Guidelines for Drinking-Water Quality - Second Edition - Volume 2 - Health Criteria and Other Supporting Information

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

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Preface

In 1984 and 1985, the World Health Organization (WHO) published the first edition of *Guidelines for drinking-water quality* in three volumes. The development of these guidelines was organized and carried out jointly by WHO headquarters and the WHO Regional Office for Europe (EURO).

In 1988, the decision was made within WHO to initiate the revision of the guidelines. The work was again shared between WHO headquarters and EURO. Within headquarters, both the unit for the Prevention of Environmental Pollution (PEP) and the ILO/UNEP/WHO International Programme on Chemical Safety (IPCS) were involved, IPCS providing a major input to the health risk assessments of chemicals in drinking-water.

The revised guidelines are being published in three volumes. Guideline values for various constituents of drinking-water are given in Volume 1, *Recommendations* together with essential information required to understand the basis for the values. Volume 2, *Health criteria and other supporting information,* contains the criteria monographs prepared for each substance or contaminant; the guideline values are based on these. Volume 3, *Surveillance and control of community supplies,* is intended to serve a very different purpose; it contains recommendations and information concerning what needs to be done in small communities, particularly in developing countries, to safeguard their water supplies.

The preparation of the current edition of the *Guidelines for drinking-water quality* covered a period of four years and involved the participation of numerous institutions, over 200 experts from nearly 40 different developing and developed countries and 18 meetings of the various coordination and review groups. The work of these institutions and scientists, whose names appear in Annex 1, was central to the completion of the guidelines and is much appreciated.

For each contaminant or substance considered, a lead country prepared a draft document evaluating the risks for human health from exposure to the contaminant in drinking-water. The following countries prepared such evaluation documents: Canada, Denmark, Finland, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom of Great Britain and Northern Ireland and United States of America.

Under the responsibility of a coordinator for each major aspect of the guidelines, these draft evaluation documents were reviewed by several scientific institutions and selected experts, and comments were incorporated by the coordinator and author prior to submission for final evaluation by a review group. The review group then took a decision as to the health risk assessment and proposed a guideline value.

During the preparation of draft evaluation documents and at the review group meetings, careful consideration was always given to previous risk assessments carried out by IPCS, in its Environmental Health Criteria monographs, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues, and the joint FAO/WHO Expert Committee on Food Additives, which evaluates contaminants such as lead and cadmium in addition to food additives.

It is clear that not all the chemicals that may be found in drinking-water were evaluated in developing these guidelines. Chemicals of importance to Member States which have not been evaluated should be brought to the attention of WHO for inclusion in any future revision.

It is planned to establish a continuing process of revision of the *Guidelines for drinking-water quality* with a number of substances of agents subject to evaluation each year. Where appropriate, addenda will be issued, containing evaluations of new substances or substances already evaluated for which new scientific information has become available. Substances for

which provisional guideline values have been established will receive high priority for re-evaluation.

Acknowledgements

Acronyms and abbreviations used in the text

AAS	atomic absorption spectrometry
A/C	asbestos-cement
ADA	ampicillin-dextrin agar
ADI	acceptable daily intake
a.i.	active ingredient
AIDS	acquired immunodeficiency syndrome
ALAD	aminolaevulinic acid dehydratase
ALAT	alanine aminotransferase
AOC	assimilable organic carbon
APHA	American Public Health Association
BOD	biochemical oxygen demand
Bq	Becquerel
BSP	bromosulfophthalein
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
cfu	colony-forming units
CHO	Chinese hamster ovary
CMC	carboxymethyl cellulose
DENA	diethylnitrosamine
DMAA	dimethylarsinic acid
DNA	deoxyribonucleic acid
DOPA	3,4-dihydroxyphenylalanine
ECG	electrocardiogram
EDTA	edetic acid
EEG	electroencephalogram
EIEC	enteroinvasive *E. coli*
EP	erythrocyte protoporphyrin
EPA	Environmental Protection Agency (USA)
ETEC	enterotoxigenic *E. coli*
FAO	Food and Agriculture Organization of the United Nations
FPD	flame photometric detection
GC	gas chromatography
GCI	general cognitive index
GEMS	Global Environment Monitoring System
GOT	glutamic-oxaloacetic transaminase
GPT	glutamic-pyruvic transaminase
h	hour
HD	Hodgkin disease
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
ICRP	International Commission on Radiological Protection
ID	infective dose
Ig	immunoglobulin
IgG	immunoglobulin G

IgM	immunoglobulin M
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
IQ	intelligence quotient
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC_{50}	lethal concentration, median
LD_{50}	lethal dose, median
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LT	heat-labile enterotoxin
MAC	*Mycobacterium avium* complex
MAIS	*Mycobacterium avium, M. intracellulare, M. scrofulaceum* complex
MDI	mental development index
MFL	million fibres per litre
MIB	2-methylisoborneol
MMAA	monomethylarsonic acid
MNCV	motor nerve conduction velocity
MS	mass spectrometry
MSCA	McCarthy Scales of Children's Abilities
MTD	maximum tolerated dose
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NAG	non-agglutinable
NCI	National Cancer Institute (USA)
NCV	non-cholera vibrios
NEU	nitrosoethylurea
NHANES	US National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NOAEL	no-observed-adverse-effect level
NTA	nitrilotriacetic acid
NTP	National Toxicology Program (USA)
NTU	nephelometric turbidity unit
Pa	Pascal
PDI	psychomotor development index
pK_a	log acid dissociation constant
PMTDI	provisional maximum tolerable daily intake
PTWI	provisional tolerable weekly intake
PVC	polyvinyl chloride
RNA	ribonucleic acid
SAED	selected-area electron diffraction
SAP	serum alkaline phosphatase
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SMR	standardized mortality ratio
ST	heat-stable enterotoxin
STS	soft tissue sarcoma
T_3	triiodothyronine

T_4	thyroxine
TCU	true colour unit
TDI	tolerable daily intake
TDS	total dissolved solids
TEM	transmission electron microscopy
TOC	total organic carbon
TPA	tetradecanoyl-phorbol-acetate
UNEP	United Nations Environment Programme
UV	ultraviolet
WHA	World Health Assembly
WHO	World Health Organization

1. Introduction

This volume of the *Guidelines for drinking-water quality* explains how guideline values for drinking-water contaminants are to be used, defines the criteria used to select the various chemical, physical, microbiological, and radiological contaminants included in the report, describes the approaches used in deriving guideline values, and presents, in the form of brief monographs, critical reviews and evaluations of the effects on human health of the substances or contaminants examined.

This edition of the *Guidelines* considers many drinking-water contaminants not included in the first edition. It also contains revised guideline values for many of the contaminants included in the first edition, which have been changed as a result of new scientific information. The guideline values given here supersede those in the 1984 edition.

Although the number of chemical contaminants for which guideline values are recommended is greater than in the first edition, it is unlikely that all of these chemical contaminants will occur in all water supplies or even in all countries. Care should therefore be taken in selecting substances for which national standards will be developed. A number of factors should be considered, including the geology of the region and the types of human activities that take place there. For example, if a particular pesticide is not used in the region, it is unlikely to occur in the drinking-water.

In other cases, such as the disinfection by-products, it may not be necessary to set standards for all of the substances for which guideline values have been proposed. If chlorination is practised, the trihalomethanes, of which chloroform is the major component, are likely to be the main disinfection by-products, together with the chlorinated acetic acids in some instances. In many cases, control of chloroform levels and, where appropriate, trichloroacetic acid will also provide an adequate measure of control over other chlorination by-products.

In developing national standards, care should also be taken to ensure that scarce resources are not unnecessarily diverted to the development of standards and the monitoring of substances of relatively minor importance.

Several of the inorganic elements for which guideline values have been recommended are recognized to be essential elements in human nutrition. No attempt has been made here to define a minimum desirable concentration of such substances in drinking-water.

1.1 General considerations

The primary aim of the *Guidelines for drinking-water quality* is the protection of public health. The guidelines are intended to be used as a basis for the development of national standards that, if properly implemented, will ensure the safety of drinking-water supplies through the elimination, or reduction to a minimum concentration, of constituents of water that are known to be hazardous to health. It must be emphasized that the guideline values recommended are not mandatory limits. In order to define such limits, it is necessary to consider the guideline values in the context of local or national environmental, social, economic, and cultural conditions.

The main reason for not promoting the adoption of international standards for drinking-water quality is the advantage provided by the use of a risk-benefit approach (qualitative or quantitative) to the establishment of national standards and regulations. This approach should lead to standards and regulations that can be readily implemented and enforced. For example, the adoption of drinking-water standards that are too stringent could limit the availability of water supplies that meet those standards - a significant consideration in regions of water shortage. The standards that individual countries will develop can thus be influenced by national priorities and economic factors. However, considerations of policy and convenience must never be allowed to endanger public health, and the implementation of standards and regulations will require suitable

facilities and expertise as well as the appropriate legislative framework.

The judgement of safety - or what is an acceptable level of risk in particular circumstances - is a matter in which society as a whole has a role to play. The final judgement as to whether the benefit resulting from the adoption of any of the guideline values given here as standards justifies the cost is for each country to decide. What must be emphasized is that the guideline values have a degree of flexibility and enable a judgement to be made regarding the provision of drinking-water of acceptable quality.

Water is essential to sustain life, and a satisfactory supply must be made available to consumers. Every effort should be made to achieve a drinking-water quality as high as practicable. Protection of water supplies from contamination is the first line of defence. Source protection is almost invariably the best method of ensuring safe drinking-water and is to be preferred to treating a contaminated water supply to render it suitable for consumption. Once a potentially hazardous situation has been recognized, however, the risk to health, the availability of alternative sources, and the availability of suitable remedial measures must be considered so that a decision can be made about the acceptability of the supply.

As far as possible, water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, and protozoan pathogens and helminth parasites. Failure to provide adequate protection and effective treatment will expose the community to the risk of outbreaks of intestinal and other infectious diseases. Those at greatest risk of waterborne disease are infants and young children, people who are debilitated or living under unsanitary conditions, the sick, and the elderly. For these people, infective doses are significantly lower than for the general adult population.

The potential consequences of microbial contamination are such that its control must always be of paramount importance and must never be compromised.

The assessment of the risks associated with variations in microbial quality is difficult and controversial because of insufficient epidemiological evidence, the number of factors involved, and the changing interrelationships between these factors. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human and animal excreta. Microbial risk can never be entirely eliminated, because the diseases that are waterborne may also be transmitted by person-to-person contact, aerosols, and food intake; thus, a reservoir of cases and carriers is maintained. Provision of a safe water supply in these circumstances will reduce the chances of spread by these other routes. Waterborne outbreaks are particularly to be avoided because of their capacity to result in the simultaneous infection of a high proportion of the community.

The health risk due to toxic chemicals in drinking-water differs from that caused by microbiological contaminants. There are few chemical constituents of water that can lead to acute health problems except through massive accidental contamination of a supply. Moreover, experience shows that, in such incidents, the water usually becomes undrinkable owing to unacceptable taste, odour, and appearance.

The fact that chemical contaminants are not normally associated with acute effects places them in a lower priority category than microbial contaminants, the effects of which are usually acute and widespread. Indeed, it can be argued that chemical standards for drinking-water are of secondary consideration in a supply subject to severe bacterial contamination.

The problems associated with chemical constituents of drinking-water arise primarily from their ability to cause adverse health effects after prolonged periods of exposure; of particular concern are contaminants that have cumulative toxic properties, such as heavy metals, and substances that are carcinogenic.

It should be noted that the use of chemical disinfectants in water treatment usually results in the formation of chemical by-products, some of which are potentially hazardous. However, the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection should not be compromised in attempting to control such by-products.

The radiological health risk associated with the presence of naturally occurring radionuclides in drinking-water should also be taken into consideration, although the contribution of drinking-water to total ambient exposure to these radionuclides is very small under normal circumstances. The guideline values recommended in this volume do not apply to water supplies contaminated during emergencies arising from accidental releases of radioactive substances to the environment.

In assessing the quality of drinking-water, the consumer relies principally upon his or her senses. Water constituents may affect the appearance, odour, or taste of the water, and the consumer will evaluate the quality and acceptability of the water on the basis of these criteria. Water that is highly turbid, is highly coloured, or has an objectionable taste or odour may be regarded by consumers as unsafe and may be rejected for drinking purposes. It is therefore vital to maintain a quality of water that is acceptable to the consumer, although the absence of any adverse sensory effects does not guarantee the safety of the water.

Countries developing national drinking-water limits or standards should carefully evaluate the costs and benefits associated with the control of aesthetic and organoleptic quality. Enforceable standards are sometimes set for contaminants directly related to health, whereas recommendations only are made for aesthetic and organoleptic characteristics. For countries with severely limited resources, it is even more important to establish priorities, and this should be done by considering the impact on health in each case. This approach does not underestimate the importance of the aesthetic quality of drinking-water. Source water that is aesthetically unsatisfactory may discourage the consumer from using an otherwise safe supply. Furthermore, taste, odour, and colour may be the first indication of potential health hazards.

Many parameters must be taken into consideration in the assessment of water quality, such as source protection, treatment efficiency and reliability, and protection of the distribution network (e.g., corrosion control). The costs associated with water quality surveillance and control must also be carefully evaluated before developing national standards.

1.2 The nature of the guideline values

Guideline values have been set for potentially hazardous water constituents and provide a basis for assessing drinking-water quality.

(a) A guideline value represents the concentration of a constituent that does not result in any significant risk to the health of the consumer over a lifetime of consumption.

(b) The quality of water defined by the *Guidelines for drinking-water quality* is such that it is suitable for human consumption and for all domestic purposes, including personal hygiene. However, water of a higher quality may be required for some special purposes, such as renal dialysis.

(c) When a guideline value is exceeded, this should be a signal: (i) to investigate the cause with a view to taking remedial action; (ii) to consult with, and seek advice from, the authority responsible for public health.

(d) Although the guideline values describe a quality of water that is acceptable for lifelong consumption, the establishment of these guideline values should not be regarded as implying that the quality of drinking-water may be degraded to the recommended level. Indeed, a

continuous effort should be made to maintain drinking-water quality at the highest possible level.

(e) Short-term deviations above the guideline values do not necessarily mean that the water is unsuitable for consumption. The amount by which, and the period for which, any guideline value can be exceeded without affecting public health depends upon the specific substance involved. It is recommended that when a guideline value is exceeded, the surveillance agency (usually the authority responsible for public health) should be consulted for advice on suitable action, taking into account the intake of the substance from sources other than drinking-water (for chemical constituents), the toxicity of the substance, the likelihood and nature of any adverse effects, the practicability of remedial measures, and similar factors.

(f) In developing national drinking-water standards based on these guideline values, it will be necessary to take account of a variety of geographical, socio-economic, dietary, and other conditions affecting potential exposure. This may lead to national standards that differ appreciably from the guideline values.

(g) In the case of radioactive substances, screening values for gross alpha and gross beta activity are given, based on a reference level of dose.

It is important that recommended guideline values are both practical and feasible to implement as well as protective of public health. Guideline values are not set at concentrations lower than the detection limits achievable under routine laboratory operating conditions. Moreover, guideline values are recommended only when control techniques are available to remove or reduce the concentration of the contaminant to the desired level.

In some instances, *provisional* guideline values have been set for constituents for which there is some evidence of a potential hazard but where the available information on health effects is limited. Provisional guideline values have also been set for substances for which the calculated guideline value would be (i) below the practical quantification level, or (ii) below the level that can be achieved through practical treatment methods. Finally, provisional guideline values have been set for certain substances when it is likely that guideline values will be exceeded as a result of disinfection procedures.

Aesthetic and organoleptic characteristics are subject to individual preference as well as social, economic, and cultural considerations. For this reason, although guidance can be given on the levels of substances that may be aesthetically unacceptable, no guideline values have been set for such substances where they do not represent a potential hazard to health.

The recommended guideline values are set at a level to protect human health; they may not be suitable for the protection of aquatic life. The guidelines apply to bottled water and ice intended for human consumption but do not apply to natural mineral waters, which should be regarded as beverages rather than drinking-water in the usual sense of the word. The Codex Alimentarius Commission has developed Codex standards for such mineral waters.[1]

[1] Codex Alimentarius Commission. *Codex standards for natural mineral waters.* Rome, Food and Agriculture Organization of the United Nations, 1994 (Codex Alimentarius Vol. XI, Part III).

1.3 Criteria for the selection of health-related drinking-water contaminants

The recognition that faecally polluted water can lead to the spread of microbial infections has led to the development of sensitive methods for routine examination to ensure that water intended for human consumption is free from faecal contamination. Although it is now possible to detect the presence of many pathogens in water, the methods of isolation and enumeration are often complex and time-consuming. It is therefore impracticable to monitor drinking-water for every

possible microbial pathogen. A more logical approach is the detection of organisms normally present in the faeces of humans and other warm-blooded animals as indicators of faecal pollution, as well as of the efficacy of water treatment and disinfection. The various bacterial indicators used for this purpose are described in Chapter 9. The presence of such organisms indicates the presence of faecal material and, hence, that intestinal pathogens could be present. Conversely, their absence indicates that pathogens are probably also absent.

Thousands of organic and inorganic chemicals have been identified in drinking-water supplies around the world, many in extremely low concentrations. The chemicals selected for the development of guideline values include those considered potentially hazardous to human health, those detected relatively frequently in drinking-water, and those detected in relatively high concentrations.

Some potentially hazardous chemicals in drinking-water are derived directly from treatment chemicals or construction materials used in water supply systems. Such chemicals are best controlled by appropriate specifications for the chemicals and materials used. For example, a wide range of polyelectrolytes are now used as coagulant aids in water treatment, and the presence of residues of the unreacted monomer may cause concern. Many polyelectrolytes are based on acrylamide polymers and co-polymers, in both of which the acrylamide monomer is present as a trace impurity. Chlorine used for disinfection has sometimes been found to contain carbon tetrachloride. This type of drinking-water contamination is best controlled by the application of regulations governing the quality of the products themselves rather than the quality of the water. Similarly, strict national regulations on the quality of pipe material should avoid the possible contamination of drinking-water by trace constituents of plastic pipes. The control of contamination of water supplies by *in situ* polymerized coatings and coatings applied in a solvent requires the development of suitable codes of practice, in addition to controls on the quality of the materials used.

Part 1. Microbiological aspects

2. Microbiological aspects: introduction

The most common and widespread health risk associated with drinking-water is contamination, either directly or indirectly, by human or animal excreta, particularly faeces. If such contamination is recent, and if those responsible for it include carriers of communicable enteric diseases, some of the pathogenic microorganisms that cause these diseases may be present in the water. Drinking the water, or using it in food preparation, may then result in new cases of infection.

The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis, or typhoid fever. Most of them are widely distributed throughout the world. Faecal contamination of drinking-water is only one of several faeco-oral mechanisms by which they can be transmitted from one person to another or, in some cases, from animals to people.

One ingested waterborne pathogen, namely guinea worm *(Dracunculus medinensis)*, is not faecal in origin and deserves special mention. Although it is of limited geographical distribution, this helminth is of major public health importance in endemic areas. It is the only human infection that is solely transmitted by the waterborne route, and the World Health Assembly has committed itself to the eradication of dracunculiasis by the end of 1995 (resolution WHA 44.5, 1991).

Other pathogens cause infection when water containing them is used for bathing or for recreation involving water contact, rather than by the oral route. Some may also cause infection by inhalation when they are present in large numbers in water droplets, such as those produced by showers and some air-conditioning systems or in the irrigation of agricultural land.

It is not only by causing infection that microorganisms in drinking-water can affect human health. In some circumstances, cyanobacteria can produce toxins that may remain in the water even when the cyanobacteria themselves have been removed. Finally, there are some organisms whose presence in water is a nuisance, but which are of no significance for public health.

2.1 Agents of significance

The human pathogens potentially transmitted in drinking-water are listed in Table 2.1. Some general information on those included in the table is given below.

Table 2.1 Waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Main route of exposure[a]	Persistence in water supplies[b]	Resistance to chlorine[c]	Relative infective dose[d]	Important animal reservoir
Bacteria						
Campylobacter jejuni, C. coli	High	O	Moderate	Low	Moderate	Yes
Pathogenic *Escherichia coli*	High	O	Moderate	Low	High	Yes
Salmonella typhi	High	O	Moderate	Low	High	No
Other salmonellae	High	O	Long	Low	High	Yes
Shigella spp.	High	O	Short	Low	Moderate	No
Vibrio cholerae	High	O	Short	Low	High	No
Yersinia enterocolitica	High	O	Long	Low	High(?)	Yes
Legionella	Moderate	I	May multiply	Moderate	High	No
Pseudomonas aeruginosa	Moderate	C, IN	May multiply	Moderate	High(?)	No
Aeromonas spp.	Moderate	O, C	May multiply	Low	High(?)	No
Mycobacterium, atypical	Moderate	I, C	May multiply	High	?	No
Viruses						
Adenoviruses	High	O, I, C	?	Moderate	Low	No
Enteroviruses	High	O	Long	Moderate	Low	No
Hepatitis A	High	O	Long	Moderate	Low	No
Hepatitis E	High	O	?	?	Low	Probable
Norwalk virus	High	O	?	?	Low	No
Rotavirus	High	O	?	?	Moderate	No(?)
Small round viruses (other than Norwalk virus)	Moderate	O	?	?	Low(?)	No
Protozoa						
Entamoeba histolytica	High	O	Moderate	High	Low	No
Giardia intestinalis	High	O	Moderate	High	Low	Yes
Cryptosporidium parvum	High	O	Long	High	Low	Yes
Acanthamoeba spp.	Moderate	C, I	May multiply	High	7	No
Naegleria fowleri	Moderate	C	May multiply	Moderate	Low	No
Balantidium coli	Moderate	O	?	Moderate	Low	Yes
Helminths						
Dracunculus medinensis	High	O	Moderate	Moderate	Low	Yes
Schistosoma spp.	Moderate	C	Short	Low	Low	Yes

? = Not known or uncertain

^a O = oral (ingestion); I = inhalation in aerosol; C = contact with skin; IN = ingestion in immunosuppressed patients.

^b Detection period for infective stage in water at 20 °C: short = up to 1 week; moderate = 1 week to 1 month; long = over 1 month.

^c When the infective stage is freely suspended in water treated at conventional doses and contact times: resistance moderate, agent may not be completely destroyed; resistance low, agent completely destroyed.

^d Dose required to cause infection in 50% of healthy adult volunteers.

[a] O = oral (ingestion); I = inhalation in aerosol; C = contact with skin; IN = ingestion in immunosuppressed patients.

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[d] Dose required to cause infection in 50% of healthy adult volunteers.

2.1.1 Agents of high health significance

Not all potentially waterborne human pathogens are of equal public health significance (Table 2.1). Some of them, including most of the ingested pathogens, present a serious risk of disease whenever they are present in drinking-water, and their elimination from it should be given high priority. Examples include strains of *Escherichia coli, Salmonella, Shigella, Vibrio cholerae, Yersinia enterocolitica,* and *Campylobacter jejuni,* the viruses described in Chapter 4, and the parasites *Giardia, Cryptosporidium, Entamoeba histolytica,* and *Dracunculus.*

While most of the pathogens of high significance in Table 2.1 are found worldwide, others are a public health problem only in limited regions of the world, e.g. guinea worm is found only in certain countries of Africa and Asia. Historically, pandemics of cholera have spread from well-defined regions where the outbreaks first occurred. Although high priority should be given to control of these pathogens in drinking-water, this is of regional significance only.

 2.1.2 Opportunistic pathogens

Some organisms, naturally present in the environment and not normally regarded as pathogens, may cause disease opportunistically. When such organisms are present in drinking-water, they cause infection predominantly among people whose local or general natural defence mechanisms are impaired. Those most likely to be at risk include the very old, the very young, and patients in hospitals, e.g. those with burns or undergoing immunosuppressive therapy, and those suffering from acquired immunodeficiency syndrome (AIDS). Water used by such patients for drinking or bathing, if it contains excessive numbers of these agents, may produce a variety of infections involving the skin and mucous membranes of the eye, ear, nose, and throat. *Pseudomonas, Flavobacterium, Acinetobacter, Klebsiella,* and *Serratia* are examples of such opportunistic pathogens, as is *Legionella,* which can infect the lungs if inhaled in droplets. Some of these, such as *Legionella* and *Aeromonas,* can also cause disease in otherwise healthy individuals when the specific conditions prevailing within a water-supply system have enabled them to multiply to unusually high concentrations. These organisms, while clearly of medical importance, only acquire public health significance under certain conditions. Their removal from drinking-water may therefore be given moderate priority.

 2.1.3 Nuisance organisms

Nuisance organisms, by definition, have no public health significance. However, they produce problems of turbidity, taste and odour or appear as visible animal life in the water. As well as being aesthetically objectionable, they indicate that both water treatment and the maintenance and repair of the distribution system are defective.

