	Outbreak	15
Indiana	40	Outbreak	15
New York, NY	20	Outbreak	15
Aspen, CO	50	Outbreak	15
Atlanta, GA	50	Outbreak	15
Minnesota	150	Outbreak	15
Hawaii	45	Outbreak	15
Boston, MA	220	Outbreak	15
Baltimore, MD	160	Outbreak	15
Philadelphia, PA	80-120	Children	15
Wyoming	220	Native American children	15

Giardia prevalence rate.[3] Beavers may be a reservoir and have been implicated in waterborne outbreaks.[31,34,35] Dogs, gerbils, guinea pigs, beavers, raccoons, and bighorn sheep have been experimentally infected with G. lamblia,[36] and muskrats in the Detroit watershed were found to be infected.[37]

MODE OF TRANSMISSION

The most common mode of transmission is from contaminated water supplies.[1,33,38] Twenty-three waterborne outbreaks of giardiasis were reported in the U.S. in the years 1965 to 1978, affecting 7000 persons. Outbreaks generally involve small municipal systems, semipublic systems, or untreated water, with only chlorine for

treatment, or no treatment at all.[39] Foodborne outbreaks have been reported.[40] Hand-to-mouth transfer of cysts from the feces of infected individuals occurs, especially in day-care centers[1] and institutions[28] and also via anal contact during sex.[1,41]

SUSCEPTIBILITY AND RESISTANCE

The mechanisms that protect humans from infection with Giardia are largely unknown.[42] There appears to be some sort of partial immunity. In a study in Bangladesh, first infections led to clinical symptoms, but most later infections had no symptoms.[20] Members of a group of campers in Utah who drank untreated mountain water 2 mo previous to an outbreak there had, upon renewed exposure, a lower attack rate than those who had not been previously exposed.[6] Residents living in Aspen, CO, for more than 2 y prior to a local outbreak had a lower attack rate than newcomers.[30] Humoral immunity possibly plays a role in host defense,[41,43] and nonimmune factors may influence duration, incidence, and severity of giardiasis.[44] Acquired resistance in mice to Giardia muris has been demonstrated.[42,45] Human milk may play a role in protection of exposed infants,[44] but the protection is not clear-cut.[20]

ENVIRONMENTAL PERSISTENCE

Giardia lamblia generally forms a resistant cyst before leaving the intestine; this is the form found in the environment.[7] Trophozoites, which may be passed in severely diarrheic feces, do not survive.[41] Giardia cysts survive for the longest time in cold water; 5°C appears to be optimal.[46] Cysts have survived for up to 10 mo in fresh water at 8°C, and 1 mo in fresh water at 21°C.[46] Cysts survived 32 d in fresh water at room temperature.[47] They cannot tolerate freezing. Cysts are at optimum viability at pH 6 to 8.[46]

DOSE-RESPONSE RELATIONSHIP

Rendtorff and Holt[47] and Rendtorff[48] performed a series of feeding studies on prison volunteers in 1954. The results are shown in Table C_2-2. It should be noted that Table C_2-2 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 the studies, 64.7% of men fed 100 cysts stored 0 to 16 d became infected[47] with no decrease in infectivity over cyst-storage time. A reexamination of Rendtorff's data, presented by the same author in 1978,[4] attributed the low infectivity of the 25-cyst

Table C_2-2. Dose response for Giardia lamblia.[47,48]

No. cysts given	No. exposed	No. 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

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-mo 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.

LATENCY

The incubation period is variable. In experimental infections, incubation ranged from 6 to 22 d.[1] Latency has been reported to range from 3 to 56 d with an average of 7 to 9 d.[3,39,49]

DISINFECTION

Studies have been performed on the resistance of Giardia to several disinfectants. Table C_2-3 shows disinfection by chlorine,[46] and Table C_2-4 displays the effects of various emergency water-disinfectant-treatment methods.[5] The cysts of this parasite are, relative to bacteria and viruses, very resistant to the effects of chlorine in water.