 2.2 Routes of exposure

For the faeco-oral pathogens, drinking-water is only one vehicle of transmission. Contamination of food, hands, utensils, and clothing can also play a role, particularly when domestic hygiene is poor. Because of this multiplicity of transmission routes, improvements in the quality and availability of water, in excreta disposal, and in general hygiene education are all important factors in achieving reductions in diarrhoea morbidity and mortality rates *(1).*

While many faeco-oral pathogens have been shown to cause waterborne epidemics, none of them causes epidemics exclusively by this means. Neither the identification of a specific pathogen in drinking-water nor the occurrence of a common-source epidemic can therefore be taken as proof of waterborne disease transmission. To obtain confirmatory evidence, an epidemiological investigation is required. Those infections for which there is epidemiological evidence of waterborne transmission are listed in Table 2.1.

The significance of the water route varies considerably both with the disease and with local conditions.

Thus, waterborne transmission of poliomyelitis has not been conclusively demonstrated, while waterborne epidemics of giardiasis, typhoid fever, and cholera have frequently been documented. One reason for this is that there are important differences between the pathogens in terms of their persistence in water, their removal by conventional water-treatment processes, and the minimum infective dose, i.e. the number of organisms needed to cause infection when taken by mouth.

2.3 Persistence in water

The persistence of a pathogen in water is a measure of how quickly it dies after leaving the body. In practice, the numbers of a pathogen introduced on a given occasion will tend to decline exponentially with time, reaching insignificant and undetectable levels after a certain period (Table 2.1).

A pathogen that persists outside the body only for a short time must rapidly find a new susceptible host. It is therefore less likely to be transmitted through a water-supply system than within a family or some other group living closely together, where lax personal cleanliness will allow the infection to be transferred from one person to another.

The persistence of most pathogens in water is affected by various factors, of which sunlight and temperature are among the most important. Lifetimes are shorter, sometimes much shorter, at warmer temperatures. For example, whereas enteric viruses may be detected for up to 9 months at around 10 °C, their maximum period of detection at 20 °C is nearer to 2 months *(2)*.

Some pathogens are more resistant than others to conventional water-treatment processes, particularly chlorination at the doses and contact times usually employed. This is also indicated in Table 2.1 and discussed in further detail in Chapter 11.

2.4 Infective dose

For several intestinal pathogens, attempts have been made to determine the number of organisms needed to produce either a clinically apparent infection or intestinal colonization in human subjects (see Table 2.2). The significance or the results of these studies is obscure. While they undoubtedly provide an order of relative infectivity, it is doubtful whether the actual infective doses obtained are relevant to natural infections. The number of subjects exposed to infection in experimental studies is necessarily small and the experiments are designed to ensure that many of them become infected. During an outbreak, the number of subjects exposed may be very large, but only a small proportion become infected. Thus the minimum infective dose in an outbreak, and the attack rate, are probably much lower than in an experimental study. In many outbreaks of typhoid fever, the case rate can be explained only by assuming that the infective dose was low.

The likelihood of ingesting very large numbers of a pathogen on a single occasion from drinking-water is relatively small, both because enteric pathogens cannot normally multiply in water and because the water tends to disperse them. On the other hand, if polluted water is permitted to contaminate food, bacterial pathogens, initially present in small numbers, can multiply to produce very large doses.

Table 2.2 Studies on the infectivity of various pathogens in human volunteers

Pathogen	Reference no.
Salmonella typhi	3, 4
Other *Salmonella* spp.	3
Escherichia coli	5-12
Shigella spp.	13
Campylobacter jejuni	14
Vibrio cholerae	15-17
Giardia intestinalis	18, 19
Echovirus 12	20
Poliovirus type 1 (attenuated vaccine strains)	21, 22
Rotavirus strain CJN	23
General methodologies	24

Bacteria that cause intestinal infections are able to invade and grow in the intestine. It is convenient, therefore, to develop a model of infection that states that, under the correct circumstances, a single infective organism must be able to initiate a clinically significant infection. The infective dose (ID) required to ensure that infection occurs in a specified proportion of subjects - for example, half the subjects (ID_{50}) - may be considered to represent, for a particular bacterial species, the probability that the single organism will reach the correct portion of intestine under the right circumstances to initiate a clinically apparent infection.

In a natural infection, the variables affecting this probability may be numerous and varied, as shown in Table 2.3. The transfer of the pathogen from one case to the next may appear simple, but numerous factors, including the ability to survive in the environment and the nature of the environment available to the host, play an important role. Socioeconomic factors, such as the practice of food hygiene, the availability of pure water, and the adequacy of excreta disposal, further complicate the picture.

After ingestion of the pathogen, the development of an infection depends on the balance between host factors, such as gastric acidity and intestinal immunity, tending to remove it, and factors aiding the bacteria in their attempt to colonize the intestine, such as the possession of colonization factors and adhesions. Studies in animals suggest that the growth phase and growth rate may be crucial.

After an infection has been initiated, its clinical expression is still not certain. The bacterial virulence factors, such as enterotoxin production and invasiveness, produce pathological changes, but their overt expression is countered by homoeostatic mechanisms in the gut. The multiplication of the bacteria extends the area of pathological changes, while the development of immunity inhibits the expression of bacterial virulence and results in the elimination of the pathogen. The infective dose, as determined in experimental situations, must, in part, represent the number of bacteria needed to produce disease before being overcome by the host defences. The effect of infective dose on incubation period has been demonstrated in a study on typhoid fever, which showed that the larger the administered dose, the shorter the incubation period *(4)*, a finding that may constitute evidence in support of that conclusion.

Table 2.3 Factors determining the probability of developing clinically significant intestinal infections

Host	Stage of infection	Pathogen
Socioeconomic factors (food hygiene, availability of potable water, excreta disposal, public health measures)	Ingestion	Environmental survival
Antimicrobial defences, gastric acidity, etc.	Infection	Growth phase, adhesins and colonization factors
Immune system, antitoxic immunity	Water loss	Enterotoxin production, mucosal invasion
Homoeostatic mechanisms of water absorption in the gut	Diarrhoea	

The physiological state of the host is another important factor. Undoubtedly the response to infection of the healthy north American adults, in whom most studies on infective dose have been performed (Table 2.2), is different from that of malnourished infants in Africa, Asia, or South America. Factors such as gastric acid production and the immune response are both influenced by nutritional status. Caution is therefore necessary in extrapolating from infective dose to epidemiological mechanism.

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3. Bacteria[1]

3.1 Pathogens excreted

3.1.1 Salmonella

General description

The genus *Salmonella* is a member of the family Enterobacteriaceae. The genus is currently subdivided into the subgenera I-IV, on the basis of biochemical characteristics. Most salmonella strains isolated from humans and warm-blooded vertebrates belong to subgenus I, while subgenera II, III (also called Arizona) and IV are more frequently associated with reptiles, in which they reside commensally. Currently more than 2000 serotypes are named. There are considerable regional variations in the prevalence of serotypes *(1)*.

The virulence of *Salmonella* spp. depends on serotype and strain specificity in host range and infective dose and on host status. *S. typhi* is a specific human pathogen. In particular, *S. typhi, S. paratyphi* A, and *S. paratyphi* B are able to invade tissues and cause septicaemia with high temperature rather than diarrhoea. This is known as enteric fever. In humans, the majority of the other serotypes give rise to a transient intestinal infection manifesting itself as acute gastroenteritis with diarrhoea. Certain serotypes are highly pathogenic for humans, while others are devoid of any pathogenic action. Many salmonella infections are asymptomatic *(2)*.

Routes of exposure

In the case of *S. typhi* and *S. paratyphi* A, human carriers are the source of infection, whereas milk-borne transmission can also occur with *S. paratyphi* B. The incidence of enteric fever decreases as a country becomes more highly developed in terms of controlled sewerage systems and drinking-water supplies, and the supply of pasteurized milk and dairy products. Most salmonellae are primarily pathogens of animals, which constitute important reservoirs for those infections *(2)*.

Salmonellae may be present in all kinds of food grown in faecally polluted environments, and are commonly isolated from poultry and livestock and foods prepared from them. Furthermore, animal feedstuffs and fertilizers prepared from animal products may be highly contaminated with salmonellae, and they are also widely distributed in the environment. The contamination of food and animal feedstuffs by water contaminated with salmonellae is considered to be an additional risk factor *(3, 4)*. Dumping of unprocessed slaughterhouse wastes into rivers has been found to be a cause of salmonellae infections. Contamination by salmonellae and conditions favouring their regrowth should be avoided at all stages of the production, transport, storage, and preparation of food, feedstuffs, and drinking-water. Sludge disposal and irrigation must always be accompanied by appropriate hygienic precautions.

The transmission routes of salmonellae are highly complex. Person-to-person transmission may occur, but the relative importance of the human and non-human reservoirs depends on the dietary, agricultural, and sanitary situation in a particular community *(2)*.

Significance in drinking-water

Waterborne outbreaks have been chiefly associated with *S. typhi* and much less frequently with *S. paratyphi* B or other *Salmonella* serotypes *(2)*. Epidemiological studies of outbreaks suggest that the ingestion of relatively few cells of *S. typhi* may cause typhoid fever, whereas studies in volunteers (Table 2.2, p. 14) indicate that, for other *Salmonella* serotypes, millions of cells are usually required to cause

gastroenteritis.

Salmonellae are excreted in the faeces of infected humans or animals. Faecal contamination of groundwater or surface waters, as well as insufficiently treated and inadequately disinfected drinking-water, are the main causes of epidemic waterborne outbreaks caused by *Salmonella* spp.

Waterborne outbreaks due to heavy contamination are usually characterized by an explosive onset. The majority of cases develop over a period of a few days, and may be followed by a secondary crop of contact cases *(2)*. The geographical distribution of infections in major outbreaks is often strongly correlated with the pattern of a waterworks pipeline network.

Salmonellae can be found in open wells as a result of the drainage or flooding of contaminated surface water into unprotected well shafts. It is uncommon for salmonellae to be isolated from piped water supplies, whether treated or untreated, and their presence suggests a serious fault in the design or management of the system *(2)*.

Penetration of pathogens into water sources must be avoided by the protection of groundwater and surface water catchment areas. A review of the literature has shown that, in general, pathogens will not travel further than the distance that the groundwater flows in 10 days, except in fissured rocks such as limestone and heavily fissured soils *(5)*.

Drinking-water must be of low turbidity after treatment if adequate chlorination is to be achieved. Furthermore, a low load of assimilable organic carbon (AOC) in the treated water is considered to be an important factor in reducing survival time and preventing the regrowth of salmonellae within the distribution system. Reported survival times for salmonellae in drinking-water range from a few days to over 100 days.

Several outbreaks have been caused by the deposition of contaminated sediments in the distribution system for drinking-water, especially in water basins and pipes. Sediments may be shifted to new positions in the system by water pressure oscillations or temporary scarcity of water. Regular flushing of the distribution system is therefore recommended.

3.1.2 Yersinia

General description

The genus *Yersinia* is currently placed in the family Enterobacteriaceae and comprises seven species. *Y. pestis, Y. pseudotuberculosis,* and certain serotypes of *Y. enterocolitica* are pathogens for humans. Atypical strains within *Y. enterocolitica,* isolated most frequently from environmental samples, are grouped together as *Y. enterocolitica*-like organisms. They are nonpathogenic for humans and can be subdivided by biochemical means into *Y. intermedia, Y. frederiksenii, Y. kristensenii,* and *Y. aldovae.*

The cells of *Y. enterocolitica* are Gram-negative rods, motile at 25 °C but nonmotile in cultures grown at 37 °C. Certain strains of *Y. enterocolitica* cause acute gastroenteritis with diarrhoea, but other human diseases caused by *Y. enterocolitica* are also known. Biochemical and serological typing of enteric *Y. enterocolitica* strains show that serotypes O:3 and O:9 are commonly found in Africa, Asia, Canada and Europe, whereas serotype O:8 is exclusively isolated in the United States *(6-8)*.

There is some evidence that *Y. enterocolitica* infection may be waterborne. The following discussion is confined to this species.

Routes of exposure

The transmission of *Y. enterocolitica* from the natural reservoirs to humans has been the subject of much debate. Many domestic and wild animals are considered to be possible reservoirs of *Y. enterocolitica*

because of the high isolation rates of the organism from such sources. It is likely that wild animals, particularly shrews, hares, foxes, and beavers, form a natural reservoir of *Y. enterocolitica.* Swine have been implicated as a major reservoir of *Y. enterocolitica* serotypes involved in human infections.

Available evidence indicates that foods, especially meat and meat products, milk and dairy products, are the major vehicles for the transmission of *Y. enterocolitica.* Furthermore, *Y. enterocolitica* has been isolated from a variety of environmental samples, especially from water, but the serotypes isolated differ from those associated with human disease.

A number of different transmission routes have been suggested for *Y. enterocolitica,* but the ingestion of contaminated food and water is probably the most likely one *(8).* Direct transmission from person to person and from animals to humans also occurs, but its relative importance has not been clarified. Further research is needed to define the epidemiological importance of "environmental" strains of *Y. enterocolitica.*

Significance in drinking-water

The apparent waterborne spread of *Y. enterocolitica* infection has been described in a number of reports. There is some evidence that pathogenic strains of *Y. enterocolitica* enter drinking-water via contaminated surface water or as a result of pollution with sewage *(9).* Recent studies have shown that human pathogenic serotypes of *Y. enterocolitica* are present in sewage and polluted surface water *(10, 11).*

In general, pathogenic types of *Y. enterocolitica* are not isolated from untreated or treated drinking-water unless faecal pollution has occurred. Occasionally, nonpathogenic serotypes of *Y. enterocolitica* and nonpathogenic *Y. enterocolitica*-like organisms *(Y intermedia, Y. frederiksenii, Y. kristensenii)* may also be isolated from drinking-water. However, none of these isolates exhibit the typical virulence markers. Such isolates are probably of environmental origin without public health importance *(12).*

Water samples yielding *Y. enterocolitica* often show only slight coliform contamination. One study indicated that 25% of *Y. enterocolitica*-positive samples were negative for both total and faecal coliforms *(9).* Intensive methods of treatment are not needed in such cases. Other studies have shown a close relationship between faecal pollution and *Y. enterocolitica* isolation rates *(13).*

Standard chlorination procedures are sufficient to avoid the transmission of *Yersinia* if the treated water is of low turbidity. Free chlorine in the range required for water disinfection (0.2 - 0.5 mg/litre) for 10 minutes at pH 7.0 completely eradicates the bacteria; 0.05 mg/litre of ozone eradicates the organism after contact for 1 minute regardless of pH *(14).*

A special feature of *Y. enterocolitica* and *Y. enterocolitica*-like organisms is their ability to grow at low temperatures, even at 4 °C. Accordingly, these organisms can survive for long periods in water habitats. For example, *Y. enterocolitica* was detected in previously sterilized distilled water after over 18 months at 4 °C *(15).* Such long survival periods make it difficult to determine the origin of contamination when *Yersinia* are detected.

3.1.3 Campylobacter

General description

In recent years, considerable attention has been given to *Campylobacter* spp. as important agents of enteritis, gastritis, and other human diseases.

Campylobacters are Gram-negative, slender, comma-shaped rods. They also appear S-shaped and gull-winged when in pairs *(7, 16).* The organisms show a characteristic corkscrew-like motion, which can be easily seen by phase-contrast microscopy. Campylobacters are microaerophilic organisms, requiring a low

oxygen tension (3-6%) for growth. Under unfavourable growth conditions, cells may form coccoid bodies.

A recent review discusses 14 *Campylobacter* species *(17).* Some are pathogens for humans and animals (e.g. *C. jejuni, C. coli, C. fetus*), while others are considered to be nonpathogenic (e.g. *C. sputorum, C. concisus) (16, 17).* Most of the members of the thermophilic group (growing at 42 °C) of Campylobacters cause enteritis in humans. Worldwide, Campylobacters are much more important than salmonellae as causes of acute gastroenteritis, but not as important as shigellas. Several major outbreaks of campylobacter enteritis were linked to the ingestion of contaminated food, milk, or water.

From the point of view of water hygiene, the thermophilic Campylobacters are of greatest significance; the following discussion is therefore confined to these organisms.

Routes of exposure

Thermophilic campylobacters are transmitted by the oral route. The reservoirs for Campylobacters include wild birds and poultry which are the most important, and other domesticated animals, such as pigs, cattle, dogs, and cats. Meat, in particular poultry products, and unpasteurized milk are therefore important sources of campylobacter infections *(16).* Milk may be contaminated with faeces or by the secretion of organisms into milk by cows with mastitis *(18).* In developing countries, the faeces of infected animals are important reservoirs. The infective dose is low *(19).*

Recent studies have shown that raw sewage frequently contains 10-10^5 thermophilic campylobacters per 100 ml *(20, 21).* However, *Campylobacter* counts in heavily contaminated sewage can be reduced considerably by wastewater treatment processes. Thermophilic campylobacters were found in crude sludge, but were not detectable in digested conditioned sludge of filter effluent *(21).* The occurrence of campylobacters in surface waters has proved to be strongly dependent on rainfall, water temperature, and the presence of waterfowl.

Significance in drinking-water

Several waterborne outbreaks of campylobacteriosis have been reported in the past decade. The numbers of persons involved ranged from a few to several thousands. In only two of these outbreaks were campylobacters isolated both from patients and from water samples. Unchlorinated surface water and faecal contamination of water storage reservoirs by wild birds were found to be the main causes. The consumption of unchlorinated or inadequately chlorinated surface waters is associated with a considerable risk of outbreaks of campylobacteriosis. Any contamination of drinking-water reservoirs by the excrement of waterfowl must be controlled. Consideration should be given to imposing stricter hygienic requirements for drinking-water, even if obtained from high-quality surface water, since it may be distributed without chlorination.

Campylobacters, like other bacterial pathogens, survive well at low temperatures, suggesting that cold water may be an effective vehicle of transmission. They are able to survive for several weeks *(22)* in cold groundwater or unchlorinated tapwater. The standard chlorination procedures are sufficient to prevent the spread of campylobacters along water mains if the water is of low turbidity.

3.1.4 Escherichia coli

E. coli is found in large numbers in the faeces of humans and of nearly all warmblooded animals; as such it serves as a reliable index of recent faecal contamination of water. This topic is fully covered in Chapters 9-11. Certain strains are pathogenic for humans and it is these that are described below.

General description

E. coli is a Gram-negative, non-spore-forming, rod-shaped bacterium which can be either motile or

nonmotile (motile cells are peritrichous); growth is aerobic or facultatively anaerobic. Metabolism is both respiratory and fermentative; acid is produced by the fermentation of glucose and lactose. Catalase is produced but not oxidase, and nitrates are reduced to nitrites. In the microbiological examination of water, a biochemical description is used (see sections 9.2.1-9.2.3).

Serological typing is based on the somatic O antigens, the capsular K antigens, and the flagellar H antigens. In practice, serogrouping by O antigen is often used alone and, within a particular epidemiological context, may be satisfactory. Biochemical tests do not reliably distinguish pathogenic strains of *E. coli.*

Health effects

E. coli is a normal inhabitant of the intestine, and most strains are nonpathogenic. However, subtypes able to cause gastrointestinal disease are also known. Such pathogenic *E. coli* strains cause intestinal disease by a variety of mechanisms. Infections may resemble cholera, dysentery, or gastroenteritis due to salmonellae. Four classes of pathogenic *E. coli* responsible for diarrhoea are recognized: enteropathogenic, enteroinvasive, enterotoxigenic, and verocytotoxin-producing *(23)*.

Enteropathogenic subtypes of *E. coli* were first recognized as a result of the serological examination of strains isolated from outbreaks of diarrhoea among infants. Associations of particular serotypes with disease were observed, but the corresponding pathogenic mechanisms are not fully understood for most of these organisms. These strains have been particularly associated with outbreaks of infantile gastroenteritis *(24)*.

Enteroinvasive strains of *E. coli* (EIEC) produce dysentery by a mechanism similar to that found with *Shigella* spp. These organisms invade the colonic mucosa and cause bloody diarrhoea. The property seems to be restricted to a few O groups. It must be remembered that *Shigella* and *E. coli* are closely related and that genetic material is readily exchanged between them.

Although enteropathogenic or enteroinvasive strains may cause serious illness, such epidemiological evidence as is available suggests that enterotoxigenic strains are responsible for most episodes of *E. coli* diarrhoea, particularly in developing countries. Enterotoxigenic *E. coli* (ETEC) can cause a cholera-like syndrome in infants, children, and adults.

ETEC produce either a heat-labile enterotoxin (LT), related to cholera enterotoxin, or a heat-stable enterotoxin (ST); some strains produce both toxins. The action of LT is the same as that of cholera toxin. The production of enterotoxin is controlled by plasmids.

The ability of ETEC to cause disease depends not only on the production of enterotoxin but also on their ability to colonize the small intestine. Various colonization factors, or adhesins, have been described, which enable the bacteria to attach themselves to the intestinal mucosa.

The fourth class, verocytotoxic *E. coli,* was first recognized by their production of a cytotoxin active against Vero cells in culture. The organisms commonly belong to the serogroup O157 and cause disease ranging from mild diarrhoea to haemorrhagic colitis characterized by blood-stained diarrhoea, usually without fever, but accompanied by abdominal pain. It is also a cause of the haemolytic uraemic syndrome, commonest in infants and young children, and characterized by acute renal failure and haemolytic anaemia.

Significance in drinking-water

Isolation of *E. coli* from a water supply indicates faecal contamination. However, *E. coli* is only one species among many in the family Enterobacteriaceae. Members of the lactose-fermenting species of this group, which may be referred to colloquially as "coliforms", occur in a variety of ecological niches, not all of which

are intestinal. Thus, for example, some species are associated with aquatic slimes and vegetation. The picture is further complicated by the fact that other members of the coliform group are also found in the intestine. Thus, the definitive identification of *E. coli* may be needed to determine the significance of "coliforms" in a water supply.

Conventionally, thermotolerant coliforms are identified by growth at 44-45 °C, but some isolates of *Klebsiella, Citrobacter,* and *Enterobacter* will also grow and ferment lactose under these conditions. The term "faecal coliforms" is often used for this group, but the term "faecal" is to be deprecated, since not all prove to be of faecal origin.

The detection of the pathogenic subtypes of *E. coli* in water supplies has seldom been attempted. Although this may be necessary in epidemiological research, the available methods are not suitable for the routine examination of water samples.

3.1.5 Vibrio cholerae

General description

Vibrio species are motile, non-spore-forming, slightly curved Gram-negative rods with a single polar flagellum; they are both aerobic and facultatively anaerobic. Their metabolism is both respiratory and fermentative without the production of gas. Both catalase and oxidase are formed, and nitrates are reduced to nitrites.

Among the vibrios, special attention has focused on the identity of those causing cholera. It was for a long time believed that *Vibrio cholerae* was a unique and distinct species associated with human disease, and recognized by possession of the O1 antigens. This species was further divided into "classical" and "El Tor" biotypes, the latter distinguished by the ability to produce a dialysable, heat-labile haemolysin, active against sheep and goat red cells *(25).*

A broader definition of *V. cholerae* has now been adopted. It has been known for many years that vibrios biochemically identical to *Vibrio cholerae* O1, but lacking the O1 antigen, could be found in the aquatic environment. These were termed non-cholera vibrios (NCV) or non-agglutinable (NAG) vibrios. The term "non-O1 *V. cholerae*" is now preferred, since some may produce cholera toxin, while investigation of DNA/DNA homology between *Vibrio cholerae,* NCVs, and NAG vibrios has conclusively demonstrated that they are all very closely related. Currently, all are considered to be a single species, *Vibrio cholerae,* divided into more than 80 serological types on the basis of the O or somatic antigens. The H or flagella antigen is common to all the O groups of *V. cholerae,* and H agglutination has been used as a diagnostic test.

Only *V. cholerae* serogroup O1 causes cholera. The O1 group contains two serotypes based on variations in the O antigen factors, namely Ogawa (factors A and B) and Inaba (factors A and C). These serotypes may exist in either the classical or El Tor biotype.

Routes of exposure

Cholera has historically been one of the major pandemic diseases. The present pandemic, unlike previous ones, is caused by *V. cholerae* O1 biotype El Tor.

Cholera is usually a waterborne disease, and numerous waterborne outbreaks have been documented. However, foodborne and nosocomial outbreaks are also important, and person-to-person transmission may occur under conditions of extreme crowding and poor hygiene. The problems of the transmission of cholera have been extensively reviewed, and although waterborne transmission is undoubtedly important, many aspects of the epidemiology of cholera are a matter for debate. Evidence has accumulated to suggest that, in some circumstances, *V. cholerae,* including serorype O1, may be part of the

autochthonous microbiota of natural waters *(25, 26)*.

Health effects

Infection with *V. cholerae* O1 involves the small and large intestine. In the small intestine, the vibrios attach themselves to the mucous layer covering the villous epithelium, chemotactic processes apparently playing a role in their migration to the epithelium. After attachment, vibrios penetrate the layer of mucus and adhere to the surface of the epithelial cells. Motility seems to be essential for mucus penetration to occur. Adherence to the mucosal surface is specific, involving a receptor-adhesion interaction analogous to a lectin-ligand reaction. When present in large numbers, *V. cholerae* O1 produce an enterotoxin (cholera toxin) that causes alterations in the ionic fluxes across the mucosa with the resulting catastrophic loss of water and electrolytes in liquid stools. Cholera toxin is very similar to the heat-labile toxin produced by enterotoxigenic *E. coli.*

It seems likely that other accessory virulence factors, such as mucinase and protease, are also important in the pathogenesis of cholera. Other toxins may also be involved. Enterotoxins similar to the heat-stable toxins of *E. coli* are known to be formed by *V. cholerae* of O groups 2-84, and *V. cholerae* O1 may also produce several toxins. Cholera toxin is not produced by all strains of *V. cholerae* O1, and nontoxigenic strains are considered to be nonpathogenic.

Significance in drinking-water

The isolation of *V. cholerae* O1 from water used for drinking is of major public health importance and is evidence of faecal contamination. However, other serogroups of *V. cholerae* may be part of the normal flora of some waters.

3.1.6 Shigella

General description

Shigellae are Gram-negative, non-spore-forming, non-motile rods, capable of growth under both aerobic and anaerobic conditions. Metabolism is both respiratory and fermentative; acid, but not usually gas, is produced from glucose. Lactose is seldom fermented. Catalase is usually produced, except by *Shigella dysenteriae* type 1, but oxidase is produced by one serotype only. Nitrates are reduced to nitrites.

Shigellae are serotyped on the basis of their somatic O antigens. Both group and type antigens are distinguished, group antigenic determinants being common to a number of related types. There is evidence that type antigen specificities among *Shigella flexneri* are determined by the presence of lysogenic bacteriophages. It seems likely that biotypic and serological variants are determined by the presence of phage or the carriage of plasmids. Plasmids (transferable, extrachromosomal genetic elements) were first described in this genus. Phage-typing systems exist for all groups, though they have not been widely applied, serological typing being adequate for all species except *Shigella sonnei*.

Health effects and routes of infection

Though shigella infection is not often spread by waterborne transmission, major outbreaks resulting from such transmission have been described. The characteristic bloody diarrhoea results from the invasion of the colonic mucosa by the bacteria. There is good reason to believe that the process is highly species-specific. Shigellae have no natural hosts other than the higher primates, and humans are the only effective source of infection. Of the enteric bacterial pathogens, shigellae seem to be the best adapted to cause human disease. Direct transmission between susceptible individuals is the usual route of infection, and the infective dose is lower than for other bacteria.

Significance in drinking-water

The isolation of shigellae from drinking-water indicates recent human faecal contamination and, in view of the extreme pathogenicity of the organisms, is of crucial public health significance. However, this is a rare event, possibly explained in part by the absence of a useful enrichment or selective medium for the isolation of these bacteria. Those generally used have been designed for the isolation of salmonellae and are not optimal for that of shigellae. Furthermore, without confirmatory testing, some anaerogenic strains of *E. coli* may be wrongly identified as shigellae.

3.2 Pathogens that grow in supply systems

All drinking-water contains assimilable organic compounds that will allow a certain degree of bacterial growth. The density of bacteria in drinking-water can and should be controlled for the reasons given in Chapter 8.

Certain bacteria in drinking-water deserve particular attention because they are opportunistic pathogens to humans, i.e. they are able to cause infections in susceptible persons. The most important organisms of this type, namely *Legionella* and *Aeromonas,* will be considered here. Other organisms, such as *Pseudomonas aeruginosa* and opportunistically pathogenic mycobacteria, have been detected in drinking-water supplies.

3.2.1 Legionella

General description

The genus *Legionella,* a member of the family Legionellaceae, has 22 currently known species, of which *L. pneumophila* serogroup 1 is most frequently associated with human disease. Other serogroups of *L. pneumophila* and occasionally other legionellae have also been reported to cause disease. Legionellae are Gram-negative, rod-shaped, non-spore-forming bacteria that require L-cysteine for growth and primary isolation. The cellular fatty acids in legionellae are unique among those found in Gram-negative bacilli in that they consist essentially of branched chains. Preconcentration of legionellae from environmental samples may be required if low levels are to be detected. Immunofluorescence techniques may also be used to detect legionellae in the environment.

Health effects

Legionella infections can lead to two types of disease, namely Legionnaires' disease (legionellosis) and non-pneumonic Legionnaires' disease (Pontiac fever). Legionnaires' disease is a form of pneumonia with an incubation period usually of 3 - 6 days. Males are more frequently affected than females, and most cases occur in the 40 - 70 year age group. Risk factors include smoking, alcohol abuse, cancer, diabetes, chronic respiratory or kidney disease, and severe immunosuppression, as in transplant recipients. The fatality rate in untreated cases may be 10% or higher, but the disease can be treated effectively with antibiotics such as erythromycin and rifampicin.

Legionnaires' disease is uncommon, but common-source outbreaks attract much attention. Between 100 and 200 cases are reported each year in England and Wales, and in Germany; in France, the incidence is somewhat higher, with over 400 cases per year *(27).* For people living in temperate climates, travelling to subtropical areas appears to be a significant risk factor, outbreaks being related to air-conditioning and hot-water systems in hotels. Hospital-associated Legionnaires' disease is the most serious form, because it usually affects debilitated persons and has a high mortality rate. The non-pneumonic form of the disease is milder, with a high attack rate, an acute onset (5 hours to 3 days) and symptoms similar to those of influenza: fever, headache, nausea, vomiting, aching muscles, and coughing. No fatal cases have been reported and few outbreaks have been recognized, possibly because the non-specific symptoms of the disease hinder its diagnosis.

Routes of exposure

Legionellae are widespread in natural sources of water and may also be found in soils. They occur commonly in man-made water systems, particularly in hot-water and cooling-water systems. Infection is the result of the inhalation of aerosols that are small enough to penetrate the lungs and be retained by the alveoli. The degree of risk depends on four key factors: (i) the density of the bacteria in the source; (ii) the extent of aerosol generation; (iii) the number of inhaled bacteria; and (iv) the susceptibility of the exposed individual.

Legionellae multiply in the laboratory at temperatures between 20 and 46 °C. At temperatures higher than 46 °C, the bacteria will survive, but at 60 °C only for a few minutes (28). Temperatures favourable for growth may be found in cooling towers, spas, cold-water systems in buildings, hot-water systems operated below 60 °C or "dead legs" of such systems operated at higher temperatures. Aerosols may be created by the spraying of water in cooling towers or its agitation in spas. Hot-water systems are also likely to create aerosols in showers, through nozzle heads or by splashing in sinks, baths, etc. The number of inhaled bacteria depends on the size of the aerosol generated (<5 μm being most dangerous), the dispersal of the aerosol in the air, and the duration of the exposure. Host defence is an important factor that determines whether exposure to legionellae will lead to clinical disease. It is primarily for this reason that high counts of *L. pneumophila* in water systems have been reported in the absence of disease, whereas similar or lower counts have been associated with epidemics. It is also likely, although not yet adequately proven, that differences in virulence between strains partly account for these observations.

It is now apparent that legionellae can be ingested by the trophozoites of certain amoebae (*Acanthamoeba, Hartmanella, Valkampfia,* and *Naegleria*) and even grow intracellularly and become incorporated in their cysts (29). This may explain the difficulty of eradicating legionellae from water systems and may be a factor in the etiology of the non-pneumonic disease (30).

The following are generally accepted as requiring disinfection:

- sites implicated in an outbreak of Legionnaires' disease or Pontiac fever;

- hospital wards housing high-risk patients, such as an organ transplant unit;

- buildings in which the water system has not been used for some time and where large numbers of legionellae are likely to be found.

Nevertheless, it is generally advisable to design and maintain systems in such a way that colonization by *Legionella* is prevented as much as possible. Detailed instructions for achieving this have been given in several publications (31-34) and focus on the following aspects:

- preventing the accumulation of sludge, scale, rust, algae and slime, and removing such deposits regularly;

- maintaining hot-water temperatures permanently above 60 °C or increasing them periodically to above 70 °C, and keeping cold-water supplies below 20 °C;

- selecting materials for contact with water that do not release nutrients that support the growth of *Legionella.*

The use of biocides is generally regarded as a less effective and less desirable means of controlling legionellae in water supplies in buildings. However, their use is essential to prevent the build-up of microbial slimes in air-conditioning systems in which wet, evaporative cooling towers are used. Such systems should be kept clean and well maintained, and should be inspected weekly for fouling,

accumulations of slime and scale, and corrosion; they should be thoroughly cleaned and disinfected twice yearly. Biocides are best used intermittently in clean systems *(33, 34).*

 3.2.2 Aeromonas

General description

Aeromonas spp. are Gram-negative, rod-shaped, non-spore-forming bacteria that are currently assigned to the family Vibrionaceae, although they also bear many similarities to the Enterobacteriaceae. MacDonnell et al. *(35)* have suggested the creation of a new family Aeromonadaceae. The genus *Aeromonas* is divided into two groups of which the first, the group of psychrophilic non-motile aeromonads, consists of only one species, *A. salmonicida,* an obligate fish pathogen that will not be considered further here. The group of mesophilic motile aeromonads has been divided by Popoff into three biochemically distinguishable groups *(36)*, namely *A. hydrophila, A. sobria* and *A. caviae.* Each of these three species consists of at least three different DNA-hybridization groups, and later workers have described new species such as *A. veronii, A. media, A. schubertii,* and *A. eucrenophila.* It may be expected that in the near future the taxonomy of the group of mesophilic aeromonads will be changed still further, but at present the classification of Popoff is that most widely accepted internationally.

Health effects

Mesophilic aeromonads have long been known to be pathogenic for coldblooded animals such as fish and amphibians, but in the last few decades greater attention has been given to their pathogenic significance for humans. Three major types of infection are described *(37)*: (i) systemic infections, usually in seriously immunocompromised persons; (ii) wound infections (mainly after contact with surface water); and (iii) diarrhoea. In particular, the significance of *Aeromonas* as an enteropathogenic organism is the subject of much discussion. In animal test models, such as the suckling mouse test and the rabbit ileal loop test, pure cultures of *Aeromonas* have been found to cause strong fluid accumulation which can partially be ascribed to the production of extracelluar cytotoxins. However, there have been reports that, while the culture filtrate of some *Aeromonas* strains is not enteropathogenic in an animal test model, a suspension of living cells does have this property. Cell-bound factors are apparently also of importance *(38).* Asao et al. *(39)* have purified and characterized an *Aeromonas* haemolysin, which was found to be a protein with a relative molecular mass of about 50 000 that was strongly enterotoxic and cytotoxic. It has not yet been possible to purify and characterize other toxins because the toxic activity disappears rapidly when culture filtrates are manipulated. Despite the marked toxin production by *Aeromonas* strains *in vitro,* diarrhoea has not yet been induced in test animals or human volunteers, and it is assumed that such strains are only poorly able to colonize the gastrointestinal tract *(40).* Little is known about the adhesion factors of *Aeromonas* or their interaction with receptors in the gastrointestinal tract.

Epidemiological investigations have also resulted in contradictory findings on the significance of *Aeromonas* as an enteropathogenic organism. In some studies, the numbers of *Aeromonas* in the faeces of patients with diarrhoea were greater than those in control groups, whereas in other studies there was no difference, or the bacteria were found in even greater numbers in the latter. In general, it can be said that the significance of *Aeromonas* as an enteropathogenic organism is greater in tropical areas than in the temperate zone. However, infections do occur in the temperate zone as well, albeit less frequently. *Aeromonas*-associated diarrhoea usually causes an acute but self-limiting gastroenteritis but chronic disease with serious complications may also occur. The incidence of *Aeromonas* in human diarrhoeal faeces in the Netherlands was found to vary between 0.5% in winter and 3% in summer. Most isolations were made in children under five years of age or in adults above 70 years of age. Young children yielded mainly *A. caviae,* whereas *A. sobria* was usually isolated from the elderly *(41).*

Routes of exposure

Aeromonas occurs in water, soil, and food, particularly meat, fish, and milk. The occurrence of *Aeromonas*

in drinking-water can be studied by a variety of methods. A membrane filtration method has been described in which a selective ampicillin-dextrin agar (ADA) is used *(42)*. If water samples from house installations are being examined, the addition to the sample of a complexing agent such as the disodium salt of edetic acid (Na$_2$EDTA) at a concentration of 50 mg/litre is necessary. *Aeromonas* is extremely sensitive to the traces of copper that may be present in water in domestic installations in which copper piping is used. Complexing of copper was also found to improve the survival of coliform bacteria and heterotrophic bacteria *(43)*.

The number of *Aeromonas* in surface waters can vary between 0.01 and 1000 cfu/ml. Small numbers are found in springs and in seawater that is not contaminated by sewage discharges. If such discharges are present, the number of *Aeromonas* can rise to 100 cfu/ml in seawater, and more than 1000 cfu/ml in fresh water, the species *A. caviae* then being dominant. In fresh waters not subject to sewage pollution, the numbers of *Aeromonas* are usually between 10 and 100 cfu/ml. In these waters, the numbers are higher in summer than in winter, and there is a relation between the eutrophication of the water and the summer density of *Aeromonas.* In stagnant fresh water, *A. sobria* is usually the dominant species. When river water is stored in reservoirs, the number of *Aeromonas* decreases, but there is also a shift in species composition from *A. caviae* to *A. sobria. Aeromonas* is not usually found in groundwater or is found only in very small numbers *(44)*.

Irrespective of the contamination level of the raw water, most drinking-water treatment processes appear to be able to reduce the numbers of *Aeromonas* to below 1 cfu/100 ml. However, treated water can contain larger numbers, with maxima of about 1000 cfu/100 ml as a result of regrowth in storage reservoirs with long retention times, polluted filter sand, or sudden changes in the quantities of water to be produced. Regrowth of *Aeromonas* occurs in the distribution network of most drinking-water treatment plants. The size of the *Aeromonas* population will depend on many factors but primarily on the organic content of the water and its temperature, the residence time in the distribution network, and the presence of residual chlorine.

Little is known about the type and concentrations of nutrients for *Aeromonas* in drinking-water. Van der Kooij *(45)* has suggested that *Aeromonas* prefers to grow on organic matter, e.g. from decaying nitrifying and methane-oxidizing bacteria that develop in drinking-water treatment plants or from biofilm material in distribution networks.

Control of aeromonads in drinking-water requires a multiple approach, which is similar to the general approach to limiting the regrowth of bacteria (see Chapters 8 and 9). The treatment process should effectively remove organic compounds serving as sources of carbon and energy for the growth of bacteria. Furthermore, the amount of biomass produced and subsequently released during the treatment process should be as small as possible. The distribution system should be designed in such a way that residence times are short, and it should be flushed regularly to prevent the accumulation of sediments in stretches with low water velocities. Materials in contact with drinking-water should not be a source of biodegradable compounds. These factors are of greatest importance in supplies that are not disinfected, or where the maintenance of a chlorine residual is not considered desirable for various reasons. Free available chlorine residuals of 0.2 - 0.5 mg/litre will generally be sufficient to control *Aeromonas* densities in water in the distribution network *(46, 47)*. Chlorine or other disinfectants should not be used to control occasional increases in *Aeromonas* densities in supplies that are normally not chlorinated because biofilms on pipe walls will be disturbed, and this will result initially in increases rather than decreases in bacterial concentrations. Also, in such systems the chlorine consumption will be rather high and residuals cannot be properly maintained, thus allowing regrowth in remote stretches of the network.

The question whether the presence of *Aeromonas* in drinking-water is a risk to human health cannot be answered with certainty; however, if there is a risk, it must be small because in many countries the bacterium is not important as a causative agent of diarrhoea and is not often able to colonize the gastrointestinal tract of humans. Also, the numbers present in drinking-water are small as compared with those in other sources. In food, for instance, the numbers usually found are of the order of 10^3-10^5 cfu/g.

However, drinking-water is a product that is consumed daily by everyone, including groups with a reduced resistance to infectious diseases. Some of this water is consumed without previous heating, in contrast to most foods contaminated with *Aeromonas.* A cautious approach therefore appears to be justified. Numbers of *Aeromonas* in drinking-water must be controlled as far as possible. Apart from the public health reasons for the control of *Aeromonas* levels in drinking-water, experience has shown that it is a useful and sensitive indicator of general hygiene within the drinking-water production and distribution process. Currently, no guideline value can be given because local conditions (temperature, raw water source) may greatly influence *Aeromonas* counts in drinking-water.

3.2.3 Pseudomonas aeruginosa

General description

P. aeruginosa is a member of the family Pseudomonadaceae and is a monotrichate. Gram-negative rod. It can be recognized by its production of a blue-green fluorescent pigment (pyocyanin), which, in agar cultures, will diffuse into the medium. Pigment may not be produced by strains of *P. aeruginosa* recovered from clinical specimens, and the ability to produce it may be lost on subculture. Like other fluorescent pseudomonads that occur in natural waters, *P. aeruginosa* strains produce catalase and oxidase, and ammonia from arginine, grow with citrate as the sole source of carbon, and are aerobic. *P. aeruginosa,* however, is capable of growth at 41 - 42 °C, and the blue-green pigment that it produces differs from the fluorescent pale green pigment (fluorescein) produced by other species of fluorescent pseudomonads found in water. It is also capable of growing anaerobically in stab cultures of nitrate agar.

P. aeruginosa is commonly found in faeces, soil, water, and sewage but cannot be used as an indicator of faecal contamination, since it is not invariably present in faeces and sewage, and may also multiply in the enriched aquatic environment and on the surface of unsuitable organic materials in contact with water. However, its presence may be one of the factors taken into account in assessing the general cleanliness of water distribution systems and the quality of bottled waters (see section 9.3.2).

Routes of exposure

P. aeruginosa is an opportunistic pathogen. Most of the illnesses in humans for which it is responsible are caused, not by drinking water, but by contact with it. Water containing these bacteria may also contaminate food, drinks, and pharmaceutical products, causing them to deteriorate and to act as secondary vehicles for transmission. Fixtures in contact with water, such as sinks and sink drains, tap fittings and showerheads, can also be contaminated by *P. aeruginosa* and can serve as reservoirs of infection in hospitals.

Health effects and significance in drinking-water

In healthy persons, the illnesses caused by *P. aeruginosa* are usually mild and trivial. Waterborne infections are usually associated with warm, moist environments; they include the skin rashes and pustules or outer ear canal infections (otitis externa) reported in users of indoor swimming-pools and whirlpools, where bacterial counts are high and disinfection is deficient *(48, 49)*. The presence of this organism in water supplied to hospitals and for the manufacture of pharmaceutical preparations and dressings is a matter of concern because *P. aeruginosa* is a common pathogen in infections of wounds and burns and has caused serious eye infections after the use of contaminated eye drops *(50)*. Hospital strains of *P. aeruginosa* can first colonize and then infect patients receiving cancer chemotherapy *(51)*.

The presence of this organism in potable water also indicates a serious deterioration in bacteriological quality, and is often associated with complaints about taste, odour, and turbidity linked to low rates of flow in the distribution system and a rise in water temperature (see section 9.3.2).

3.2.4 Mycobacterium

General description

Mycobacterium spp. are rod-shaped bacteria with cell walls having a high lipid content; this enables them to retain certain dyes in staining procedures that employ an acid wash, and they are therefore often referred to as acid-fast bacteria. The characteristics of the cell wall structure also result in a relatively high resistance to disinfectants. All mycobacteria are characterized by slow growth (generation times under optimal circumstances 2-20 hours), but within this range they are divided into "slow" and "rapid" growers. Most pathogenic species are found among the slow growers, which include the strictly pathogenic species *M. tuberculosis, M. bovis, M. africanum,* and *M. leprae;* these are not transmitted by water and have only human or animal reservoirs. Other mycobacterial species, often referred to as "atypical", have environmental reservoirs. Although many are considered to be nonpathogenic, several species are opportunistic pathogens for humans, the most important being the slow growers *M. kansasii, M. marinum, M. avium, M. intracellulare, M. scrofulaceum,* and *M. xenopi,* and the rapid growers *M. chelonae* and *M. fortuitum.* Some of these species are closely related, and the literature often describes a number of complexes rather than individual species. Examples are the "*M. bovis* complex" (which includes *M. africanum*), the "*M. avium* complex" (or MAC, which includes *M. intracellulare*), or the "*M. avium, M. intracellulare, M. scrofulaceum* complex" (or MAIS) and the "*M. fortuitum-chelonae* complex."

Health effects

The strictly pathogenic mycobacteria are associated with classical infectious diseases such as tuberculosis and leprosy. The environmental mycobacteria may cause a range of diseases including tuberculous lung disease and disseminated infections which may also involve the skeleton (*M. kansasii, M. avium* complex), infections of the lymph nodes (MAIS complex), and infections of the skin and soft tissues (*M. marinum, M. fortuitum-chelonae* complex) *(52, 53).* Diseases caused by opportunistic pathogenic mycobacteria are not normally transmitted from person to person but are usually the result of environmental exposure in combination with predisposing factors, such as dust retained in the lungs, surgical wounds, or immunosuppression produced by medication (transplant patients) or by underlying disease (AIDS, malignancies). Mycobacteria are generally resistant to many antimicrobial agents, hence effective treatment may be difficult.

Routes of exposure

An extensive review of the occurrence of mycobacteria in environmental sources has been published *(54).* Tapwater has long been known to harbour saprophytic mycobacteria; in fact, one of the most commonly occurring species, *M. gordonae,* is known as the tapwater bacillus. The occurrence of opportunistic pathogenic species in tapwater has also been demonstrated by various authors *(55, 56).* These organisms may accidentally contaminate clinical specimens during and after collection, or during processing in the laboratory; this may falsely suggest that the patients concerned are suffering from a mycobacterial infection *(57, 58).* A link between the occurrence of mycobacteria in drinking-water and disease has sometimes been suggested. Endemic *M. kansasii* infections in Czechoslovakia were studied from 1968 onwards, the peak incidence being found in a small, densely populated district in which workers were engaged in mining, heavy industry, and power generation. *M. kansasii* could also be isolated from shower outlets in collieries, and it was later shown that the drinking-water system in the entire region was widely contaminated. It was suggested that mycobacteria from drinking-water were spread via aerosols *(59).* The high isolation frequency of *M. kansasii* from clinical specimens in Rotterdam, the Netherlands, led to an investigation of the water supply system. The organisms were frequently isolated from tapwater, and were of the same phage type and showed the same weak nitratase activity as clinical strains *(60).* The increase in the isolation frequency of the *M. avium* complex in Massachusetts, USA, has also been attributed to their presence in drinking-water *(61).* It should be noted that in all these cases there is only circumstantial evidence of a causal relationship between the occurrence of mycobacteria in drinking-water and human disease. Certainly, the low infectivity of environmental mycobacteria does not warrant the setting of

standards or the institution of eradication programmes.

The ecology of opportunistic mycobacteria in water supplies is poorly understood. The bacteria have been isolated infrequently from treated water or mains water *(52, 57)* but appear to multiply within the plumbing systems in buildings as well as in taps. Increased isolation frequencies have been associated with higher temperatures (hot-water systems or cold-water pipes in the vicinity of central heating). Older buildings appear to be more frequently colonized than new ones *(61)*, and transport of drinking-water over long distances also seems to increase the content of mycobacteria *(58)*. Haas et al. *(62)* attempted to correlate total microscopic counts of acid-fast bacteria (hence including both pathogenic and saprophytic species) with a range of physicochemical parameters. A negative correlation with total chlorine residual and a positive correlation with turbidity and total organic carbon (TOC) was established, but these variables only accounted for a small proportion of the overall variance of counts. It might also be expected that materials used for plumbing would have an effect on mycobacterial densities, but no experimental evidence of such an effect has yet been presented.

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4. Viruses[1]

4.1 General description

The viruses of greatest significance in the waterborne transmission of infectious disease are essentially those that multiply in the intestine of humans and are excreted in large numbers in the faeces of infected individuals. Although viruses cannot multiply outside the tissues of infected hosts, some enteric viruses appear to have a considerable ability to survive in the environment and remain infective. Discharges of sewage and human excreta constitute the main source of human enteric viruses in the aquatic environment. With the various analytical methods currently available, wide variations are found in the numbers of viruses present in sewage. These belong to the families shown in Table 4.1. The numbers of viruses and the species distribution will reflect the extent to which they are being carried by the population. Sewage treatment may reduce the numbers of viruses 10-1000-fold, depending on the nature and extent of the treatment given. However, it will not eliminate them entirely, and the sludge produced during sewage treatment will often contain large numbers. As sewage mixes with receiving water, viruses are carried downstream, remaining detectable for varying periods of time, depending on the temperature, the degree to which they are adsorbed on to sediments, the depth to which sunlight penetrates into the water, and other factors. Consequently, enteric viruses can be found in sewage-polluted water at the intakes to water-treatment plants.

The relationship between the occurrence of viruses in water and risks to health is not a simple one; the factors involved are discussed in section 4.3. Table 4.1 lists those viruses, infective for humans, which have been found in sewage-polluted water and the illnesses with which they have been associated.

4.1.1 The nature of viruses

Viruses are replicating infectious agents that are among the smallest of all microorganisms. In essence, they are nucleic acid molecules that can enter cells and replicate in them, and code for proteins capable of forming protective shells around them. The following characteristics are shared by all viruses:

1. The virus particle or virion consists of a genome, either RNA or DNA, that is surrounded by a protective protein shell called the capsid. This shell is itself often enclosed within an envelope that contains both protein and lipid.