Table C_2-3. Disinfection of *Giardia* cysts with chlorine.[40]

Temperature (°C)	pH	Chlorine (mg/L)	Time (min)	Percent killed
25	6,7,8	1.5	10	100
15	6	2.5	10	100
15	7,8	3.0	10	100
15	7,8	2.5	30	99.5
15	7,8	2.5	60	100
15	7,8	2.5	10	98
5	6,7	1.0	30	90
5	6	1.0	60	99.8
5	7	1.0	60	98
5	8	1.0	30	80
5	8	1.0	60	95
5	6	2	10	90
5	6,7	2	30	95
5	6,7	2	60	99.9
5	7,8	2	10	80
5	8	2	30	93
5	6	2	60	99.8
5	6	4	10	96
5	6	4	30	99.6
5	6,7,8	4	60	99.9
5	7	4	10	92
5	7,8	4	30	98
5	8	4	10	90
5	6,7	8	10	99.9
5	8	8	30	99.9
5	8	8	10	99.7

Table C_2-4. Disinfection of Giardia cysts by emergency methods (% killed in water).[50]

Treatment	Temperature			
	3°C		20°C	
	Water type			
	Clear	Cloudy	Clear	Cloudy
Control	0.0	0.0	0.0	0.0
Halazone	> 99.8	> 99.8	>99.8	> 99.8
Chlorine bleach[a]	> 99.8	91.7	> 99.8	> 99.8
Globaline[a,b]	> 99.8	97.5	> 99.8	> 99.8
EDWGT[c,b]	> 99.8	> 99.8	> 99.8	> 99.8
Iodine (2% tincture)	> 99.8	74.6	> 99.8	> 99.8
Iodine (saturated)	77.3	96.5	> 99.8	> 99.8

[a] Contents not specified in this paper.

[b] Used as recommended by manufacturer.

[c] Emergency drinking-water germicide tablet (EPA Reg. No. 34161-1-37257), contents not specified.

Giardia cysts were found to be very resistant to UV irradiation. Less than 90% of cysts were killed by up to 63,000 $\mu W \cdot s/cm^2$, whereas Escherichia coli was inactivated 99.9% by a UV level of 3000 $\mu W \cdot s/cm^2$. Commercial UV treatment units usually cannot attain the high doses necessary to kill Giardia cysts.[51]

In the process of treatment of municipal drinking water from sources possibly contaminated with Giardia cysts, it is necessary to filter the water after chlorination to be certain the cysts have been eliminated.[39]

MONITORING METHODS

Standard Methods for the Examination of Water and Wastewater[52] states that the following method should be considered tentative since recovery-efficiency data are limited. A yarn-wound Orlon filter apparatus is used, and a suggested volume of 1900 L, collected over 18 to 24 h, is filtered. The filter itself is homogenized, the homogenate is filtered through a coarse screen, and excess liquid is squeezed out. The filtrate is settled, decanted, and either filtered or flocculated with HCl and formalin and then refiltered. Filter washings are stained with Lugol's iodine and scanned under 10X magnification for

Giardia cysts. Suspect cysts are confirmed under 43X examination. It is suggested that three preparations per sample be examined, and if all three contain no cysts, to consider the sample negative. The Environmental Protection Agency has determined the Orlon filter method to be 58% efficient.[53]

Because microscopic methods require that only a very small number of organisms be examined, efficiency of Giardia detection methods can be very low. Some samples containing less than 4,000 cysts/L (a high concentration) may not be detected at all.[54]

Although trophozoites can be cultured, there is no in vitro method for cultivating Giardia cysts at present. Animal testing is one method of testing infectivity of Giardia cysts, but requires a specialized laboratory.[52] Excystment can be achieved under laboratory conditions and is often used as an indicator of viability in chlorination studies.[46,55]

INDICATOR-PATHOGEN RELATIONSHIP

Frequently there is no apparent correlation between coliform numbers and the presence of cysts.[35] 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.[39] 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.[6] (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.[31]

CONCENTRATION IN THE ENVIRONMENT

Concentration in the environment can vary widely. This may reflect intermittent contamination of water, poor sampling recovery efficiency, insufficient sample volume, or sampling frequency.[53] In an outbreak in Rome, NY, only one cyst was isolated from 1 million liters of raw water from the plant intake.[39] This is about one acre-foot of water, a tremendously large amount to sample. At the Androscoggin Water Treatment Plant associated with the Berlin, NH, outbreak, three cysts were recovered per 100 mL treated water.[53] Fifteen percent of water samples collected in open drains in the city of Ibadan, Nigeria, contained Giardia cysts.[49]

Jakubowski and Erickson[53] estimated that raw sewage may contain from 96,000 to 2,400,000 cysts/L when 1 to 25% of the population is infected. Infected persons may shed 1,000,000 cysts/g of feces.[52] In some outbreaks, no cysts have been recovered at all.