2. Viruses replicate only inside specific host cells. They are totally dependent on the host cell's synthetic apparatus and energy sources, and are thus parasites at the genetic level.

Table 4.1 Viruses pathogenic to humans which can occur in polluted water and diseases attributed to them

Virus family	Members	No. of serotypes	Diseases caused
Picornaviridae	Human polioviruses	3	Paralysis, meningitis, fever
	Human echoviruses	32	Meningitis, respiratory disease, rash, fever, gastroenteritis
	Human coxsackie-viruses A1-22,24	23	Enteroviral vesicular pharyngitis, respiratory disease, meningitis, enteroviral vesicular stomatitis with exanthem (hand, foot and mouth disease)
	Human coxsackie-viruses B1-6	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, epidemic myalgia (pleurodynia)
	Human enteroviruses 68-71	4	Meningitis, encephalitis, respiratory disease, rash, acute enteroviral haemorrhagic conjunctivitis, fever
	Hepatitis A virus	1	Hepatitis A
Reoviridae	Human reoviruses	3	Unknown
	Human rotaviruses	5	Gastroenteritis, diarrhoea
Adenoviridae	Human adenoviruses	41	Respiratory disease, conjunctivitis, gastroenteritis
Parvoviridae	Adeno-associated viruses	4	Latent infection following integration of DNA into the cellular genome
Caliciviridae	Human caliciviruses	5	Gastroenteritis in infants and young children
	Small round structured viruses (including Norwalk virus)	14	Gastroenteritis, acute viral gastroenteropathy (Winter vomiting disease)
Caliciviridae (?)	Hepatitis E virus	?	Hepatitis E
Unknown	Astroviruses	1	Gastroenteritis, neonatal necrotizing enterocolitis
Papovaviridae	Papillomaviruses	2	Plantar warts

4.1.2 Classification of animal viruses

The present universal system for virus taxonomy is set arbitrarily at the hierarchical levels of family, genus, and species by the International Committee on Taxonomy of Viruses *(1)*. The fundamental criteria used for classification purposes are the type and strandedness of the nucleic acid of the viral genome and the presence or absence of a lipoprotein envelope.

Virus families, designated by terms ending in -viridae, represent clusters of genera of apparently common evolutionary origin. Virus genera are designated by terms ending in -virus and are based on common evolutionary origin and biophysicochemical or serological properties (see Table 4.1). Virus species have not been designated formally except for the family Adenoviridae, where the term is now defined on the basis of immunological distinctiveness.

Picornaviruses are 27 - 28 nm particles consisting of positive-sense single-stranded RNA enclosed in a protein coat of icosahedral symmetry, which are stable at pH 3; one member of this family, hepatitis A virus, is particularly stable, e.g. it can survive for some hours at pH 1. They resist inactivation by various environmental factors for a number of weeks, particularly when associated with sediments in natural waters. The genus *Enterovirus,* which is one of the three genera of the Picornaviridae family pathogenic to humans, contains six major groups: human polioviruses, human echoviruses, human coxsackievirus groups A and B, the new enterovirus serotypes 68-71, and, as mentioned above, hepatitis A virus.

The family Reoviridae contains six genera, two of which - human reoviruses (orthoreoviruses) and human rotaviruses - have been detected in polluted water. The virus particles are approximately 70 nm in diameter and have both an inner capsid 50 - 65 nm in size, of icosahedral symmetry, enclosing a double-stranded, segmented genome, and an outer one, in which striking differences are apparent in the different genera. The orthoreoviruses have a well-defined outer capsid, composed of hexagonal and pentagonal subunits. The rotavirus outer capsid lacks visible subunit structures. Both genera lose infectivity relatively slowly even at ambient temperatures and are stable over a wide range of pH values.

The family Adenoviridae contains two genera. The mammalian adenoviruses include 41 human species, subdivided on the basis of their biophysical, biochemical, biological, and immunological characteristics into six subgenera (A-F). The virion is a non-enveloped regular icosahedron (20 triangular surfaces and 12 vertices), which is 65 - 80 nm in diameter. A fibre-like structure projects from each of the vertices. The genome is a single linear molecule of double-stranded DNA.

Parvoviridae are among the smallest of the DNA animal viruses. The virion is 18 - 26 nm in diameter, is of icosahedral symmetry and has a single-stranded DNA genome. The family Parvoviridae contains three genera, for two of which, *Parvovirus* and *Dependovirus,* waterborne transmission is a possibility. The virion is extremely resistant to inactivation; it is stable between pH 3 and 9, and at 56 °C for 60 minutes. The genus *Dependovirus* (adeno-associated virus, AAV), has a relatively wide host range; infection is common in the general population. AAV only infects human cells cryptically; no overt disease has been observed.

The so-called "small round structured viruses", which include Norwalk virus, contain RNA and a single capsid polypeptide typical of caliciviruses; they are therefore currently included in the Caliciviridae family (see also p. 47).

Hepatitis E virus is an important cause of acute hepatitis in tropical and subtropical countries. Classification of this virus is difficult, but many have placed it among the Caliciviridae *(2).*

The Papovaviridae consist of several genera, among them the papilloma viruses. Papovaviridae are non-enveloped, icosahedral particles, 45 - 55 nm in diameter, which contain one molecule of double-stranded DNA. They are highly resistant to inactivating environmental factors. Natural transmission is presumed to be through contact, and the diseases that they cause have been associated with swimming-pools.

4.2 Routes of exposure

4.2.1 General considerations

Acute gastrointestinal and diarrhoeal illnesses continue to be the major water-borne diseases throughout the world. Rapid methodological advances have recently been made in the study of their etiology that have revolutionized the diagnosis of viral diarrhoeal diseases. Waterborne outbreaks due to viruses have now been recorded from developed and developing countries all over the world *(2-5).* Many different strains of viruses have been isolated from raw and treated drinking-water *(6).* Isolation from water does not prove beyond all possible doubt that water is a vehicle for the transmission of disease, although it

does indicate that a hazard exists. Proper treatment and disinfection should result in drinking-water that is essentially virus-free. Epidemiological proof of the water-borne transmission of viral diseases is very difficult to obtain for a variety of reasons *(7)*, including the following:

- the symptoms may not resemble those of typical waterborne diseases;

- asymptomatic carriage and excretion occur in a large proportion of those infected;

- some infections have long incubation periods, e.g. hepatitis caused by hepatitis A virus;

- waterborne transmission may be at a low level, and secondary spread may occur by other routes;

- suitably sensitive methods for detecting the infectious agent in water may be lacking.

Waterborne transmission has been unequivocally demonstrated for hepatitis A and hepatitis E viruses, rotaviruses and Norwalk virus, and the explosive epidemics that they cause have been well documented. For the other viruses included in Table 4.1, waterborne transmission is a probability but has not been definitely established.

Low-level transmission may occur in which small numbers of viruses present in drinking-water, either sporadically or continuously, produce asymptomatic infections that remain unrecognized. The person-to-person spread of such infections in the community could lead to disease outbreaks apparently unconnected with water. However, the existence of such a mechanism has not been confirmed.

In a prospective epidemiological study among city dwellers receiving bacteriologically satisfactory drinking-water, it was found that the group receiving water not treated by reverse osmosis at the point of use had 25% more gastrointestinal symptoms than those receiving water treated by this process *(8)*. The symptoms observed were compatible with infection caused by the Norwalk virus or astroviruses, which were probably incompletely removed from the sewage-contaminated river water used as the source.

In some areas, water sources may be heavily polluted, and the water-treatment processes used may not be reliable. For this reason, and because of the large number of persons at risk, drinking-water must be regarded as having a very significant potential as a vehicle for the environmental transmission of enteric viruses. As with other microbial infections, enteric viruses may also be transmitted by contaminated food and aerosols, as well as by direct contact, the usual mode of transmission.

Schemes for the recycling of wastewater for domestic use are being considered in some cities, while in many others, water for potable supplies is obtained from contaminated surface sources containing a significant proportion of waste-water. The risk of viruses penetrating the water-treatment processes - including pretreatment storage and disinfection - must be carefully evaluated whenever wastewater is to be reused in this way.

4.2.2 Specific families of viruses

Enteroviruses have a worldwide distribution, their prevalence increasing during the warm months of the year in temperate climates. The epidemiology of these infections suggests that faecal-to-oral transmission is the major means of spread and that various types of enterovirus can give rise to large outbreaks when they are transmitted by the water route.

Rotaviruses and orthoreoviruses have been detected in sewage, rivers, and lakes and in treated drinking-water in some countries *(9-12)*. Transmission occurs via the faecal-to-oral route. The infection is usually associated with sporadic cases, but several large waterborne outbreaks have been well documented *(13, 14)*. The rotaviruses are of considerable public health importance as a common cause of acute diarrhoea, particularly in young children. They infect and multiply in mature or differentiated enterocytes located on

the villi of the duodenum and small intestine, and are excreted in large numbers; as many as 1000 virus particles may be present per gram of faeces for approximately 8 days after the onset of symptoms.

Adenoviruses generally infect conjunctival, respiratory, and intestinal epithelium in addition to regional lymphoid tissue. Prolonged excretion of viruses both from the pharynx and from the intestinal tract has been described. Several species, particularly subgroups B, C, D and E, and serotypes 1, 2, 3, 4, 5, 6, 7 and 15, have been isolated from sewage, rivers, lakes, groundwater, and water used for drinking and swimming. Waterborne transmission occurs by the faecal-to-oral route, by inhalation of adenovirus aerosols into the lower respiratory tract, and by eye contact when the conjunctival surface is mildly irritated. Several large outbreaks of pharyngoconjunctival fever have been associated with swimming-pools *(15, 16)*.

The use of electron microscopy for the examination of faecal specimens from persons with nonbacterial gastroenteritis resulted in many observations of small viruses ranging in size from 20 to 40 nm, the "small round structured viruses" already mentioned on p. 45. The first of these viruses to be described was the Norwalk agent which was detected in volunteers fed filtered faecal suspension obtained from patients in an outbreak of winter vomiting disease. Morphologically similar viruses known as the Hawaii, Wollan, Ditching, Parramatta, Snow Mountain and Montgomery County agents were subsequently found. Failure to culture any of these agents satisfactorily delayed definitive classification but, as previously noted, they are now assigned to the Caliciviridae family.

Norwalk virus infects the villi of the jejunum. Virus shedding in stools occurs during the first 72 hours after the onset of illness. The virus is transmitted by the faecal-to-oral route. Of all Norwalk-related outbreaks, water seems to be responsible for about 40%, the type of water involved including drinking-water supplies, recreational bathing water, and shellfish-harvesting water *(17)*.

4.3 Health effects

Enteric viruses are capable of producing a wide variety of syndromes, including rashes, fever, gastroenteritis, myocarditis, meningitis, respiratory disease, and hepatitis (Table 4.1). In general, asymptomatic infections are common and the more serious manifestations rare. However, when drinking-water is contaminated with sewage, gastroenteritis and hepatitis may occur in epidemic proportions. Apart from these infections, there is little, if any, epidemiological evidence to show that adequately treated drinking-water is involved in the transmission of virus infections.

Gastroenteritis of viral origin may be associated with a variety of agents (Table 4.1). It is usually of 24-72 hours' duration with nausea, vomiting and diarrhoea; it occurs in susceptible individuals of all ages, but is most serious in the very young and very old, where dehydration and electrolyte imbalance can occur rapidly and threaten life if not corrected without delay.

Dependoviruses (adeno-associated virus), together with adenoviruses, have been recovered from surface water *(18)*; it is therefore suspected that waterborne transmission of these viruses can occur.

Hepatitis A virus (human enterovirus 72) and enterically transmitted hepatitis E virus cause infections of the liver typically accompanied by lassitude, anorexia, weakness, nausea, vomiting, headache, abdominal discomfort, fever, dark urine, and jaundice. Hepatitis, if mild, may require only rest and restricted activities for a week or two, but when severe may cause death from liver failure, or may result in chronic disease of the liver. Severe hepatitis is tolerated less well with increasing age, and the fatality rate increases sharply beyond middle age. The mortality rate is higher among those with pre-existing malignancy and cirrhosis *(19)*. A fulminant form leading to death within days occurs in 0.1-0.6% of cases. Hepatitis E infection in pregnant women has a high mortality rate. Local epidemics are usually traceable to contaminated food or water. The virus has been detected in polluted rivers *(20)* and in drinking-water *(21)*. Several very large outbreaks of drinking-water-transmitted hepatitis have occurred in India *(2)*, China (Mendong, personal communication), Algeria *(22)* and the former Soviet Union *(23)*.

Adenoviruses are among the viral agents associated with acute nonbacterial infectious gastroenteritis. Of the various species, two (types 40 and 41) cannot routinely replicate in cell cultures and are called fastidious variants. Such fastidious adenoviruses have been found in many parts of the world and are probably second only to rotaviruses as a cause of gastroenteritis in young children. They tend to be endemic rather than epidemic although outbreaks have occurred. Cytopathogenic adenoviruses can easily be detected in all kinds of water, so that waterborne transmission of the fastidious variants has also been suspected *(6)*.

Rotaviruses are responsible for a large proportion of severe episodes of diarrhoea in small children and infants, and may also cause gastroenteritis in the elderly *(24).* They are responsible for as much as 50% of the gastroenteritis in infants and children admitted to hospital during the cooler months of the year in temperate climates. Rotaviruses have occasionally been isolated from drinking-water in some countries, but more often from sewage *(9, 25).* Acute infection is characterized by the abrupt onset of severe watery diarrhoea with fever and vomiting. Dehydration and metabolic acidosis may develop, resulting in death if untreated. Those most severely infected and affected are between 6 and 24 months old.

The Norwalk virus usually causes self-limiting explosive epidemics of gastroenteritis that last for 24-48 hours, are community-wide, and involve school-age children, family contacts, and adults. Roughly one-third of such outbreaks of gastroenteritis can be attributed to the Norwalk virus. Infections result in delayed gastric emptying, nausea, vomiting, and abdominal cramps. About 50% of infected persons have associated diarrhoea; some have fever and chills. A transient lymphopenia has been observed. Norwalk and Norwalk-like viruses (small round structured viruses) primarily infect and cause disease in older children and adults, and have been responsible for a large number of outbreaks of acute infectious nonbacterial gastroenteritis. Infection may be spread by municipal water systems, semi-public water supplies, recreational swimming, and stored water *(4, 26, 27)* although other modes of transmission, including person-to-person spread, are usually more important.

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5. Protozoa

Drinking-water plays a major role in the spread of three of the intestinal protozoa pathogenic for humans, namely *Giardia intestinalis* (syn. *G. lamblia,* the etiological agent of human giardiasis), *Cryptosporidium parvum* (human cryptosporidiosis), and *Entamoeba histolytica* (amoebic dysentery). *Balantidium coli* infection (balantidiasis) is uncommon, although the parasite has a worldwide distribution. These pathogenic intestinal protozoa can be transmitted to humans by any mechanism whereby material contaminated with faeces containing viable organisms from infected individuals can reach the mouth. However, infections with pathogenic *Naegleria fowleri* (naegleriasis or primary amoebic meningoencephalitis) and *Acanthamoeba* spp. (meningitis, keratitis) are associated primarily with recreation and the inhalation of warm soil-contaminated water, and are comparatively rare.

5.1 Giardia

5.1.1 General description

Life cycle

Organisms in the genus *Giardia* (also called *Lamblia*) are flagellated protozoa that parasitize the intestines of humans and animals. These flagellates have a simple two-stage life cycle consisting of the reproductive trophozoite stage and the environmentally resistant cyst stage. When ingested by a susceptible host, the cysts are induced to excyst by exposure to acid in the stomach and perhaps also by contact with enzymes or other as yet undefined digestants *(1).* After excysting, the trophozoite leaves the cyst wall behind and rapidly undergoes cytokinesis, splitting by binary fission into two daughter trophozoites *(2)* which are bilaterally symmetrical and vary in shape from ellipsoidal to pyriform *(3).* The anterior end is rounded and contains two nuclei, while the posterior end tends to be pointed. The dorsal side is convex, and the ventral side contains an adhesive or sucking disc by which the organism attaches itself to intestinal surfaces. Each trophozoite has two slender median rods or axostyles, four pairs of flagella, and a pair of median bodies. The trophozoites may be 9-21 μm long, 5-15 μm wide, and 2-4 μm thick.

Perhaps in response to population pressures, the trophozoites release their hold on the intestinal epithelium and enter the lumen. As they travel down the intestines, they are apparently induced to encyst by exposure to bile, alkaline pH, and possibly bacterial metabolites *(4).* The cysts are ovoid, 8-12 μm long by 7 - 10 μm wide, and contain the same structures (nuclei, axostyles, median bodies) as the trophozoites; however, up to four nuclei may be visible within each cyst. The cysts are discharged with the faeces and thereby returned to the environment.

The length of time that cysts can survive depends on the temperature. *G. intestinalis* cysts have survived for at least 77 days and *G. muris* cysts for at least 84 days when suspended in water at less than 10 °C. Above 20 °C, cyst in-activation is relatively rapid. Sharp decreases in cyst viability have been noted after 3 days' storage in water at 20 °C or after only 1 day at 37 °C. The thermal death point for *G. muris* cysts has been reported to be 54 °C, and *G. intestinalis* have been inactivated by exposure to 55 °C for 5 minutes. Cysts may be inactivated in water by bringing the temperature to boiling point *(5-8).*

Host range

Giardia organisms are widely distributed in nature and have been reported as occurring in more than 40 species of animals including amphibians, birds, and mammals *(9).* However, whether or not giardiasis is, or can be, a zoonosis is debatable. Some investigators have reported infecting a variety of animals - including dog, beaver, muskrat, gerbil, and rat - with cysts from human sources *(10-12)*, but others have

been unable to infect mice, hamsters, rats, cats, and dogs with such cysts *(13)*. However, all of them have been able to infect some species of animals with cysts derived from different ones. While there are anecdotal reports to suggest that humans may become infected with cysts from deer, beavers, and muskrats *(14)*, no controlled studies on human volunteers inoculated with organisms from animal sources have yet been reported. It appears that some species of *Giardia* may be host-specific while others may not be. In addition, since at least some animals that inhabit watersheds can become infected with cysts from humans, they may act as intermediaries for human *Giardia* infection rather than as primary reservoirs. Methods are needed capable of differentiating between the cysts causing human infections and those found in environmental samples. Until such methods are developed, it would seem prudent, as has been suggested *(15)*, to assume that humans may be susceptible to many of the *Giardia* infecting lower animals.

The North American literature strongly supports the concept that animal vectors have been the source of the contamination of watersheds and of waters all but inaccessible to humans.

5.1.2 Routes of exposure

As with other pathogenic intestinal protozoa, *Giardia* can be transmitted by any mechanism whereby material contaminated with faeces containing viable organisms from infected individuals can reach the mouth. Documented routes of exposure include drinking-water, recreational water, food, and person-to-person contact.

Water

Epidemic giardiasis associated with contaminated drinking-water has been reported in the United States of America *(16)*, Canada *(17)*, England *(18)*, Scotland *(19)*, and Sweden *(20)*. Drinking-water has also been implicated as the vehicle of transmission in outbreaks occurring among travellers in the former Soviet Union *(21)*. The USA has experienced a great number of reported water-borne outbreaks, over 25 occurring between 1986 and 1988 *(22)*. In some of the outbreaks, water supplies had been contaminated with human sewage; in others, faecal discharges from watershed animals were the suspected sources of the contamination. Surveys of such animals have shown very high *Giardia* prevalence in aquatic voles *(23)* and muskrats *(24)*. Most of the outbreaks in the USA have been attributed to contaminated surface water treated only by disinfection *(16)*. *Giardia* cysts can be inactivated by disinfection, but are among the most resistant waterborne pathogens; effective disinfection calls for consideration of the water pH, turbidity, and temperature, as well as controlling the disinfectant dose and contact time *(25)*. The wide distribution of *Giardia* in humans and animals, the uncertainty concerning cross-species infectivity, the resistance of the cysts to inactivation by disinfection, and experience with the outbreaks led the USA to develop regulations on the disinfection of all surface water supplies in the country *(26)*. Risk analysis, using a probabilistic model, suggests that an annual risk of infection of less than one per 10 000 population can be achieved for source waters with 0.7-70 cysts per 100 litres, when treatment to achieve a 10^3-10^5-fold reduction is applied *(27)*.

Endemic giardiasis has also been associated with the consumption of contaminated drinking-water in such diverse locations as the USA *(16)* and South Africa *(28)*. In addition to endemic and epidemic giardiasis from drinking-water supplies, there have been reported outbreaks in the USA *(29)* and in Canada *(30)*, affecting children and adults, caused by the ingestion of swimming-pool water. The source of contamination in these outbreaks was apparently related to defecation in the water by infected children.

Relative significance of routes of exposure

Quantifying the degree of significance of the various routes of transmission of giardiasis is difficult because of a lack of information on the total prevalence or incidence of infection or disease. Bennett and co-workers *(31)*, using published material and survey data from the National Center for Health Statistics, estimated that 60% of the cases of giardiasis occurring in the USA were waterborne. Kappus & Juranek

(32) suggested that 45-50% of giardiasis cases in the USA were associated with drinking unfiltered municipal water. They also suggested that 40-45% of cases were associated directly or indirectly with person-to-person transmission at day-care centres, and that the remaining cases (about 10%) involved exposure while travelling, engaging in sexual practices that involve faecal exposure, or ingesting untreated surface water while hiking or camping. The contribution of waterborne as opposed to person-to-person transmission may be expected to vary from country to country depending on a number of factors including the extent of water treatment, the sanitation facilities, and local customs. However, apart from *Cryptosporidium* (see p. 56), *Giardia* probably has the greatest potential for transmission through drinking-water of all the waterborne parasitic protozoa since:

- cysts from humans are infective for a wide variety of domestic and wild animals and are widely distributed in the environment;

- some waterborne outbreaks have been attributed to the contamination of drinking-water by cysts of nonhuman origin;

- the cysts are highly resistant to disinfection.

 5.1.3 Health effects

Although the pathogenicity of the organisms was for long controversial, it is now widely accepted that *Giardia* can cause disease, and Koch's postulates have been satisfied by experimental human infections *(33).* Much of the controversy apparently arose from the highly variable illness-to-infection ratio observed. Asymptomatic infections with *Giardia* have been reported to account for up to 76% of the total under epidemic conditions *(34).* The time between ingestion of the organism and the appearance of the parasite in the stool is about 9 - 14 days, while the incubation period may range from 1 to 75 days with a median value of 8 - 15 days *(35)*. Symptomatic infections may be acute, subacute, or chronic, and the condition may last for months if not diagnosed and treated. Symptoms that have been commonly reported include diarrhoea, flatulence, foul-smelling stools, cramps, distension, fatigue, anorexia, nausea, weight loss, and vomiting. Intolerance to lactose may develop during the infection and persist even after the organism has been eradicated *(35)*. Infection in children may interfere with growth and normal development *(36)*, but mortality has rarely been reported in patients of any age.

The pathophysiological mechanisms in giardiasis remain to be clarified. As with the clinical effects, histopathological changes in the intestinal mucosa can cover a wide spectrum ranging from minimal to significant enteropathy with enterocyte damage, villus atrophy, and crypt hyperplasia *(37)*.

No explanation can be given for the broad range of clinical and pathological effects observed but both parasite and host factors are probably involved. Strain variation in pathogenicity has been demonstrated in humans *(33)*, while strain and host variations have been observed in animals *(38)*. In addition to local effects that can be produced directly by the parasites, their metabolic activity *(39)*, and secretion products, host factors that could contribute to the degree of tissue damage include nutritional status, systemic immune responses, and mucosal immunity *(37, 40)*. *Giardia* isolated from humans and animals have been found to be associated with bacteria, virus-like particles and mycoplasma-like organisms *(41)*. It has been suggested that these apparent symbionts may be transmitted via *Giardia* cysts. In addition, a double-standed RNA virus has been found in *Giardia (42)*. Some isolates of *G. intestinalis* are susceptible to infection with this virus while others are not *(43)*. What effect, if any, these associated organisms might have on the virulence of *Giardia* or on the pathogenesis of the disease is not known.

 5.2 Cryptosporidium spp.

 5.2.1 General description

Cryptosporidium spp. are intracellular coccidian parasites of the gastrointestinal and respiratory tracts of

numerous animals, including mammals, birds, and fish, and have a worldwide distribution. At present, six species are known, namely *C. parvum* and *C. muris,* which infect mammals, *C. baileyi* and *C. meleagridis,* which infect birds, and *C. serpentis* and *C. nasorum,* which infect reptiles and fish, respectively. *C. parvum* is the major species responsible for clinical disease in humans and domestic animals *(44).* As with both *E. histolytica* and *G. intestinalis,* infection occurs by ingestion of the transmissive phase which, for *Cryptosporidium* spp., is the oocyst. Person-to-person transmission occurs *(45)*, and oocysts from humans are infective for numerous mammals, including cattle and sheep *(46)*, while both domestic and feral animals may be reservoirs of human infection *(47)*. Infected humans can excrete 10^9 oocysts a day, and calves and lambs can excrete up to 10^{10} oocysts daily for up to 14 days *(48).* The average density of oocysts in raw sewage has been estimated at 5000 per litre *(49).* The broad host range together with the high output of oocysts ensures a high level of contamination in the environment. *Cryptosporidium* is an obligate parasite that develops only within a living host cell; unlike the other protozoa transmitted by drinking-water, but in common with other coccidia, it has several characteristic developmental stages *(44).* Infection is initiated following ingestion of the oocyst, which contains four naked, motile sporozoites. These are released through the suture in the oocyst wall following exposure to trypsin and bile salts, and attach themselves intimately to the surface of adjacent epithelial cells. They develop within a parasitophagous vacuole which is intracellular but extracytoplasmic, initially as a fixed trophozoite, then through asexual and sexual stages to finally become oocysts.

Cryptosporidium completes its life cycle within a single host; however, unlike *E. histolytica* and *G. lamblia,* endogenous reinfection (autoinfection) occurs which, together with recycling of the asexual stage, allows parasite numbers to build up to a high level. In addition, external maturation of oocysts is not required, and the thin-walled oocysts, which account for up to 20% of the total, excyst during passage through the intestine, releasing sporozoites which further increase the infection. The majority of the oocysts become detached and sporulate during passage through the gut to become thick-walled oocysts which are infective when excreted. *C. parvum* oocysts are spherical; their modal size is 4.5 × 5.0 μm (range 4-6 μm).

In various surveys conducted throughout the world, *Cryptosporidium* infection in immunocompetent persons has been found in 26 countries, with a reported prevalence of 0.6-20% in developed countries and 4-20% in developing ones. The infection is more common in children than in adults *(50).* Among AIDS patients, cryptosporidiosis has a prevalence of 3-4% in the USA and over 50% in some African countries and Haiti. An asymptomatic carrier state exists, but the ratio of cases to carriers has not been determined. At present, no effective drug is available for the treatment of cryptosporidiosis.

5.2.2 Routes of exposure

As with other pathogenic intestinal protozoa, *Cryptosporidium* can be transmitted by any mechanism whereby material contaminated with faeces containing viable organisms from infected humans or animals can reach the mouth.

Drinking-water

Humans and other mammals are reservoirs for infection, and the contamination of water supplies with either human or animal sewage can lead to the transmission of *Cryptosporidium* through drinking-water. Outbreaks have been traced to the contamination of drinking-water by both human and animal wastewaters *(51 - 54).* Oocysts can survive several months in water at 4 °C and are among the most chlorine-resistant pathogens known *(55)*. Waterborne outbreaks of cryptosporidiosis have been reported from both the USA and the United Kingdom and, in most of the recently documented outbreaks, oocysts have been identified in drinking-water. Outbreaks have been associated with untreated drinking-water, water treated by chlorination only, and water subjected to conventional treatment (coagulation, sedimentation, sand filtration and chlorination). Because oocysts are only 4 - 6 μm in size, the extent to which those present in raw water are removed by various water-treatment processes is still unclear. As with other intestinal protozoa pathogenic to humans, the infective dose is thought to be small. When two primates were given a dose of 10 oocysts, disease was produced in both *(56).* Information both on oocyst

survival in the environment and on resistance to disinfection is incomplete at present; however, oocysts lose their infectivity at temperatures below 0 °C or when kept at above 45 °C for 5-20 minutes *(55-57)*.

Apart from *Giardia* (see p. 52), *Cryptosporidium* probably has the greatest potential for transmission through drinking-water of all the waterborne parasitic protozoa since:

- oocysts from humans are infective for a wide variety of domestic and wild animals, and are widely distributed in the environment;

- some waterborne outbreaks have been attributed to the contamination of drinking-water by oocysts of nonhuman origin;

- among the protozoa under consideration, *Cryptosporidium* spp. have the smallest and most chlorine-resistant oocysts.

Other routes of exposure

Swimming-pools have been incriminated in the transmission of cryptosporidiosis *(54)*, but the evidence for the outdoor recreational water route for the transmission of infection is circumstantial *(52, 53)*. However, as oocysts can be detected in recreational waters, and such waters are being increasingly used for immersion sports, it is likely that the importance of this route of infection will increase in the future.

Since both animals and humans are reservoirs of infection and both *E. histolytica* and *G. intestinalis* can be transmitted by food, it seems likely that this may also be true for *Cryptosporidium* spp.

The transmission of *Cryptosporidium* infection between children and adults appears to be rare where good personal hygiene is practised. However, the transmission of infection among preschool children in day-care centres *(46, 58)* and similar institutions is probably common.

5.2.3 Health effects

Immunocompetent patients

While infection may be asymptomatic, it is usually associated with diarrhoea (80 - 90% of cases). Gastrointestinal symptoms, which may be accompanied by an influenza-like illness (20 - 40% of cases), include vomiting, anorexia, and flatulence. Symptoms typically last 7 - 14 days, and prolonged excretion of oocysts is unusual.

Immunocompromised patients

In patients with AIDS, other acquired abnormalities of T-lymphocytes, congenital hypogammaglobulinaemia, severe combined immunodeficiency syndrome, those receiving immunosuppressive drugs, and those with severe malnutrition, a severe cholera-like illness is produced, resulting in intractable nausea, weight loss, and severe dehydration (as much as 20 litres of liquid stool may be lost per day).

Except in those patients in whom the suppression of the immune system can be relieved by stopping immunosuppressant drugs, symptoms persist unabated until the patient dies *(59).*

5.3 Entamoeba histolytica

5.3.1 General description

E. histolytica is distributed worldwide and exists in trophozoite and cyst stages. Infection occurs by

ingestion of cysts; these range in size from 10 to 20 μm (average 12 μm). Since *E. histolytica* is primarily a parasite of primates, humans are the reservoir of infection. Dysenteric individuals pass only trophozoites, which are adversely affected by environmental factors such as drying and changes in temperature and salt concentration, while most or all of the parasites in this active amoeboid stage are destroyed by gastric juice *(60)*. Consequently, chronic cases and carriers who excrete cysts are more important sources of infection. Various surveys throughout the world have indicated a prevalence of 10-45% for *E. histolytica* infections and carriers can discharge up to 1.5×10^7 cysts daily *(61)*.

5.3.2 Routes of exposure

Since humans are the primary reservoir for infection with *E. histolytica,* the contamination of water supplies with domestic sewage can lead to the transmission of this organism through drinking-water. Outbreaks have been traced to sewage contamination of drinking-water *(61)*. The potential for waterborne transmission may be greater in the tropics, where the carrier rate sometimes exceeds 50%, as compared with more temperate regions where the prevalence in the general population is generally less than 10%. The cysts can survive for several months in water at 0 °C, 3 days at 30 °C, 30 minutes at 45 °C, and 5 minutes at 50 °C *(55)*, and are extremely resistant to chlorination *(62)*.

E. histolytica may also be transmitted by food, including raw vegetables, and food handlers may be important in transmission *(61)*. Although swimming-pools have not been definitely incriminated, they are a potential source.

Of the intestinal protozoan pathogens, *E. histolytica* is the most prevalent worldwide. Person-to-person spread and contamination of food by infected food handlers appear to be the most significant means of transmission, although contaminated drinking-water also plays a role.

5.3.3 Health effects

Though most infections with *E. histolytica* are asymptomatic or cause only minor symptoms, deaths can occur. The usual clinical manifestations are gastroenteritis with symptoms ranging from mild diarrhoea to fulminating bloody dysentery. Liver abscess is the most common metastatic complication. Pathogenicity appears to depend both on strain virulence and on host factors, including the nutritional status of the individual and the associated bacterial flora *(61)*.

5.4 Balantidium coli

5.4.1 General description

Balantidium coli is a ciliated organism of worldwide distribution; both the trophozoite and cyst stages can be infective for humans. The spherical to ovoid cysts are 40-60 μm in diameter, yellowish to greenish in colour, and have a two-membrane wall. Human infections usually occur as a result of the ingestion of food or water contaminated with faecal material from infected swine. Other hosts include lesser primates and, rarely, dogs and rats. *B. coli* is very common in swine but is considerably less prevalent in humans. Asymptomatic carrier infections can occur in humans and the world incidence is estimated at less than 0.7% *(60)*.

5.4.2 Route of exposure

The only reported waterborne outbreak of balantidiasis occurred in the Truk District of Micronesia in 1971. It was concluded that the epidemic probably resulted from the contamination of water supplies by pig faeces when a devastating typhoon destroyed pig pens and precarious water-catchment facilities *(63)*.

5.4.3 Health effects

The incidence of balantidiasis in humans is low, and direct contact with pigs appears to be the main route of transmission of the causative organism. The potential exists for the transmission of the organism in food and water contaminated with pig faeces.

Balantidiasis can present as an acute bloody dysentery, but an asymptomatic carrier state also occurs in humans *(60).*

 5.5 Naegleria and Acanthamoeba

 5.5.1 General description

Free-living amoebae cause severe human disease of waterborne origin. *Naegleria fowleri* is the etiological agent of primary amoebic meningoencephalitis *(64).* Although another species of *Naegleria, N. australiensis,* is known to produce fatal brain infection in experimental animals, no human cases due to this species have been reported *(65).* Various species of the genus *Acanthamoeba* cause keraritis, skin and pulmonary infections, and granulomatous amoebic meningitis *(66).* Infections by *Hartmannella* reported in the older literature were all due to *Acanthamoeba.* Infections with *N. fowleri* are almost always associated with recreational contact rather than with the drinking of water. *Acanthamoeba* eye infections are mostly related to inadequate cleaning or disinfection of contact lenses.

Naegleria spp. exist in three forms, namely as a trophozoite, a flagellate, and a cyst stage *(67)*. The trophozoites (10-20 μm) move by eruptive pseudopod formation. They have a single nucleus with a central nucleolus, although binucleated and multinucleated forms do occur. A sexual stage is unknown, and reproduction is by simple binary fission. The trophozoite can transform into a flagellate stage with two anterior flagella. The flagellate does not divide but reverts to the trophozoite stage. Under adverse conditions, the trophozoite transforms into a circular cyst, 7 - 15 μm in diameter. Although the cyst is quite resistant to chlorination, prolonged contact does kill it.

Acanthamoeba spp. have two forms *(67)*. The trophozoites (10-30 μm) are characterized by needle-like projections called filopodia or acanthopodia. Like *Naegleria,* they usually have a single nucleus with a central nucleolus, and reproduce by binary fission. In most species, the cyst stage (14-25 μm) is typically polygonal or starlike and has two easily distinguished cell walls. In some species, including the most virulent ones, the cyst is more or less rounded, and the two cell walls are difficult to discern. Cysts of *Acanthamoeba* are extremely resistant to chlorination.

Pathogenic species can be differentiated from nonpathogenic ones by prescreening on cell lines and then by intranasal instillation of the cultured amoebae into mice. Different species of pathogenic *Naegleria* and *Acanthamoeba* can be identified by antigen, isoenzyme and/or DNA studies. *Naegleria fowleri* is typically thermophilic, growing in water at temperatures up to 45 °C. Pathogenic *Acanthamoeba* rarely thrive at such high temperatures.

 5.5.2 Routes of exposure

Because of its thermophilic nature, *N. fowleri* is distributed worldwide in surface waters that are naturally heated by the sun or in industrial cooling waters and geothermal springs *(68)*. Most infections are reported in industrialized countries. In Australia, many fatal cases occurred through the use of unfiltered, chlorinated water for washing and bathing *(69)*. Cases in developing countries are most probably under-reported.

Some *Acanthamoeba* infections are related to water, but most, except for keratitis, occur in debilitated persons. Keratitis can occur following a minor trauma to the eye and subsequent washing, or as a result of wearing contact lenses. In particular, inadequate cleaning and disinfection of contact lenses favour the

occurrence of *Acanthamoeba* keratitis. Contact lens cases appear to be breeding places for this organism.

Acanthamoeba can be found in all environments, and particularly frequently in chlorinated swimming-pools and drinking-water. Although airborne transmission of free-living amoebae does occur, the evidence for infection by this route is controversial.

 5.5.3 Health effects

Naegleria fowleri causes fatal meningoencephalitis particularly in young and healthy individuals after swimming or activities causing infected water to be inhaled. The amoeba enters the brain by penetrating the olfactory mucosa and cribriform plate *(70).* The infection is very severe, and patients often die (5-10 days after penetration) before the infectious agent can be diagnosed. In addition, treatment is difficult, as only amphotericin B appears to be effective. Administration of other antibiotics together with amphotericin B might increase success rates. Although the infection remains rare (about 100 cases had been described up to 1980), new cases are encountered every year.

Acanthamoeba can cause diseases ranging from meningitis to pulmonary and wound infections, but few cases have been reported. However, the number of cases of keratitis increased considerably in the 1980s. While only 20 cases of keratitis were reported up to 1984, the number of cases in the USA had increased to over 200 by 1989 *(71).* Very few treatments are effective against *Acanthamoeba* infections, although keratitis cases can now be treated effectively; corneal transplants were usually necessary in the past.

Legionella bacteria can grow inside the cells of *Naegleria, Acanthamoeba (72)* and other free-living amoebae, and are protected against disinfection when inside the cysts of these amoebae. This is discussed further on p. 29.

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6. Helminths

The helminths or parasitic worms comprise two unrelated groups of organisms, namely flatworms belonging to the phylum Platyhelmintha, and roundworms belonging to the phylum Nematoda. Apart from the guinea worm, *Dracunculus medinensis,* which is transmitted solely by drinking-water, it is rare for any of those listed in Table 6.1 to be so transmitted. On the other hand, the continued use of poor-quality borehole or piped water is a major factor in the risk of acquiring the other helminth infections.

Table 6.1 Helminths potentially transmitted by drinking-water

Zoological classification	Species	Infective stage and usual mode of infection
Phylum Nematoda (roundworms)	*Dracunculus medinensis*	Larvae in Cyclops ingested in water
	Ascaris lumbricoides	Eggs ingested from soil
	Toxocara canis	Eggs ingested from soil
	Trichuris trichiura	Eggs ingested from soil
	Necator americanus	Penetrative larvae in soil
	Ancylostoma duodenale	Penetrative larvae in soil
	Strongyloides stercoralis	Penetrative larvae in water
Phylum Platyhelmintha, class Trematoda (flukes)	*Schistosoma* spp.	Free-swimming cercarial larvae penetrate skin
	Fasciola spp.	Cercarial larvae encysted and ingested on vegetation
Class Cestoidea, subclass Cestoda (tapeworms)	*Taenia solium*	Cysticerci consumed in raw pork or wild boar
	Echinococcus spp.	Eggs ingested from soil
	Spirometra spp.	Larvae in cyclops ingested in water or from soil

6.1 Dracunculus medinensis[1]

[1] The valuable contribution made by Dr P.J.A. Ranque, Dracunculiasis Eradication, WHO, Geneva, in the preparation of this section is gratefully acknowledged.

6.1.1 General description

The guinea worm is the longest nematode parasite of humans, the female worm measuring up to 700 mm in length. When the female is ready to discharge its embryos, its anterior end emerges from a blister, usually on the foot or lower limb, and releases many thousands of embryos when the affected part of the body is immersed in water. The male worms measure only 25 mm in length, remain in the tissues and so are never seen. Embryos can be released on several occasions on contact with water in ponds, or in large open step-wells, used as sources of drinking-water. After a few weeks the entire worm is expelled from the body. Embryos can live in water for about 3 days but, when ingested by certain species of freshwater cyclopoid Copepoda (Crustacea), penetrate into the haemocoelom, moult twice, and are infective to a new host in about 2 weeks. If the cyclops, which measure 0.5 - 2 mm in length, are swallowed in drinking-water, the larvae are released in the stomach, penetrate the intestinal and peritoneal walls, and inhabit the subcutaneous tissues. Mature gravid female worms emerge about 1 year after infection *(1)*.

Infection with guinea worm is geographically limited to rural areas of India, Pakistan, and 16 countries in sub-Saharan Africa (Benin, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Ethiopia, Ghana, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, Sudan, Togo, and Uganda). The annual incidence of dracunculiasis is estimated to be less than 2 million cases; approximately 140 million people are at risk *(2)*.

6.1.2 Routes of exposure

Drinking-water containing infected cyclops is the only source of infection with *Dracunculus,* which is therefore the only human parasite that can be eradicated solely by the provision of safe drinking-water. The eradication of guinea worm infection from the world by 1995 was a target of the International Drinking Water Supply and Sanitation Decade (1981-1990), and the World Health Assembly formally committed itself to this goal in 1991 (resolution WHA 44.5).

The disease occurs in rural areas where piped water supplies are not always available. Control is based principally on the provision of boreholes and safe wells, but also includes measures aimed at preventing contamination of water sources, filtering of water by consumers, and in some situations chemical treatment of ponds and open wells. There are no effective antihelminthic drugs for the clinical treatment of the infection.

Transmission is usually highly seasonal, depending on changes in water sources. For instance, transmission is highest in the early rainy season in a dry savanna zone of Mali with under 800 mm annual rainfall, but in the dry season in the humid savanna area of southern Nigeria with over 1300 mm annual rainfall.

6.1.3 Health effects

As previously mentioned, when a female guinea worm emerges, it causes the formation of a blister, which bursts, and a portion of the worm is extruded. In about 50% of all cases, the whole worm is extruded in a few weeks, the lesion then heals rapidly, and disability is of limited duration. However, in the remaining cases, complications ensue, and the track of the worm becomes secondarily infected, leading to morbidity, which lasts for months. Mortality is extremely rare, but permanent disability can result from contractures of tendons and chronic arthritis; in 1988, it was estimated that, in Nigeria, there were 12 000 such cases annually out of more than 600 000 infections each year.

Usually only one worm emerges, but there may be two, three, or occasionally many. Worms do not survive for more than one transmission season, but there does not appear to be any acquired immunity, and the same individuals can be reinfected many times. Incidence rates in infected communities can be very high, 30% of the 14 - 45-year age group often becoming infected each year. The economic effect on agricultural productivity can be important; for instance, an 11% annual reduction in rice production has been reported from an area of eastern Nigeria, at a cost of US$ 20 million *(3)*.

6.2 Schistosoma[1]

[1] The valuable contribution made by Dr K.E. Mott, Schistosomiasis Control, WHO, Geneva, in the preparation of this section is gratefully acknowledged.

6.2.1 General description

Schistosoma spp. belong to the class of trematodes or flukes, whose infective larvae are able to penetrate the human skin or mucous membranes, causing schistosomiasis. They may be transmitted through drinking-water, but are more of a hazard when water is used for washing or bathing.

Schistosome eggs are excreted in the urine or faeces of an infected person, and break open on reaching fresh water, releasing a tiny parasite (a miracidium). This must penetrate a freshwater snail within 8-12 hours if it is to develop further. Once it has penetrated the snail, the parasite divides many times until, within 4-7 weeks or longer, depending on the type of parasite, thousands of new forms (cercariae) break out of the snail into the water. The cercariae can live for up to 48 hours, and can penetrate human skin within a few seconds.

After penetration, the young parasites migrate through the lymphatic system to the blood vessels of the portal system, affecting the intestine (intestinal schistosomiasis) or the blood vessels around the bladder (urinary schistosomiasis), and develop into male or female adult worms within about 4 weeks. The adult worms live less than 5 years on average, although they can live for up to 40 years. Of the eggs produced by the female worm - over 200 per day for some species - only about half leave the body in the faeces (intestinal schistosomiasis) or in the urine (urinary schistosomiasis), the rest remaining embedded in the body, where they damage important organs. Heavy infections with schistosomes, which occur mainly in

children, cause the actual disease.

Intestinal schistosomiasis caused by *Schistosoma mansoni* occurs in 52 countries in Africa, the Eastern Mediterranean Region, the Caribbean, and South America. Oriental or Asiatic intestinal schistosomiasis, caused by the *S. japonicum* group of parasites (including *S. mekongi* in the Mekong river basin), is endemic in seven countries in the South-East Asia and Western Pacific Regions. (Another form of intestinal schistosomiasis caused by *S intercalatum* has been reported from ten central African countries.) Urinary schistosomiasis, caused by *S. haematobium,* is endemic in 54 countries in the African and Eastern Mediterranean Regions.

6.2.2 Routes of exposure

Schistosome infections are acquired when infected water is used for domestic activities, bathing or washing, or while working in contact with water. Ingested cercariae can penetrate the buccal mucous membranes, but this is a relatively unimportant route of entry. If safe drinking-water is readily available it will be used for washing, thus reducing the need to use contaminated surface water.

While there is a real possibility of piped untreated surface water transmitting schistosomiasis, most transmission is from unpiped sources such as pools, wells, and cisterns. In regions where schistosomiasis is endemic, the construction of dams and large reservoirs often leads to an increase in the population of the aquatic snail host and thus favours the spread of the disease. There are also many examples of increased transmission of schistosomiasis as a result of irrigation, the most dramatic being found along the Nile valley in Egypt and Sudan *(4).*

Schistosome infections are a hazard of recreational and irrigational water use rather than of drinking-water. However, improvements in community water supplies will reduce the incidence of schistosomiasis, particularly in communities where incidence is high *(4, 5).*

6.2.3 Health effects

The human schistosomes are a cause of severe morbidity and sometimes death in the 200 million people infected worldwide. In terms of socioeconomic and public health importance in tropical and subtropical areas, the disease now ranks second to malaria *(4).*

In communities where it is endemic, the prevalence of infection is greatest in 10-14-year-old children; in many African communities over 70% of village children may be infected. Pathology is due mainly to the host's reaction to eggs that fail to escape. Primary lesions are mainly in the liver, intestine, and around the bladder, but the most severe pathological effects are the consequence of secondary damage to the upper urinary tract, bladder cancer, and liver fibrosis and its haemodynamic consequences.

Schistosomiasis has a significant effect on health *(6).* In infected people without clinical evidence of disease, it is estimated that 30 work days are lost per year as a result of *S. japonicum* infection and 4 work days per year as a result of *S. haematobium* infection. After a latency period of 5-15 years, approximately 10% of infected people will develop severe disease. An 18% reduction in the work output of persons with severe *S. mansoni* infection can be expected. A reduction of 12% or more in exercise capacity was found in children with *S. haematobium* infection in Zimbabwe, but this was recovered by 1 month after treatment. Similarly, a 7-10% improvement in exercise capacity was found in children with *S. haematobium* infection 1 month after treatment in Kenya (KE Mott, personal communication).

A specific type of bladder cancer occurs in countries where urinary schistosomiasis is endemic, and is the leading cause of death due to cancer in Egypt among men aged 20-44 years. Diseases of the central nervous system, affecting the spinal cord, are more frequent and cause more debility than is widely recognized, especially among migrants into endemic areas of *S. mansoni* transmission.

In persons with schistosomiasis and intercurrent hepatitis B or typhoid fever, the severity and duration of both increase markedly, with an increased risk of chronic liver disease.

Since a single cercaria is infective, there is no safe level and cercariae should be absent from drinking-water. In the absence of routine monitoring assays, reliance must be placed on preventive measures if a significant risk from drinking-water is suspected in an area. The cercariae have a free-living life of under 48 hours, and storage for this period renders water safe *(7)*. It is likely that storage for 24 hours will greatly reduce infectivity. Slow sand filters, provided that they are properly operated, will remove the majority of cercariae, and disinfection at a residual level of 0.5 mg of free chlorine per litre for 1 hour will kill cercariae of the human schistosomes *(8). A* sounder approach is to use a source that does not contain the host snails and is not subject to excretal contamination.

6.3 Other helminths

A great variety of helminth eggs and larvae have been detected in drinking-water, and it is clear that none of those infective to humans should be present if the drinking-water is to be safe. However, the vast majority of such helminths are not primarily waterborne, and it is neither feasible nor necessary to monitor water for them on a routine basis *(9).*

Helminths that could conceivably be transmitted through drinking-water are listed in Table 6.1. *Fasciola* spp., which belong to the same class as the schistosomes (the Trematoda), are principally parasites of farm and domestic animals. The cercariae which emerge from freshwater snails encyst on water plants and infect humans if these are ingested. Some tapeworms (Cestoda) have very resistant eggs, and those of *Taenia solium* (the pork tapeworm) and *Echinococcus* spp. can develop in humans. Eggs are liberated from the gravid proglottids (segments), and are passed out in faeces. They can then be ingested from soil or on salad vegetables, although the normal route of infection with *T. solium* is the ingestion of raw pork containing the larval cysticercus stage. Eggs of *Echinococcus* have been recovered from wells in an area of East Africa and might be transmitted in drinking-water. Another tapeworm, *Spirometra,* has its tapeworm stage in carnivores, and two intermediate hosts, the first being a cyclopoid copepod and the second an amphibian, reptile, rodent or herbivore, depending on species. Humans occasionally act as intermediate hosts for the larvae (spargana) by ingesting first-stage larvae inside cyclops when drinking water from ponds.

The resistant eggs of various common, ubiquitous, intestinal nematode parasites of humans, such as *Ascaris* and *Trichuris* (and the common dog ascarid, *Toxocara,* the eggs of which can hatch in humans and the larvae cause visceral damage), are passed in faeces and normally ingested in soil or on salad vegetables. The eggs occasionally enter water but have a high relative density and settle quickly; drinking-water does not play an important part in their transmission.