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APPENDIX D

UNCERTAINTIES AND RESEARCH RECOMMENDATIONS

During the process of gathering and reviewing information on the disease organisms discussed in Appendices A through C, it became apparent that there are several areas where more information is needed. Table D_1-1 presents a summary of the key areas where insufficient or no information is available, thereby identifying data gaps and potential areas for future research.

Review of Table D_1-1 for bacterial organisms indicates the following:

1. For the more recently identified etiologic agents of waterborne diarrhea reviewed in this text, such as Yersinia spp. and Campylobacter spp., all categories of research need to be explored or improved. There are no adequate enumeration techniques or monitoring methods, the fate or role of these organisms in the environment is not well-defined, and the effectiveness of disinfectants in the control of these agents should be studied further. The clinical symptomology and pathogenicity of these organisms have recently been described; however, there have been few studies concerning the parameters of dose response, latency, or immunity.
2. Information on the other bacterial pathogens reviewed (i.e., Salmonella spp., S. typhi, Shigella spp., V. cholerae, E. coli) is generally available in some detail, but needs better definition in order to improve the confidence of the risk estimates. Clinically, much information has been collected on these organisms because they have been of major concern for many years. Areas such as occurrence and carrier rates, concentration in raw water, and secondary attack rates still need to be better defined. Vaccine development is progressing for cholera, typhoid, and pathogenic E. coli, but the vaccines currently offer limited protection and variable efficacy.
3. One of the most important but neglected areas is the relationship between indicators and pathogens. Frequently, the correlation between coliform numbers in water and numbers of pathogens or the disease rate in those exposed to contaminated water is confused and incomplete. It was also noted that a serious question exists as to the advisability of using coliforms as indicators of water quality in tropical areas of the world. Research is needed to (a) demonstrate which microorganism(s) would best serve as indicators of water quality under a variety of conditions; (b) determine the relationship between indicator organisms and the numbers of infectious agents that may be present; and (c) develop methods for the rapid detection and enumeration

Table D_1-1. Summary of potential topics for future research and indication of research areas where adequate data are available. NOTE: (--) = few to no data; (+) = limited data, needs improvement; (++) = adequate data available.

Organism	Occurrence in water	Dose response	Latency	Environmental persistence	Disinfection	Monitoring method	Indicator-pathogen	Environmental concentration
Salmonella spp.	+	+	++	+	+	+	--	+
S. typhi	+	++	++	+	+	+	+	+
Shigella spp.	+	+	++	+	+	+	--	--
V. cholerae	+	++	++	+	+	+	+	+
E. coli	+	++	++	+	+	+	--	--
Yersinia spp.	+	--	--	--	--	--	--	--
Campylobacter spp.	+	--	--	--	--	--	--	--
Hepatitis A	--	--	++	--	+	--	--	--
Enteroviruses	+	+	++	++	++	+	+	+
Rotavirus	--	--	++	--	--	--	--	--
Norwalk agent	--	--	++	--	--	--	--	--
E. histolytica	--	--	++	+	+	+	--	--
G. lamblia	+	+	++	+	++	+	+	+

in water of appropriate indicators or specific pathogens. These data are essential to improving the confidence of disease-risk estimates based on water-quality criteria.

4. There is limited information concerning the survival of bacterial pathogens in water under various environmental conditions (pH, temperature, salinity, organic loading, effect of indigenous microflora, etc.). Data concerning the environmental concentration of bacterial pathogens in water systems are generally inadequate. Additional research is needed to monitor seasonal or annual fluctuations and the effects of rural, suburban, and urban areas on bacterial pathogen concentrations and survival rates in priority waters in selected geographic areas.

Review of Table D_1-1 for viral and parasitic pathogens indicates the following:

1. There is little to no information available on human dose-response relationships, occurrence and concentration values in water, and indicator organism-pathogen relationships for the organisms reviewed in this study. Additional research should be conducted in these areas to improve our understanding of these organisms, as well as confidence in the estimates of risk.
2. Monitoring techniques are not available for the isolation and enumeration of hepatitis A, Norwalk agent, and rotavirus, all of which appear to be the most important viruses associated with waterborne diseases. Additionally, the techniques available for isolating and enumerating E. histolytica and G. lamblia are difficult, time-consuming, and require highly trained laboratory personnel to perform. Research should be performed to develop reasonable quantitative techniques for rapidly monitoring these organisms in the environment.
3. Reliable information is needed concerning the survival rates in water and during water treatment of the above-mentioned viruses and parasites.

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