Other intestinal nematode parasites which infect many millions of people in the tropics and subtropics are *Necator* and *Ancylostoma* (the hookworms) and *Strongyloides.* Eggs (or, in the case of *Strongyloides,* larvae) are passed in the faeces, and the larvae develop in the soil into an infective stage which can penetrate the skin of a new host. While the larvae of *Ancylostoma* are sometimes ingested on salad vegetables, there is little evidence that drinking-water is ever a source of infection for these soil-transmitted nematodes.

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7. Toxins from cyanobacteria

Blooms of cyanobacteria (commonly called blue-green algae) are very common in lakes and reservoirs used for potable water supply. These bacteria are capable of producing various toxins which fall into the following three categories: (i) hepatotoxins produced in fresh water by *Microcystis, Oscillatoria* and *Anabaena,* and by *Nodularia* in brackish water; (ii) neurotoxins produced by species of *Anabaena, Oscillatoria, Nostoc, Cylindrospermum* and *Aphanizomenon;* (iii) lipopoly-saccharides from a number of species *(1)*.

The most commonly encountered are the hepatotoxins, which induce death by circulatory shock as a result of massive liver haemorrhage within 2-24 hours of oral intake of a sufficiently large quantity *(2-7)*. At present, there are thought to be more than 13 variants of the toxin, which is a cyclic structure containing seven amino acids of relative molecular mass varying from about 800 to 1050. The best studied of these hepatotoxins is microcystin LR:R, which has a relative molecular mass of 994.

The LD_{50} of microcystin LR:R has been shown to be about 30 µg/kg of body weight in mice by intraperitoneal injection *(8).* A lethal dose is about 1-2 µg of pure toxin per mouse; however, the toxicity by the oral route appears to be about an order of magnitude less. There appear to be no other toxicity data available on the pure toxin, although studies with diluted extracts of a toxic bloom, reported to contain 56.6 µg/ml of an unknown variant of microcystin, showed that liver damage could be induced in mice given a one-quarter dilution of the extract in their drinking-water for 1 year *(9)*. Microcystin was not mutagenic in the Ames test *(10)*, but purified microcystin LR inhibited protein phosphatase *in vitro* with the same potency and specificity as the tumour promoter okadaic acid *(11)*.

The hepatotoxic cyclic peptide from *Nodularia,* termed nodularin, has a structure similar to that of microcystin, but contains only five amino acids. The oral LC_{50} for mice has been determined as 67 µg/ml for females and 73 µg/ml for males receiving 4.5-7 ml of drinking-water per day containing crude extracts *(7)*.

There are a number of unconfirmed reports of algal toxins in drinking-water supplies causing health problems, including an outbreak of hepatoenteritis in Palm Island, Australia *(12).* However, the most convincing evidence comes from an epidemiological study by Falconer et al. *(13)* of an Australian

community in which raised serum enzymes indicative of mild, reversible liver damage were observed in hospital patients who drank water from a local reservoir with a very large toxic bloom of *Microcystis aeruginosa.* Recent surveys of large numbers of fresh waters worldwide, which produce heavy growths of cyanobacteria, have shown the presence of cyanobacterial toxins at over 60% of sites *(14).* Algal blooms may change from being nontoxic to toxic in a very short time, but there is at present no well established method for analysis of the toxin in drinking-water.

It has been reported that activated carbon removes microcystin to a significant extent (*15-17*) and that ozone at a dose of 1.0 - 1.5 mg/litre destroys toxicity *(17)* by converting microcystin into a less toxic substance *(18).* The use of algicides such as copper sulfate at the height of the bloom is not recommended, since this leads to a massive release of toxin into the water, and may have been responsible for the unusual problems seen on Palm Island *(12).*

At present, it is not clear how great a hazard algal toxins pose in drinking-water, and the data are insufficient to enable any guidelines to be drawn up. However, problems resulting from the progressive eutrophication of inland waters appear to be increasing and with them the likelihood of cyanobacterial blooms. This emphasizes the need for the protection of sources, and particularly of lakes and reservoirs, from discharges of nutrient-rich effluent.

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8. Nuisance organisms

Nuisance organisms constitute a morphologically and physiologically diverse group, including planktonic and benthic cyanobacteria (blue-green algae), actinomycetes, iron, manganese, and sulfur bacteria, Crustacea, and protozoa. These organisms cause problems when the conditions in reservoirs or distribution systems are such as to support their growth. Thus organic matter in drinking-water supports the growth of bacteria and fungi, which in turn will help to maintain populations of protozoa and Crustacea. Many invertebrate animals can feed on bacteria, fungi, and protozoa. The content of organic compounds in treated water should therefore ideally be so low as to inhibit the growth of bacteria and to prevent that of other organisms during distribution.

8.1 Microbiological problems

Although the raw water itself does not usually contain large numbers of nuisance organisms, problems may develop during the water-treatment process. Nuisance organisms become concentrated on the surfaces and inside the beds of filters, where they autolyse and release cellular compounds that cause colour, turbidity, tastes, and odours. Activated carbon filters will, after a while, contain large amounts of organic matter, thus providing an excellent substrate for bacteria, which can create problems in the water supply, either by causing taste, odour, and turbidity, or microbiologically by increasing the colony counts of aerobic heterotrophic bacteria. Significant amounts of organic carbon can cause the growth of *Aeromonas* spp. in the distribution system during the warmer months of the year (see section 3.2.2). Large numbers of aerobic, heterotrophic bacteria in treated water can interfere with the interpretation of the tests for the coliform group by masking their presence or giving false positive reactions. A particular problem exists with some strains of *Aeromonas* spp., which produce acid and gas with coliform media, even at 44 °C.

Most of these nuisance organisms can be controlled relatively easily by care in operating water-treatment processes. Nutrient-rich raw water should be avoided if proper water treatment cannot be applied.

The compounds produced by nuisance organisms have low taste and odour thresholds, e.g. the earthy taints of geosmin (*trans*-1,10-dimethyl-*trans*-9-decadole) and MIB (2-methylisoborneol) produced by

actinomycetes and cyanobacteria. These compounds cause problems in drinking-water at threshold values of 10 and 25 ng/litre respectively, and are therefore often the cause of complaints by consumers before they are detected by analytical methods. It is therefore advisable to use panels of trained judges of taste and odour so that the compounds can be detected and the necessary measures taken before they become a problem in the drinking-water supply. Another way to prevent nuisance organisms from causing taste and odour problems is by means of regular microscopic examination of the organisms present in the water. As soon as a group of organisms known to cause these problems becomes dominant, appropriate measures should be taken to deal with them.

Some of these organisms can also produce colour in drinking-water. Pigmented organisms, such as cyanobacteria and algae, can be crushed on filters, resulting in the release of pigments, while microalgae can pass through the filters and cause both coloration and turbidity.

If water contains ferrous or manganous salts, these can be oxidized by iron or manganese bacteria, resulting in rust-coloured or black deposits in storage tanks and on the walls of pipes in parts of the distribution system where the flow rate is low. If the flow rate is subsequently increased, however, these deposits can be loosened and transported to consumers. Rust-coloured deposits can stain laundry. The slurry will also contain organic deposits which can decompose to produce tastes and odours. Manganese-oxidizing microorganisms (bacteria, fungi, and, very rarely, protozoa) produce deposits in aquifers, wells, and water conduits, the problems caused by such deposits including reduced yield, clogging of slots in well pipes, increased turbulence in pipes resulting in reduced flow velocity, damage to equipment for measuring water flow, black-coloured water, stains on laundry, and problems with food-handling establishments. The deposits can contain heavy metals such as arsenic, lead, zinc, and copper. Bacteria can become attached to them, so that, if they are disturbed, the colony count of the water will be increased. Prevention is based on the removal of Mn(II) from raw water, if a value of about 0.1 mg/litre is exceeded.

Iron and sulfur bacteria may contribute to the corrosion of iron and steel well pipes and drinking-water mains. Such microbially mediated corrosion can occur as a consequence of:

- the adsorption of nutrients and the depletion of dissolved oxygen by the colonies of microorganisms that have accumulated at the metal surface;

- the liberation of corrosive metabolites, such as organic acids and other complex-forming compounds;

- the production of sulfuric acid from sulfides or elemental sulfur; and

- the inclusion of sulfate-reducing bacteria in the cathodic process under anaerobic conditions.

The presence of certain organisms in water may be an indication either of the corrosion of cast iron or of the biodeterioration of construction materials to form substances that support the growth of microorganisms. The latter include non-metallic materials, such as plastics, rubber-jointing compounds, and pipe-lining materials, which can provide organic nutrients and thus encourage the growth of microorganisms, sometimes including coliform organisms other than *Escherichia coli* and *Pseudomonas aeruginosa*. Deterioration can occur in pipelines carrying groundwater or surface water. Unchlorinated waters, or water in which the chlorine residual has disappeared, appear to support higher rates of attack than those in which a residual can be detected.

Nuisance organisms may also cause problems in groundwater sources by encrusting well screens, thus reducing yield and impairing the aesthetic quality of the supply. Their presence may also indicate organic pollution of the aquifer.

Routine monitoring of such nuisance organisms cannot be recommended because of their diverse nature and unpredictable occurrence, although bacteriologists should be aware that they can impair water quality.

It is not practicable to specify any quantitative guideline values for nuisance microorganisms.

8.2 Problems caused by invertebrate animals

Invertebrate animals often infest shallow, open wells, from which supplies are drawn by bucket, but problems are not uncommon in large, public supplies. The animals derive their food from the bacteria, algae, and protozoa in the water or present on slimes on pipe and tank surfaces.

The types of animal concerned can be considered, for control purposes, as belonging to two groups. Firstly, there are free-swimming organisms in the water itself or on water surfaces, such as the crustacea *Gammarus pulex* (freshwater shrimp), *Crangonyx pseudogracilis, Cyclops* spp. and *Chydorus sphaericus.* Secondly, there are other animals that either move along surfaces or are anchored to them (such as *Asellus aquaticus* (water louse), snails, *Dreissena polymorpha* (the zebra mussel) and other bivalve molluscs, and the bryozoan *Plumatella* sp.), or inhabit slimes (such as *Nais* spp., nematodes and the larvae of chironomids) *(1)*. In warm weather, slow sand filters can sometimes discharge the larvae of gnats (*Chironomus* and *Culex* spp.) into the water, if the top layer of the bed collapses, causing draw-down of unfiltered water.

The only health hazard positively identified arises in tropical countries where water fleas *(Cyclops)* are the intermediate host of the guinea worm *(Dracunculus medinensis)* (see section 6.1).

Penetration of waterworks and mains is more likely to be a problem when low-quality raw waters are abstracted and high-rate filtration processes used. Pre-chlorination assists in destroying animal life and in its removal by filtration but, if excessive, may produce chlorinated organic compounds and convert total organic carbon into a biodegradable form. Maintenance of chlorine residuals in the distribution system, the production of high-quality water, and the regular cleaning of water mains by flushing or swabbing will usually prevent infestation.

Bryozoan infestation can be treated with a shock dose of chlorine, maintained at 10 mg/litre for about 24 hours, followed by flushing. Permethrin treatment of water at an average dose of 0.01-0.02 mg/litre for 24-48 hours has been used to destroy *Asellus* and other Crustacea, but treated water must not be discharged into watercourses, as it is rapidly toxic to fish and other aquatic life at this concentration *(2, 3)*. The most effective procedure is to draw treated water into the main by opening hydrants downstream of the injection point. These are then closed, allowing sufficient contact time (ideally 24 hours) to paralyse the Crustacea, after which the mains are cleared by flushing and swabbing. Persons using renal dialysis should not be supplied with permethrin-treated water, and those rearing fish should be warned not to replenish the culture tanks with mains water while it is being treated. The treated water can be safely discharged into sewers for treatment at sewage works *(2)*.

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9. Microbial indicators of water quality

9.1 Rationale

The recognition that faecally polluted water is responsible for spreading enteric diseases led to the development of sensitive methods of verifying that drinking-water is free from faecal contamination. Even though many waterborne pathogens can now be detected, the methods are often difficult, relatively expensive, and time-consuming. Furthermore, pathogens are shed into water only from infected people and animals, and it is not possible to examine water for every possible pathogen that might be present. It is prudent to regard as unsafe all water that contains bacteria indicating faecal pollution, because of the risk that enteric pathogens may be present. The bacteria selected as indicators of faecal pollution should be universally present in the faeces of humans and warm-blooded animals in large numbers. Other desirable properties of faecal indicators are that they should be readily detected by simple methods and that they do not grow in natural waters. Furthermore, it is essential that their persistence in water and the extent to which they are removed by water treatment are similar to those of waterborne pathogens.

Examination for faecal indicator bacteria in drinking-water provides a very sensitive method of quality assessment. It is also important to determine the quality of the raw water, not only to assess the degree of pollution but also to enable the best local source to be selected and the best form of treatment chosen. Microbiological examination for faecal indicators is the most sensitive and specific method for detecting recent faecal pollution, i.e. pollution that is potentially dangerous, since simple chemical analysis is not adequate for this purpose. Water must be examined regularly and frequently because pollution is often intermittent and may not be detected if examination is limited to only one or a small number of samples. For this reason it is better to examine drinking-water frequently by means of a simple test rather than less often by several tests or a more complicated test. When personnel and facilities are limited, routine microbiological examination for evidence of faecal contamination must always be given first priority *(1)*.

Microbiological examinations can also be carried out with other objectives than assessing the degree of faecal contamination. They may give information on the effectiveness with which specific groups of microorganisms have been removed by treatment processes; thus, if bacteriophages are present this may indicate that viruses have not been removed, and the presence of spores of sulfite-reducing clostridia also shows highly persistent microorganisms may have survived. Colony counts of aerobic, heterotrophic bacteria, or microscopic or indirect chemical methods (e.g. the assay of adenosine triphosphate by luminometry) may provide information on the availability of nutrients in the water that support bacterial growth, which may result in aesthetic problems or in the presence of opportunistic pathogens. For some of these latter organisms, specific culture methods are also being used, namely for *Pseudomonas aeruginosa, Legionella* and *Aeromonas* (see section 3.2); however, these should not be used routinely, but only when necessary to solve problems related to the occurrence of the organisms concerned.

9.2 Indicators of faecal contamination

The use of normal intestinal organisms as indicators of faecal pollution rather than the pathogens themselves is universally accepted for monitoring and assessing the microbial safety of water supplies. In practice, the criteria to be satisfied by an ideal indicator (see section 9.1) cannot all be met by any one organism. However, many of them are best fulfilled by *Escherichia coli,* and to a lesser extent by the thermotolerant coliform bacteria; *E. coli* is thus the indicator of choice when resources for supplementary microbiological examination are limited. Other microorganisms that satisfy some of these criteria, though not to the same extent as *E. coli* and the thermotolerant coliform organisms, can also be used as supplementary indicators of faecal pollution in certain circumstances.

Because enteroviruses and the cysts of some parasites are known to be more resistant than *E. coli* and coliform organisms to disinfection, the absence of these organisms in surface water that has only been disinfected will not necessarily indicate freedom from enteric viruses and the resting stages of *Cryptosporidium, Giardia,* amoebae, and other parasites.

The significance that can be attached to the presence or absence of particular faecal indicators varies with each organism and particularly with the degree to which that organism can be specifically associated with faeces. For example, some of the genera detected by the methods for enumeration of thermotolerant and total coliform bacteria have nonfaecal sources in the environment, e.g. in soil or decaying vegetation, or can even grow in the aquatic environment, thus limiting their usefulness as indicators of faecal contamination. Other bacterial indicators have useful properties which enable them to be used for particular purposes. For example, although the faecal streptococci and enterococci and the spores of sulfite-reducing clostridia, typified by *Clostridium perfringens,* are less numerous than coliforms in faecally polluted water, they have greater powers of survival and so may be used to confirm the presence of faecal contamination when *E. coli* is not found or to assess the efficiency of treatment processes. Anaerobic bacteria, such as bifidobacteria and the *Bacteroides fragilis* group, are more abundant than coliform organisms in faeces, but decay rapidly in water, and accepted standard methods for their detection and enumeration are not yet available. Full identification of these indicator organisms would require such an extensive series of tests as to be impracticable in routine monitoring.

9.2.1 Escherichia coli

Escherichia coli is abundant in human and animal faeces, where numbers may attain 10^9 per gram of fresh faeces. It is found in sewage, treated effluents, and all natural waters and soils subject to recent faecal contamination, whether from humans, farm animals, or wild animals and birds. The presence of *E. coli* in water always indicates potentially dangerous contamination requiring immediate attention. Complete identification of *E. coli* is too complicated for routine use, hence certain tests have been evolved for identifying this organism rapidly with a high degree of certainty. Some of them are the subject of international and national standards and have been accepted for routine use, whereas others are still being developed or evaluated. Detection of *E. coli* on complex media entails incubation at the restrictive temperature of 44-45 °C in combination with demonstration of the production of acid and gas from lactose and of specific biochemical reactions such as indole production and β-glucuronidase activity, and the absence of urease activity. In other tests, chemically defined media with specific substrates for the growth and detection of enzymatic activities of *E. coli,* such as β-galactosidase and β-glucuronidase, are used. Confirmation of the presence of *E. coli,* as indicated by these methods, requires extensive biochemical identification or the use of alternative, commercially available test systems. Such confirmation is not recommended as a routine, but may be necessary to validate the use of routine tests under specific conditions.

9.2.2 Thermotolerant (faecal) coliform organisms

These are defined as the group of coliform organisms that are able to ferment lactose at 44-45 °C. They comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella, Enterobacter,* and *Citrobacter.* Of these organisms, only *E. coli* is specifically of faecal origin, being always present in the faeces of humans, other mammals, and birds in large numbers, and rarely found in water or soil that has not been subject to faecal pollution. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. In tropical and subtropical waters, thermotolerant coliform bacteria may occur without any obvious relation to human pollution and have been found on vegetation in a tropical rainforest *(2).* This means that the occurrence of the thermotolerant coliform group in subtropical or tropical waters or those enriched with organic wastes does not necessarily indicate faecal contamination by humans since they can originate from wild animals, including birds. However, their presence in waters in warm climates should not be ignored, as the basic assumption that pathogens may be present and that treatment has been inadequate still holds good. Regrowth of thermotolerant coliform organisms in the distribution system is unlikely unless sufficient bacterial nutrients are present (biochemical oxygen demand (BOD) greater than 10 mg/litre) or unsuitable materials are in contact with the treated water, the water temperature is above 15 °C, and there is no free chlorine residual.

Thermotolerant coliforms are less reliable indicators of faecal contamination than *E. coli,* although under most circumstances their concentrations are directly related to *E. coli* concentrations in water. Their use for water-quality examination is therefore considered acceptable. Internationally standardized methods and media for their detection have been validated, and are relatively simple and widely available. When necessary, thermotolerant coliform isolates can be subjected to further confirmatory tests to detect those that are presumptive *E. coli.* Normally, a test for the ability to produce indole from tryptophan at 44 ± 0.5 °C is sufficient. The detection and identification of these organisms as faecal organisms or presumptive *E. coli* provide strong evidence of recent faecal contamination and of the need for immediate investigation.

Because thermotolerant coliform bacteria are readily detected by single-step methods, they have an important secondary role as indicators of the efficiency of individual water-treatment processes in removing faecal bacteria. They may therefore be used in assessing the degree of treatment necessary for waters of different quality and for defining performance targets for bacterial removal (see section 11.3).

9.2.3 Coliform organisms (total coliforms)

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water. The term "coliform organisms (total coliforms)" refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, and able to ferment lactose at 35-37 °C with the production of acid, gas, and aldehyde within 24-48 hours. They are also oxidase-negative and non-spore-forming. These definitions have recently been extended by the development of rapid and direct enzymatic methods for enumerating and confirming members of the coliform group. By definition, coliform bacteria display β-galactosidase activity. Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia, Citrobacter, Enterobacter,* and *Klebsiella.* However, the group of coliform bacteria, as defined by modern taxonomical methods, is heterogeneous and includes lactose-fermenting bacteria which can be found in both faeces and the environment, namely in nutrient-rich waters, soil, decaying vegetation and drinking-water containing relatively high levels of nutrients. Examples of such species are *Enterobacter cloacae* and *Citrobacter freundii.*

The coliform group also contains species that are rarely, if ever, found in faeces and which can multiply in relatively good-quality drinking-water, e.g. *Serratia fonticola, Rahnella aquatilis,* and *Buttiauxella agrestis.* Several lactose-fermenting species of *Serratia* and *Yersinia* can be isolated from uncontaminated water or soil. There are also many reports of the existence of non-lactose-fermenting but otherwise characteristic coliform bacteria. Lactose-negative strains which otherwise resemble the traditional coliform genera lack the lactose permease enzyme. They do, however, possess the β-galactosidase enzyme and will appear as coliform bacteria if a β-galactosidase test is applied. The existence of nonfaecal bacteria that fit the definition of coliform bacteria and of lactose-negative coliform bacteria limits the applicability of this group of bacteria as indicators of faecal pollution.

Coliform bacteria should not be detectable in treated water supplies and, if found, suggest inadequate treatment, post-treatment contamination, or excessive nutrients. In this sense, the coliform test can be used to assess treatment efficiency and the integrity of the distribution system. Although coliform organisms may not always be directly related to the presence of faecal contamination or pathogens in drinking-water, the coliform test is still useful for monitoring the microbial quality of public water supplies. If there is any doubt, especially when coliform organisms are found in the absence of faecal coliforms and *E. coli,* secondary indicator organisms may be used to determine whether faecal contamination is present; these include the faecal streptococci and sulfite-reducing clostridia, especially *Clostridium perfringens.*

9.2.4 Faecal streptococci

The term "faecal streptococci" refers to those streptococci generally present in the faeces of humans and

animals. All possess the Lancefield group D antigen. Taxonomically, they belong to the genera *Enterococcus* and *Streptococcus.* The genus *Enterococcus* has recently been defined to include all streptococci sharing certain biochemical properties and having a wide tolerance of adverse growth conditions. It includes the species *E. avium, E. casseliflavus, E. cecorum, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii,* and *E. solitarius.* Most of these species are of faecal origin and can generally be regarded as specific indicators of human faecal pollution under many practical circumstances. They may, however, be isolated from the faeces of animals, whereas certain species and subspecies, such as *E. casseliflavus, E. faecalis* var. *liquefaciens, E. malodoratus,* and *E. solitarius* occur primarily on plant material. The taxonomy of enterococci has recently undergone important changes, and detailed knowledge of the ecology of many of the new species is lacking.

In the genus *Streptococcus,* only *S. bovis* and *S. equinus* possess the group D antigen and are members of the faecal streptococcus group. They occur mainly in animal faeces. Conventional media for the isolation and identification of faecal streptococci, such as m-enterococcus agar, KF-streptococcus agar, and azide-glucose broth, generally support the growth of all faecal streptococci. However, particularly in warm climates, other cocci may also develop on these media, so that confirmatory tests are needed. More restrictive media that support the growth of the enterococci in particular have also been proposed and have been widely used in the USA *(3)*. The applicability and specificity of these media need to be further tested under a wide range of conditions. Faecal streptococci rarely multiply in polluted water and are more persistent than *E. coli* and coliform bacteria. Their main value in assessing water quality is therefore as an additional indicator of treatment efficiency. Furthermore, streptococci are highly resistant to drying and may be valuable for purposes of routine control after new mains have been laid or distribution systems repaired, or for detecting pollution by surface run-off to groundwater or surface waters.

9.2.5 Sulflte-reducing clostridia

These are anaerobic, spore-forming organisms, of which the most characteristic, *Clostridium perfringens (C. welchii)*, is normally present in faeces, though in much smaller numbers than *E. coli.* However, they are not exclusively of faecal origin and can be derived from other environmental sources. Clostridial spores can survive in water much longer than organisms of the coliform group and will resist disinfection. Their presence in disinfected waters may thus indicate deficiencies in treatment *(4)*. In particular, the presence of *C. perfringens* in filtered supplies may be a sign of deficiencies in filtration practice, while *C. perfringens* spores may indicate the presence of protozoan cysts; because of their longevity, they are best regarded as indicating intermittent or remote contamination and thus are of special value. However, they are not recommended for the routine monitoring of distribution systems. Because they tend to survive and accumulate, they may be detected long after pollution has occurred and far from the source, and thus give rise to false alarms.

9.2.6 Bacteriophages

Bacteriophages are viruses that infect bacterial host cells. They usually consist of a nucleic acid molecule (genome) surrounded by a protein coat (capsid). Bacteriophages may contain either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) as the genome and may have a very simple, cubic structure or a more complex one with heads, tails, tail fibres, or other attachments. They are in the size range 25-100 nm. Bacteriophages have been proposed as indicators of water quality, particularly with respect to human enteric viruses, both because of their similar nature and because they are relatively easy to detect in water samples *(5)*. Furthermore, data are accumulating showing the similarities between certain groups of bacteriophages and human enteric viruses in terms of survival in the aquatic environment and responses to water- and wastewater-treatment processes. Two groups of bacteriophages have been studied extensively in the context of viral indicators in water, namely the somatic coliphages, which infect standard *E. coli* host strains via cell wall (somatic) receptors, and the F-specific RNA bacteriophages which infect *E. coli* and related bacteria through the F- or sex-pili. Neither of these groups of organisms occurs in high concentration in faeces, but they are invariably found in sewage. They are used, therefore, primarily as an index of sewage contamination and, because of their high persistence as compared with bacterial

indicators, as an additional indicator of treatment efficiency or groundwater protection.

9.2.7 Miscellaneous indicators

The bifidobacteria and the *Bacteroides fragilis* group are anaerobes which are specific to faeces, where they outnumber the coliform group. They do not survive or multiply in natural waters, and have been seen as an alternative to the coliform group in tropical and semitropical regions, where the latter can multiply in warm and organically enriched water *(6)*. However, their numbers decline more rapidly then those of thermotolerant coliforms and *E. coli* in passing from faeces through sewage and into polluted waters, indicating that their rate of decay is greater than that of other bacterial indicators *(6)*. This is a disadvantage, since bacteria in the coliform group are themselves more sensitive to decay than viral and protozoal pathogens. In addition, the methods of detecting them in water are not very reliable and have not been standardized.

9.3 Indicators of water quality and treatment efficacy

9.3.1 Heterotrophic plate counts (colony counts)

Heterotrophic plate counts may be used to assess the general bacterial content of water. They do not represent all the bacteria present in the water but only those able to grow and produce visible colonies on the media used and under the prescribed conditions of temperature and time of incubation. Colony counts are often determined following incubation at 22 °C and 37 °C to assess the relative proportions of naturally occurring water bacteria unrelated to faecal pollution and of bacteria derived from humans and warm-blooded animals, respectively. The count at 22 °C is of little sanitary value, but is useful in assessing the efficiency of water treatment, specifically the processes of coagulation, filtration, and disinfection, where the objective is to keep counts as low as possible. The 22 °C count may also be used to assess the cleanliness and integrity of the distribution system and the suitability of the water for use in the manufacture of food and drink, where high counts may lead to spoilage. Any increase in counts in the test at 37 °C as compared with those normally found may be an early sign of pollution.

9.3.2 Aeromonas spp. and Pseudomonas aeruginosa

Apart from heterotrophic plate counts, the use of other microorganisms, including *Aeromonas* and *Pseudomonas aeruginosa,* has been advocated as a means of assessing the hygienic quality of drinking-water *(7, 8)*. However, neither examination for these organisms nor heterotrophic plate counts are essential for the routine monitoring of hygienic quality. They are of value in certain circumstances in giving an indication of the general cleanliness of the distribution system and in assessing the quality of bottled water. However, high heterotrophic plate counts and counts of these bacteria may interfere with the detection of *E. coli,* coliforms, and other bacterial indicators of faecal pollution.

9.4 Methods

9.4.1 Standard methods

Microbiological examination provides the most sensitive, although not the most rapid, indication of the pollution of drinking-water supplies. Because the growth medium and the conditions of incubation, as well as the nature and age of the water sample, can influence the species isolated and the count, the accuracy of microbiological examinations may vary. This means that the standardization of methods and of laboratory procedures is of great importance if uniform criteria for the microbiological quality of water are to be used in different laboratories and countries. International standard methods should be evaluated in local circumstances before being adopted in national surveillance programmes. Information is given here on established standard methods, particularly those of the International Organization for Standardization (ISO), Geneva (Table 9.1), to encourage their use. There are also other well established national standards, such as those of the American Public Health Association (APHA) *(3)* and of the United

Kingdom *(9)*. Established standard methods should be used for routine examinations.

Whatever method is chosen for the detection of *E. coli* and the coliform group, some means of "resuscitating" or recovering environmentally or disinfectant-damaged strains must be used, such as preincubation for a short time at a lower temperature *(3, 9)*.

9.4.2 Methods for pathogenic bacteria, protozoa, and cytopathic enteroviruses

Although the direct search for specific pathogenic bacteria has no place in the routine microbiological examination of water, there are occasions when examination for intestinal pathogens may be necessary as, for example, during an epidemic, when pollution is suspected, or in the evaluation of a new source. The chances of detecting pathogens will be greater if large samples of water are examined, and if media selective for certain intestinal pathogens are used. Examination for bacterial pathogens will include some, if not all, of the following steps: concentration of the organisms in the sample, inoculation into enrichment broth, subculture onto selective agar media, and biochemical and serological examination of suspect colonies. Rather than rely on a single method, it is better to use as many methods as possible so that no opportunity to detect a pathogen is missed *(3, 9)*. This is especially true for the detection of *Salmonella,* since no single method is suitable for all serotypes.

Table 9.1 ISO standards for water quality

Standard no.	Title
5667-1:1980	Sampling - Part 1: Guidance on the design of sampling programmes
5667-2:1982	Sampling - Part 2: Guidance on sampling techniques
5667-3:1985	Sampling - Part 3: Guidance on the preservation and handling of samples
5667-4:1987	Sampling - Part 4: Guidance on sampling from lakes, natural and man-made
5667-5:1991	Sampling - Part 5: Guidance on sampling of drinking-water and water used for food and beverage processing
5667-6:1990	Sampling - Part 6: Guidance on sampling of rivers and streams
6222:1988	Enumeration of viable micro-organisms - colony count by inoculation in or on a nutrient agar culture medium
6461-1:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 1: Method by enrichment in a liquid medium
6461-2:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 2: Method by membrane filtration
7704:1985	Evaluation of membrane filters used for microbiological analyses
7899-1:1984	Detection and enumeration of faecal streptococci - Part 1: Method by enrichment in a liquid medium
7899-2:1984	Detection and enumeration of faecal streptococci - Part 2: Method by membrane filtration
8199:1988	General guide to the enumeration of micro-organisms by culture
8360-1:1988	Detection and enumeration of *Pseudomonas aeruginosa* - Part 1: Method by enrichment in liquid medium
8360-2:1988	Detection and enumeration of *Pseudomonas aeruginosa* - Part 2: Membrane filtration method
9308-1:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* - Part 1: Membrane filtration method
9308-2:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli* - Part 2: Multiple tube (most probable number) method
6579:1990	General guidance on methods for the detection of *Salmonella*

Isolation methods are available for *Salmonella* spp. *(3, 9), Shigella* spp. *(10), Vibrio cholerae (11), Yersinia enterocolitica (12), Campylobacter* spp. *(13-15), Legionella* spp. *(16, 17), P. aeruginosa (3, 9), Aeromonas* spp. *(18), Giardia* spp. *(19-22), Cryptosporidium* spp. *(21-23),* and *Naegleria* spp. *(24).*

Standard methods are now available for concentrating and recovering cytopatic enteroviruses from large volumes of water (i.e. in the range 10-1000 litres) *(3, 25-27).* A method for enumerating male-specific bacteriophages in water has been described *(28),* and some of the factors influencing their recovery have been reviewed *(5).*

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10. Microbiological criteria

10.1 Rationale

10.1.1 Overall strategy

A supply of drinking-water should be sufficient in quantity, wholesome, and not injurious to health. These requirements are all inter-related. The history of water-supply engineering has repeatedly shown that the provision of safe drinking-water is the most important step which can be taken to improve the health of a community by preventing the spread of waterborne disease.

Microbiological monitoring provides a sensitive indication of the extent to which source protection, treatment, and distribution are effective barriers to the transmission of infectious agents of waterborne disease at the time that the samples were taken. It is important to realize at the outset that microbiological integrity is provided by source protection and treatment, and that sudden loss of this integrity or steady deterioration may be missed if monitoring is not frequent enough. Proper design of sampling schemes is important.

The task of monitoring is properly that of the water-supply agency whereas surveillance - keeping public health and the safety and acceptability of water supplies under continuous review - is the duty of the local and national health authorities. Good communications between bodies responsible for monitoring and surveillance are essential. The user has an important role in preserving the quality of the water delivered to the premises through the proper design, construction, and maintenance of storage tanks, taps, and associated plumbing so as to prevent deterioration.

10.1.2 Treatment objectives and microbiological criteria

It is difficult with the epidemiological knowledge currently available to assess the risk to health presented by any particular level of pathogens in water, since this will depend equally on the infectivity and invasiveness of the pathogen and on the innate and acquired immunity of the individuals consuming the water. It is only prudent to assume, therefore, that no water in which pathogenic microorganisms can be detected can be regarded as safe, however low the concentration.

Furthermore, only certain waterborne pathogens can be detected reliably and easily in water, and some cannot be detected at all. This has led over many years to the adoption of the concept of faecal indicator species (see section 9.2) and to universal agreement that the most specific and suitable bacteriological indicator of faecal pollution is *Escherichia coli.* Any water that contains *E. coli* must be regarded as faecally contaminated and unsafe, and requiring immediate remedial action.

Only strict attention to source protection and to the design and operation of efficient treatment and distribution will guarantee the exclusion of pathogens from drinking-water delivered to the consumer. For each water supply, the quality of the source water must guide the selection of the treatment processes, and due attention must be given to the ability of these processes to eliminate different pathogens (see Chapter 11). The microbiological water criteria presented here provide the means for demonstrating that these measures have been satisfactory at the time of sampling. The selection and design of water-treatment processes capable of achieving the necessary reductions in faecal and pathogenic agents will ensure that, if properly operated, these systems will always be able to produce water of the desired quality. This strategy is the only one that can be adopted in the case of pathogens, such as *Giardia, Cryptosporidium,* and viruses, that are more resistant than *E. coli* to terminal disinfection.

10.1.3 Water supplies for small remote communities

The provision of water supplies to small remote communities encounters particular problems worldwide, in that location, available facilities, and financial constraints often mean that only untreated water can be supplied, treatment is limited in extent because only local resources are available, or monitoring is infrequent or impossible. In such circumstances, sanitary assessment of the supply is all-important, and it is recommended that such assessments should be carried out periodically and at least yearly. In addition, the guideline values recommended here should be regarded as a goal for the future, not an immediate requirement. The guideline values recommended for the elimination of hazards may be very difficult to

achieve under some conditions, and must then be applied, together with adequate methods of excreta disposal, in an appropriate manner depending on those conditions and on the availability of resources. Unless other sources of risk are adequately controlled, the effect of providing pure water on the transmission of diarrhoeal diseases may not be achieved.

The particular problems of supplies for small remote communities and their management are the subject of Volume 3 of *Guidelines for drinking-water quality.*

 10.2 Bacteriological quality

Guideline values for bacteriological quality are summarized in Table 10.1, but they should not be used uncritically without reference to the information given in the text. It is most important that the reasons for adopting them are properly understood.

Table 10.1 Bacteriological quality of drinking-water[a]

Organisms	Guideline value
All water intended for drinking	
E. coli or thermotolerant coliform bacteria[b,c]	Must not be detectable in any 100-ml sample
Treated water entering the distribution system	
E coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100-ml sample
Total coliform bacteria	Must not be detectable in any 100-ml sample
Treated water in the distribution system	
E. coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100-ml sample
Total coliform bacteria	Must not be detectable in any 100-ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period.

[a] Immediate investigative action must be taken if either *E. coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.

[b] Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

[c] It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 of *Guidelines for drinking-water quality.*

It is self-evident and unquestionable that water intended for drinking must not contain agents of waterborne disease. However, many pathogens, including bacteria, viruses, and parasites, are difficult or even impossible to detect. For this reason, as already explained in Chapter 9, microbial indicators of water quality, i.e. bacteria indicating either the potential for faecal pollution or that such pollution has occurred, are used, since their presence shows that pathogens could also be present. The most numerous of the faecal indicator bacteria is the coliform group, and the most suitable member of this group is *Escherichia coli,* since it alone is derived exclusively from the faeces of humans and warm-blooded animals. In practice, thermotolerant coliform organisms or *E. coli* should not be detectable in any 100-ml sample of

any water intended for drinking.

A further reason for adopting this criterion is that it is readily achievable by water treatment. Efficient treatment, together with terminal disinfection, should yield water free from coliform organisms, no matter how polluted the original water may have been. Furthermore, in nearly all epidemics of waterborne disease, it can be shown that the bacteriological quality of the water was unsatisfactory and that there was evidence of contamination or a failure of terminal disinfection *(1, 2)*.

In practice, the fact that *E. coli* can be found in wild and domestic animals and birds is not important because they can also carry pathogens infectious for humans.

During the passage of water from the treatment works to the consumer, its bacteriological quality may deteriorate. Members of the coliform group may be present in inadequately treated supplies or those contaminated after leaving the treatment plant, as a result either of growth on unsuitable materials in contact with the water (those used for washers, packing materials, lubricants, plastics and plasticizers, for example) or of entry from soil or natural water through leaky valves and glands, repaired mains, or back-siphonage. This type of post-contamination will most probably be found when the water is untreated or undisinfected, or where there is limited or no residual disinfectant. The occasional occurrence of coliform organisms in water in the distribution system or untreated supplies in up to 5% of samples taken over any 12-month period, in the absence of *E coli,* can be regarded as acceptable. It should be stressed that the regular occurrence of these organisms, as opposed to their occasional and sporadic detection, in such samples must be a cause of concern.

Bottled natural mineral waters constitute a special case; their quality is the subject of Codex Alimentarius standards. The water must be collected and bottled under conditions such that it will retain its original quality. When such water is marketed, the Codex standard specifies that it shall not contain *E. coli,* coliform bacteria, group D streptococci, or *Pseudomonas aeruginosa* in 250-ml samples, provision being made for re-examination if not more than two coliforms (but not *E. coli*) or group D streptococci are found in 250 ml *(3)*.

10.3 Virological quality

10.3.1 Rationale

It is essential that drinking-water supplies should be essentially free of human enteric viruses so that the risk of transmission of waterborne viral disease is negligible. It must be assumed that any drinking-water supply subject to faecal contamination exposes consumers to the risk of viral disease. There are thus two approaches to preventing viral contamination of drinking-water, namely: (i) providing drinking-water from a source that is free of faecal contamination; and (ii) producing drinking-water from a faecally contaminated source by treating it in a manner capable of reducing enteric viruses to a negligible level.

Although methods of concentrating and detecting low levels of viruses in water are available, they are too complex, expensive, and time-consuming for routine monitoring. Furthermore, not all relevant viruses can be detected by the methods currently available. As a result, failure to detect viruses in even very large volumes of water does not prove that the water is virus-free and that consumers are not at risk of viral disease. In fact, a recent epidemiological - virological study indicated that drinking-water produced by conventional treatment from a faecally contaminated surface source might have been responsible for 25% of all gastrointestinal illness of probable viral etiology, even though the created water was of acceptable microbiological quality *(4)*.

Progress has recently been made in modelling, assessing, and predicting the risks of waterborne disease associated with drinking-water containing different concentrations of viruses and protozoan cysts *(5)*. Although this has provided estimates of the health risks linked to the consumption of contaminated drinking-water, the modelling and risk analyses are based on limited dose-response data and require

further refinement and verification; they are therefore not sufficiently developed to provide quantitative criteria for virus concentrations in drinking-water. Even if such health risk assessments for waterborne viruses were possible, the inability to monitor viruses in drinking-water reliably would preclude their practical application.

In the light of the foregoing, the guidelines for viruses in drinking-water presented in Table 10.2 are based on the probable virological quality of the source water and the required degree of treatment for source waters containing different levels of faecal contamination and hence different levels of viruses. The aim of these source-protection and water-treatment guidelines is to ensure that no viruses are present even in very large volumes of drinking-water.

10.3.2 Guidelines for groundwaters

If groundwater is obtained from a protected source and found to be free of faecal contamination, it can be assumed that the water is of acceptable virological quality for drinking if other essential criteria are met. The water source and its delivery system (casing, pump, pipes, and other appurtenances) must be free of faecal contamination from either surface (e.g. waste infiltration) or subsurface (e.g. septic tanks) sources. Specifically, the water must meet the guideline criteria for turbidity and pH (Table 10.2), bacteriological quality (Table 10.1), and para-sitological quality (section 10.4)

Groundwater obtained from a protected source showing evidence of faecal contamination (1-20 *E. coli* per 100 ml) or exposed indirectly to obvious surface or subsurface sources of faecal contamination (e.g. wastewater infiltration of septic tanks) must be adequately disinfected to reduce enteric viruses to negligible levels. Adequate disinfection is defined (see Table 10.2) as the application of chlorine to achieve a free residual of at least 0.5 mg/litre after a minimum contact time of 30 minutes in water having a median turbidity not exceeding 1 NTU and a pH of <8.0, or an equivalent disinfection process in tems of virus inactivation. All such disinfection processes must produce at least 99.99% reduction of enteric viruses.

Groundwater sources and their delivery systems not adequately protected from either surface or subsurface faecal contamination, such as shallow dug wells or other unsealed or uncased wells, may be used as drinking-water sources if their *E. coli* count does not exceed 2000 per 100 ml and the water is treated by filtration and disinfection. Unprotected groundwaters containing more than 2000 *E. coli* per 100 ml are considered grossly faecally contaminated and are not recommended as water-supply sources regardless of treatment, unless no higher-quality water sources are available. In this situation, the water must be treated by at least three unit processes each of which is individually capable of reducing viruses, as prescribed for contaminated surface water (see Table 10.2).

Table 10.2 Recommended treatments for different water sources to produce water with negligible virus risk[a]

Type of source	Recommended treatment
Ground water	
Protected, deep wells; essentially free of faecal contamination	Disinfection[b]
Unprotected, shallow wells; faecally contaminated	Filtration and disinfection
Surface water	
Protected, impounded upland water; essentially free of faecal contamination	Disinfection
Unprotected impounded water or upland river; faecal contamination	Filtration and disinfection
Unprotected lowland rivers; faecal contamination	Pre-disinfection or storage, filtration, disinfection
Unprotected watershed; heavy faecal contamination	Pre-disinfection or storage, filtration, additional treatment and disinfection
Unprotected watershed; gross faecal contamination	Not recommended for drinking-water supply

[a] For all sources, the median value of turbidity before terminal disinfection must not exceed 1 nephelometric turbidity unit (NTU) and must not exceed 5 NTU in single samples.

Terminal disinfection must produce a residual concentration of free chlorine of ≥ 0.5 mg/litre after at least 30 minutes of contact in water at pH<8.0, or must be shown to be an equivalent disinfection process in terms of the degree of enterovirus inactivation (>99.99%).

Filtration must be either slow sand filtration or rapid filtration (sand, dual, or mixed media) preceded by adequate coagulation-flocculation (with sedimentation or flotation). Diatomaceous earth filtration or a filtration process demonstrated to be equivalent for virus reduction can also be used. The degree of virus reduction must be >90%.

Additional treatment may consist of slow sand filtration, ozonation with granular activated carbon absorption, or any other process demonstrated to achieve >99% enterovirus reduction.

[b] Disinfection should be used if monitoring has shown the presence of *E. coli* or thermotolerant coliform bacteria.

10.3.3 Guidelines for surface water sources

In general, surface waters are never completely pure and will always be subject to some degree of faecal contamination, so that treatment to reduce viruses to negligible levels will always be required. The quality of the source water in terms of the degree of faecal contamination (as defined by *E. coli* counts in the raw source water) will determine the degree of treatment required (see Table 10.2).

For source waters derived from protected watersheds essentially free of human faecal contamination, possibly subject only to low levels of faecal contamination from indigenous animals, and containing fewer than 20 *E. coli* per 100 ml, the required degree of treatment is adequate disinfection. However, it is essential that the source water should have a median turbidity not exceeding 1 NTU and a maximum turbidity not exceeding 5 NTU in single samples in order to ensure adequate virus inactivation by disinfection. Surface water sources that meet these virological criteria may nevertheless be contaminated with unacceptable levels of *Giardia* cysts and *Cryptosporidium* oocysts, neither of which can be

adequately controlled by disinfection treatment to control viruses. If there is a risk of protozoal contamination, the source water may have to be treated by means of strictly controlled coagulation and filtration to ensure that these agents are removed. Where necessary, special investigations can be conducted to determine whether source water contamination by protozoan cysts or oocysts is probable or demonstrable.

Surface waters from inadequately protected watersheds contaminated by both human and animal faeces and containing 20-2000 *E. coli* per 100 ml must be treated by both filtration and disinfection in order to reduce enteric viruses to negligible levels. Because such waters are likely to contain protozoal cysts or oocysts as well as enteric viruses, filtration and disinfection must be adequate to control both of these classes of pathogens.

Surface waters from inadequately protected watersheds heavily contaminated by human and animal faeces and having *E. coli* counts of over 2000 per 100 ml but not more than 20 000 per 100 ml, will require extensive treatment consisting of filtration, disinfection, and at least one other process (e.g. long-term storage or an additional filtration or disinfection process) capable of producing additional reduction of viruses of >99%. Such surface waters are clearly inferior as sources of drinking-water and should be used only when no other source of higher quality is available. If such a source is used, the local authorities will have to bear the considerable burden of ensuring that the treatment is properly designed, operated, and maintained and that there is adequate monitoring and surveillance of the water system and its water quality to ensure a continuous supply of acceptable virological quality.

Surface waters from inadequately protected sources containing more than 20 000 *E. coli* per 100 ml are considered to be grossly faecally contaminated and hence unsuitable for drinking-water supply regardless of the extent and type of treatment. Production of drinking-water from such a source carries a great risk of inadequate virological quality and would be undertaken only under the most extraordinary circumstances.

10.4 Parasitological quality

It is not possible to set guideline values for pathogenic protozoa, helminths, and free-living organisms, other than that these agents should not be present in drinking-water, because only one or very few organisms are required for humans to become infected. The analytical methods for protozoan pathogens are expensive and time-consuming and cannot be recommended for routine use. Methods for concentrating the resting stages of *Giardia* and *Cryptosporidium* from large volumes of water are being standardized. When facilities are available for studying the incidence of these parasites in surface water, these methods could be used for measuring the efficiency of different water-treatment processes in removing them and the incidence of carriage of these parasites by animal vectors in the watershed. A better understanding of information on the epidemiology and zoo-notic relationships of these parasites from the point of view of human health will then be possible.

The control of parasitic disease and of invertebrate animal life in water mains is best accomplished by means of appropriate treatment.

10.5 Monitoring

10.5.1 Approaches and strategies

The monitoring of drinking-water quality ideally consists of the following components:

- the control of quality on a routine basis to verify that treatment and distribution comply with the prescribed objectives and regulations;

- the surveillance, usually at specified intervals, of the entire water-supply system from source to consumer from the point of view of microbiological safety.

Continuous control is an integral part of the responsibilities of the water-supply agency, through which the waterworks management ensures the satisfactory performance of the treatment processes, the quality of the water produced and the absence of secondary contamination within the distribution network. In principle, an independent body should verify that the waterworks correctly performs its duties. This surveillance is usually the responsibility of the local, regional and national health authorities.

10.5.2 Sampling frequencies and procedures

The frequency of sampling will be determined by the resources available. The more frequently the water is examined, the more likely it is that chance contamination will be detected. Two main points should be noted. Firstly, the chance of detecting pollution which occurs periodically, rather than randomly, is increased if samples are taken at different times of the day and on different days of the week. Secondly, frequent examination by a simple method is more valuable than frequent examination by a complex test or series of tests. Sampling frequencies for raw water sources will depend on their overall quality, their size, the likelihood of contamination, and the season of the year. They should be established by local control agencies and are often specified in national regulations and guidelines. The results, together with information from the sanitary inspection of the gathering grounds, will often indicate whether increased vigilance is needed.

Sampling frequencies for treated water leaving the waterworks depend on the quality of the water source and the type of treatment. Minimum frequencies are one sample every 2 weeks for waterworks with a groundwater source, and one sample every week for waterworks with a surface water source.

The frequency of sampling should be greater where a large number of people are supplied because of the larger number of people at risk. Advice on the design of sampling programmes and on the frequency of sampling is given in ISO standards (see Table 9.1) and in many national regulations. The minimum number of samples to be taken each month for water in the distribution system is given for different population sizes in Table 10.3.

Table 10.3 Minimum sampling frequencies for drinking-water in the distribution system

Population served	Samples to be taken monthly
Less than 5000	1 sample
5000-100 000	1 sample per 5 000 population
More than 100 000	1 sample per 10 000 population, plus 10 additional samples

Samples should be taken at random intervals in each month from fixed points, such as pumping stations and tanks, from random locations throughout the distribution system and from taps connected directly to the mains in houses and large multioccupancy buildings, where there is a greater risk of contamination through cross-connections and back-siphonage. The frequency of sampling should be increased at times of epidemics, flooding, and emergency operations, or following interruptions of supply or repair work. With systems serving small communities, periodic sanitary surveys are likely to yield more information than infrequent sampling.

No general recommendation can be made for unpiped supplies and untreated water because the quality and likelihood of contamination will vary seasonally and with local conditions. The frequency should be established by the local control agency and reflect local conditions, including the results of sanitary surveys.

Detailed advice on the procedures to be used for sampling different sources of water or treatment plants and distribution systems and at the tap are given in Volume 3 of *Guidelines for drinking-water quality* and in standard methods *(6, 7)* (see Table 9.1). However, the following general points should be noted.

Care must be taken to ensure that samples are representative of the water to be examined and that no accidental contamination occurs during sampling. Sample collectors should therefore be trained and made aware of the responsible nature of their work. Samples should be clearly labelled with the site, date, time and other relevant information, and sent to the laboratory for analysis without delay.

If the water to be examined is likely to contain chlorine, chloramine, chlorine dioxide, or ozone, sodium thiosulfate should be added to neutralize any residual disinfectant. Properly controlled, the concentration of thiosulfate has no significant effect on the coliform organisms, including *E. coli,* either in chlorinated or in unchlorinated water samples during storage *(6)*. If heavy metals, particularly copper, are present, chelating agents (e.g. edetic acid (EDTA) or nitrilotriacetic acid (NTA)) should also be added.

When samples of disinfected water are taken, the concentration of residual disinfectant at the sampling point and the pH should be determined at the time of collection.

When a number of samples are to be taken for various purposes from the same location, the sample for bacteriological examination should be collected first to avoid the risk of contamination of the sampling point.

Samples must be taken from different parts of the distribution system to ensure that all parts of it are tested. When streams, lakes, or cisterns are being sampled, the water must be taken from below the surface, away from banks, sides of tanks, and stagnant zones, and without stirring up sediments. Taps, sampling ports, and the orifices of pumps should, if possible, be disinfected and a quantity of water run to waste to flush out the stagnant water in the pipe before the sample is taken. Sampling ports in treatment processes and on water mains must be carefully sited to ensure that samples are representative. The length of pipework to the tap should be as short as possible.

The changes that may occur in the bacterial content of water on storage can be reduced to a minimum by ensuring that samples are not exposed to light and are kept cool, preferably between 4 and 10 °C, but not frozen. Examination should begin as soon as possible after sampling and certainly within 24 hours. If samples cannot be cooled, they must be examined within 2 hours of sampling. If neither condition can be met, the sample should not be analysed.

10.5.3 Surveillance programme requirements

Surveillance is the continuous and vigilant public health assessment and review of the safety and acceptability of drinking-water supplies. Each component of the drinking-water system - the source, treatment, storage, and distribution - must function without risk of failure. A failure in one part will jeopardize and nullify the effects of other parts that function perfectly, as well as the care that has been taken to ensure that they do so. Water is liable to contamination at all stages in the process of supply, hence the need for constant vigilance. At the same time, careful and intelligent assessment of likely sources of risk and breakdown is necessary before a supply system is planned and installed and, indeed, continously thereafter, because of possible changes in conditions and potential sources of contamination. Contingency plans must be made to deal with any emergencies that may arise through natural or man-made disasters, such as accidents, wars, and civil commotions, or the cessation of supplies of essential chemicals used in treatment.

An essential part of surveillance is the establishment of a proper network for regulation and command. At government level, this means the establishment and enforcement of national standards, and the promulgation of national guidelines for achieving compliance with the laws and standards; for the water-supply agency, it means promotion of local codes of good waterworks practice together with formal instruction and training. A national inspectorate should be established to ensure that the legal requirements are met and standards complied with. This body should be separate from that representing the interests of the water provider.

Both the water provider and the inspectorate should have properly equipped laboratory facilities staffed by trained and properly qualified personnel, adequate facilities for sustaining at all times the level of monitoring required, and sufficient capacity to carry out additional examinations as required to meet special needs. Operational staff at the waterworks, should also be appropriately trained and qualified.

Lines of communication and command must be established at the outset and must be properly understood by all staff up to the highest levels. This will ensure the effective functioning of day-to-day operations and also that immediate remedial action is taken in emergencies and when contamination is discovered. Bacteriological failures must be acted on as soon as discovered, so that the micro-biologist must have the authority to instruct the engineer and the operational staff to take the necessary action. The lines of communication needed in an emergency will be complex, involving not only different public bodies but also authorities responsible for different geographical regions. Appropriate instructions must be laid down and understood at each site.

The scope of surveillance, together with examples covering the points made in this section, has been considered in a separate WHO publication, which should be consulted *(8)*. The importance of surveillance is highlighted repeatedly in official reports of serious outbreaks of waterborne disease, which usually reveal deficiencies in more than one area *(1, 2, 9)*. Surveillance procedures are described further in Volume 3 of *Guidelines for drinking-water quality.*

The levels of surveillance of drinking-water quality differ widely in developing countries in line with the differences in economic development and the provision of community water supplies in those countries. Surveillance should be progressively developed and expanded by adapting the level to the local situation and economic resources, with gradual implementation, consolidation, and development to the level ultimately desired.

10.6 Action to be taken when contamination is detected

No surface water can be assumed to be free from enteric pathogens, including viruses and parasites, since they can be derived from wild or farm animals living in the catchment area as well as from human faecal contamination. The geographical and seasonal distribution of specific pathogens in natural waters can provide valuable information on the epidemiology of disease in animal populations and the routes of transmission to the human population. Such information also indicates the precautions needed to safeguard the sources of water and the degree of treatment needed.

The occurrence of any pathogenic agent, bacterial, viral, or animal, in a drinking-water supply is always a matter for the gravest concern, demanding immediate attention to treatment and to determining the cause. The examination of drinking-water for a particular pathogen will most probably be required when the water is suspected to be the cause of an outbreak of disease in the community supplied with the water. The finding of the causal agent of the disease in the water, together with the distribution of primary cases among those using it, prove that the supply is implicated, particularly if the disease is not found among those not using the water and not acquiring infection secondarily.

10.6.1 Bacterial indicators of faecal contamination

The finding of *Escherichia coli* in any sample of water intended for drinking is a matter for concern, since it indicates that the water has been recently contaminated by faecal material from humans or animals, and that there is a likelihood that pathogens will also be present. If the water is a treated piped supply, there is also the strongest reason for suspecting that there has been a breakdown in disinfection, treatment before disinfection has failed, or contaminated water has entered the system. Immediate action must be taken to discover the source of contamination and to increase the dosage of disinfectant so as to ensure that an adequate residual is present in the water delivered to the consumer, until the problem is overcome. Consideration should be given to telling consumers to boil water intended for drinking. If the quality of

water leaving the works is satisfactory, ingress of contaminated water will most usually arise as a result either of damage to the distribution system or repairs to it, or through infiltration into underground service reservoirs or directly into the mains as a result of low pressure and back-siphonage or cross-connections. Where treatment is minimal, because source water is normally of high quality, sudden deterioration resulting from storms, flooding, or massive pollution incidents will mean that the disinfection applied is inadequate. Prior assessment of hazards by sanitary survey or by the establishment of "early-warning" monitoring systems in the catchment area will help avoid such events.

A finding of thermotolerant or total coliform bacteria in the presumptive test demands instant attention. The positively reacting tubes or colonies must be examined further using confirmatory tests, and for the presence of *E. coli.* The water must immediately be resampled from the same source. The waterworks engineer must be informed at once, so that investigations can be made to discover the source of the contamination. Such action must be regarded as the minimum. Where the failure concerns water leaving the treatment works, investigations and corrective action as outlined in the previous paragraph are necessary immediately and before the results of the confirmatory test are known.

The finding of total coliform organisms, in the absence of thermotolerant coliforms or *E. coli,* in a treated piped supply usually indicates post-treatment contamination, or growth on pipes or fittings, when the treated water entering the supply system is satisfactory. It suggests either that materials coming into contact with water, such as those used for pipes, washers, pipe sealants, and packings, or rubber and plastics used for other purposes, are supporting growth or that untreated water is entering the distribution network. Because total coliforms of nonfaecal origin can exist in natural waters, their presence can occasionally be tolerated in unpiped or untreated water, if thermotolerant coliform bacteria and *E. coli* are absent. If they are present repeatedly or in consecutive samples, as indicated in Table 10.1, action must be taken to improve the sanitary protection of the source.

In temperate or cold climates, the finding of total or thermotolerant coliform bacteria in the presumptive test leads in a high proportion of cases to confirmation of the presence of *E. coli* and therefore of evidence of faecal contamination. This may be less common in tropical and semitropical regions, particularly where the water is untreated. Nevertheless, the indication must not be ignored for the reasons given in section 9.2. If desired, the faecal origin of such coliform organisms can be confirmed using the faecal streptococcus and sulfite-reducing clostridia tests.

10.6.2 Miscellaneous indicators

Occasionally, and particularly where the source water is derived from lowland rivers and where the water temperatures in the distribution system are 20 °C or higher, *Aeromonas* spp. can occur and will interfere with the interpretation of the total coliform tests. At these temperatures and where the free chlorine residual is below 0.2 mg/litre, these bacteria are able to grow on assimilable organic carbon in the water. Similar significance attaches to the finding of *Pseudomonas aeruginosa* in supply systems. Both these organisms can occur in the absence of coliform bacteria and will interfere with the interpretation of the coliform tests. Their sanitary significance is unclear, although they can be opportunistically pathogenic and are undesirable where water is used in the manufacture of food and drink, or is supplied to hospitals. Measures to eradicate them must be taken, and may include eliminating unsatisfactory materials in contact with the water, cleaning distribution systems and plumbing in the buildings affected, and maintaining adequate residual disinfectant in the supply.

Bacteria recovered in colony counts at 22 °C are without sanitary significance. However, the occurrence of such bacteria in numbers exceeding 100 per 100 ml in piped water may indicate enrichment of the water with assimilable organic carbon. Large numbers are undesirable in water used for preparing food and drink or for bottling. Any increase in the numbers of colonies above normal levels when counts are made at 37 °C should be regarded with suspicion, since it may be caused by the onset of polluting conditions, particularly if not accompanied by an increase in the count at 22 °C. An increase in the count at 37 °C is often a valuable indication of undesirable changes and should prompt an investigation of the supply or of

the gathering grounds, if the water is untreated.

The presence of macroscopic animal life in drinking-water is aesthetically objectionable if nothing else. With piped supplies, it is an indication that flushing and cleaning of the distribution system are needed (see section 8.2). Their occurrence may sometimes reflect unusually high water temperatures as, for example, when chironomid larvae are discharged from slow sand filters into the treated water.

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11. Protection and improvement of water quality

The emphasis in this section is on protecting and improving the microbiological quality of drinking-water. It is a basic principle, long established as a result of the lessons learned from serious outbreaks of waterborne disease, that a single barrier to the spread of pathogenic organisms is not sufficient to ensure the purity of drinking-water *(1, 2).* Purity is not the only requirement, however; the drinking-water supply must also be capable of meeting the anticipated demand. Inadequate supply, together with geographical factors, often means that raw water of poor microbiological quality and possibly containing significant amounts of wastewater has to be used. A second principle is that to ensure that the drinking-water delivered to the consumer is free from pathogens, the level of treatment should be related to the degree of pollution expected in the source water. In the case of contaminated water sources, several treatment processes, designed primarily for such water will be necessary. Together, these processes will progressively remove pathogens and other contaminants from raw water and consistently produce a safe and wholesome supply of drinking-water. Ideally, safety should be achieved before the final treatment step, so that the failure of any one process will not result in waterborne disease, i.e. the system is fail-safe.

The protection of the source from pollution and the provision of adequate and properly operated treatment processes constitute the essential barriers to the transmission of disease on which the supply of wholesome water depends *(1, 2).*

11.1 Water sources

11.1.1 Selection of sources

Before a new source of drinking-water supply is selected, it is important to ensure that: (1) the quality of the water is satisfactory or can be improved by treatment to make it suitable for drinking; (2) the source will yield enough water to meet the needs of the community not only under the normal conditions of the average annual cycle but also under conditions which are unusual but can be expected, say, once in 10 years; (3) under normal abstraction conditions, the change in local water flow patterns will not cause any unacceptable deterioration in the quality of the water abstracted; and (4) the water to be abstracted can be protected against pollution.

A full sanitary and microbiological survey of the catchment area should be carried out to locate all the sources of pollution. It may be that the treatment or diversion of a small polluting discharge could make a large potential source acceptable *(3).* If remote upland sources of surface waters are being considered, it is important to assess whether access by people or livestock can be prevented and whether wild animals are likely to be vectors of salmonellae, *Giardia,* and *Cryptosporidium.*

11.1.2 Source protection

In the past, isolation of the watershed from human activity was an important means of protecting a waterway or aquifer from contamination. The rising cost of land and increases in population have made this procedure more costly and difficult, especially when new sources must be found in an area that is already developed. It is still desirable for water suppliers to own or control the land to the extent that this is feasible *(1).*

Another line of defence is to prevent polluting activities in the area which may be a source of infection. This means, for instance, defining areas where sewage sludge may not be applied, and exercising strict control over discharges of sewage effluents and agricultural wastes, the location of sites for the dumping of garbage and toxic wastes, and drilling, mining, and quarrying. Control of such activities does not necessarily mean that they should be banned, but that, in the interests of public health, they should be licensed and open to inspection and monitoring whenever water quality could be affected. Where potentially harmful substances are handled or made, steps should be taken to ensure that any effluents are either adequately treated or conveyed safely over the catchment area *(3).*

Sources of groundwater such as springs and wells should be sited and constructed so as to be protected from surface drainage and flooding. Zones of groundwater abstraction should be fenced off to prevent public access, kept free from rubbish, and sloped to prevent the formation of pools in wet weather. Animal husbandry should be adequately controlled in such zones *(1, 3).*

Protection of open surface water is difficult. It may be possible to protect a reservoir from major human activity, but the protection of a river, if possible at all, may be feasible only over a limited stretch. Often it is necessary to accept existing and historical uses of a river or lake and to design the treatment accordingly. Adequate sewage treatment is important in preserving water quality at downstream intakes *(3).*

11.2 Treatment processes

The fundamental purpose of water treatment is to protect the consumer from pathogens and from impurities in the water that may be injurious to human health or offensive. Where appropriate, treatment should also remove impurities which, although not harmful to human health, may make the water

unappealing to drink, damage pipes, plant, or other items with which the water may come into contact, or render operation more difficult or costly.

These purposes are achieved, as previously mentioned, by introducing successive barriers, such as coagulation, sedimentation (or flotation), and filtration, to remove pathogens and impurities. The final barrier is disinfection. The function of the entire system, and indeed of much of water treatment, may with some justification be regarded as that of conditioning the water for effective and reliable disinfection *(3).*

11.2.1 Storage

The storage of water in reservoirs creates favourable conditions for the self-purification of the stored water, but may also cause undesirable changes in water quality. The benefits of storage include the provision of a continuous supply of water, reduction in turbidity, reduction in pathogens through the action of sunlight and sedimentation, dilution of undesirable substances that may accidentally enter the intake, and oxidation of impurities. It also provides a buffer should pollution occur in the river. Undesirable conditions created by storage include those associated with the production of algae, pollution by birds and animals, evaporation, and the leaching of iron and manganese from soils and rocks *(2, 3).*

Reservoirs should either be constructed in series or designed to prevent short-circuiting, since this will enhance removal of pathogens and self-purification. The benefits of reservoir storage are greatest in the summer and when residence periods are about 3 - 4 weeks.

11.2.2 Presedimentation

Highly turbid surface water may require presedimentation before further treatment. Presedimentation basins are constructed in excavated ground or of steel or concrete. Such basins may be preceded by equipment for the addition of chemicals to provide partial coagulation during periods when the water is too turbid to clarify by sedimentation alone.

11.2.3 Prechlorination

Prechlorination to breakpoint has been widely used as an alternative to storage for water derived from lowland rivers and is also used when stored water contains much planktonic life. Its purpose is to reduce counts of faecal bacteria and pathogens, destroy animal life and algae, and oxidize ammoniacal nitrogen, iron, and manganese, thereby assisting in their removal. The combined and free chlorine which remains effectively discourages microbial activities, such as protozoal predation and nitrification, as well as microbial growth during subsequent filtration. When used to disinfect raw water, the oxidative effect of chlorine and even more of ozone will result in the partial conversion of total organic carbon into biodegradable organic carbon which, if not removed by biological activity during treatment (e.g. during slow sand or granular activated carbon filtration), can result in the growth of nuisance organisms during distribution. Prechlorination of organically enriched surface waters has been shown to produce a substantial increase in the content of trihalomethanes and is often a wasteful use of chlorine. It is important to balance the maintenance of the microbiological safety of drinking-water against possible hazards associated with the formation of such disinfection by-products.

11.2.4 Coagulation and flocculation

To remove particulate matter, a water-treatment plant will generally include equipment for coagulation and flocculation, followed by sedimentation and filtration.

Coagulation involves the addition of chemicals (e.g. aluminium sulfate, ferric sulfate) to neutralize the charges on particles and facilitate their agglomeration during the slow mixing provided in the flocculation step *(1)*. Floes thus formed co-precipitate, adsorb and entrap natural colour and mineral particles, and can bring about major reductions in counts of protozoa, faecal bacteria, pathogens, and viruses.

Coagulation and flocculation require a high level of operational skill. Chemical dosages and pH control must be correct, and the plant must be designed to ensure proper floe formation. Before it is decided to use coagulation as part of a treatment process, careful consideration must be given to the likelihood of process stability, the availability of regular supplies of chemicals, and the need for qualified personnel *(3)*.

11.2.5 Sedimentation or flotation

The purpose of sedimentation is to permit settleable floe to be deposited and thus reduce the concentration of suspended solids that must be removed by filters *(2)*. Flotation is an alternative to sedimentation, and has advantages when the amount of floe is small.

The factors that influence sedimentation include the size, shape, and weight of the floe, viscosity and hence the temperature of the water, the detention time, the number, depth, and areas of the basins, the surface overflow rate, the velocity of flow, and the inlet and outlet design *(1, 2)*.

Arrangements must be made for the collection and safe disposal of sludge from sedimentation tanks.

11.2.6 Rapid filtration

Typically, rapid sand filters consist of 0.4-1.2 m of sand, usually of an effective size of 0.5-1.0 mm, supported by gravel and underdrains. In recent years, single-medium filters have often been replaced by dual-medium or multimedia ones. During filtration, residual particles of floe not removed by sedimentation are trapped in the interstices of the bed, and may induce further flocculation of particles. A limited amount of biological activity may also occur, if it is not suppressed by prechlorination or by high flow rates. Both sand and mixed-media filters are normally cleaned by reversal of the flow though the bed (backwashing). Backwash water is either discharged to the sewer or drying beds or recycled after removal of sludge.

The performance of rapid filters in removing microorganisms and turbidity varies over the duration of the run between backwashings. Immediately after backwashing, performance is poor, until the bed has compacted. In some plants, water is filtered and diverted for recycling for 15-30 min at the start of each filter run. In some waterworks, a 30-min slow start for each filter run is included to prevent the initial breakthrough. Performance will also deteriorate progressively when backwashing is needed, since floe may escape through the bed into the treated water, thereby increasing its turbidity. In view of the foregoing, proper supervision and control of filtration at the waterworks are essential.

11.2.7 Slow sand filtration

Typically, slow sand filters consist of 0.5-1.5 m of silica sand with an effective size of 0.3-0.6 mm. The upper layer of fine sand is supported on gravel and a system of underdrains.

Slow sand filtration is simpler to operate than rapid filtration, as frequent backwashing is not required *(4, 5)*. It is therefore particularly suitable for developing countries and small rural systems, but is applicable only if sufficient land is available. On the other hand, the filters are readily clogged by algal blooms and do not remove heavy metals and many micropollutants efficiently. They effectively remove biodegradable organic carbon and oxidize ammonia.

When the filter is first brought into use, a microbial slime community *(schmutzdecke)* develops at the surface of the bed. This consists of bacteria, free-living ciliated protozoa and amoebae, Crustacea, and invertebrate larvae acting in food chains, resulting in the oxidation of organic substances in the water and of ammoniacal nitrogen to nitrate. Pathogenic bacteria, viruses, and resting stages of parasites are removed, principally by adsorption on to the *schmutzdecke* and by subsequent predation. When correctly loaded and operated, slow sand filtration is capable of bringing about a great improvement in water quality *(6)*. Slow sand filters, operated at a filtration rate of 1.1-4.2 m/day, were able to remove 97-99% of

enteroviruses at water temperatures of 6-11 °C. This was somewhat greater than for *E. coli,* and removal was greatest when the water was warmest *(7)*. A slow sand filter is more efficient than any other process in removing parasites (helminths and protozoa). Nevertheless, the effluent from such a filter might well contain a few *E. coli* and viruses, especially during the early phase of a filter run. It is usual to divert or recycle the filtered water produced immediately after commissioning or cleaning a filter bed until the *schmutzdecke* has been established and become effective.

11.2.8 Infiltration

Surface water can also be treated by infiltration of the raw or partly treated water into river banks or sand dunes, followed by underground passage; this is an effective means of removing undesirable microorganisms and viruses. Infiltration is applicable only in areas where suitable geological conditions exist. Pretreatment is required to prevent clogging of the infiltration area. In addition, water abstracted from the aquifer usually needs some additional treatment, such as aeration and filtration, to remove, e.g. iron and manganese present in anaerobic groundwater. The residence time underground should be as long as possible to obtain a water comparable in quality to groundwater.

11.2.9 Disinfection

The overall objective of disinfection is to ensure that the quality criteria specified in Table 10.1 (see p. 95) are always met.

Terminal disinfection of piped drinking-water supplies is of paramount importance and is almost universal, since it is the final barrier to the transmission of waterborne bacterial and viral diseases. Although chlorine and hypochlorite are most often used, water may also be disinfected with chloramines, chlorine dioxide, ozone, and ultraviolet irradiation *(3, 8)*.

The efficacy of any disinfection process will depend on the degree of purity achieved by prior treatment, as disinfectants are highly active and will be neutralized to a greater or lesser extent by organic matter and readily oxidizable compounds in water. Microorganisms that are aggregated or adsorbed on to particulate matter will also be partly protected from disinfection. It is therefore recommended that the median turbidity of water before disinfection should not exceed 1 NTU; it should not exceed 5 NTU in any individual sample.

Practical experience has shown that the kinetics of the disinfection of drinking-water follow the first-order model of Chick's law, in which the fraction of the original population surviving, x_t/x_o, after treatment for a time *t* is given by

$$x_t/x_o = e^{-kt},$$

where *k* is the specific death rate. This law is based on the assumption that all the agents being removed are equally sensitive to the disinfectant and that they are randomly distributed and not clumped together.

The specific death rate with disinfection, *k*, or the contact time, *t*, required to kill a given percentage of the original population is usually proportional to the concentration, *C*, of disinfectant, as in Watson's empirical dilution law:

$$C^n t = k$$

where *k* is a constant of proportionality and *n* is the dilution exponent. For water disinfection, the value of *n* is close to 1, and it is therefore convenient to express the product of the concentration and the time required to bring about 99% removal of a given agent as a *C.t* value. This must be done with caution, because it is assumed that Chick's law is followed and that the conditions of disinfection (temperature, pH, chemical composition of the water, its disinfectant demand, and the physiological state of the agents being

disinfected) are constant *(9).* Table 11.1 lists *C.t* values for different agents and disinfectants, and shows that, of the microorganisms listed, *E. coli* is generally the most sensitive, that the three viruses differ in sensitivity not only among themselves but also to the different disinfectants, and that the parasites *Giardia* and *Cryptosporidium* are the most resistant *(10-12).* Table 11.1 also indicates that normal chlorination conditions (i.e. free chlorine residual of 0.5 mg/litre, a contact time of 30 min, pH less than 8.0, and water turbidity less than 1 NTU) can be expected to bring about reductions greatly in excess of 99% for *E. coli* and the viruses specified but not for the parasitic protozoa.

Table 11.1 C.t values (mg.min/l) for 99% inactivation of various agents by disinfectants at 5 °C[a]

Agent	Disinfectant			
	Free chlorine pH 6-7	Preformed chloramine pH 8-9	Chlorine dioxide pH 6-7	Ozone pH 6-7
C. coli	0.034 - 0.05	95-180	0.4 - 0.75	0.02
Poliovirus type 1	1.1 - 2.5	768-3740	0.2 - 6.7	0.1 - 0.2
Hepatitis A virus	1.8	ca 590	1.7	-
Rotavirus	0.01 - 0.05	3810-6480	0.2 - 2.1	0.006 - 0.06
Giardia lamblia cysts	47 - >150	-	-	0.5-0.6
Giardia muris cysts	30 - 630	-	7.2 - 18.5	1.8 - 2.0
Cryptosporidium parvum oocysts	-	-	6.5 - 8.9	< 3.3 - 6.4
Cryptosporidium oocysts from human faeces	7.7×10^6 - 8.7×10^6	-	-	-

[a] Calculated from data in references 10-12.

It is thus clear that great care is needed to ensure that the treatment processes preceding terminal disinfection are operated correctly to ensure the effective removal of pathogens. There are many instances of disinfection failure when turbidity was 5 NTU or more. It is therefore axiomatic that, for successful disinfection, turbidity should always be less than 5 NTU and preferably less than 1 NTU.

As with chemical disinfection, ultraviolet disinfection is more effective against vegetative bacteria than against viruses and bacterial spores, while protozoal cysts are the most resistant. Data show that the minimum dosage recommended, 16 m W.s/cm^2, is sufficient to inactivate more than 99.9% of vegetative bacteria but not the other agents *(10).* Disinfection by ultraviolet radiation is applicable only to clear waters, since appreciable turbidity or dissolved organic carbon will attenuate the radiation. Although there is no residual effect of disinfection by ultraviolet radiation, this is not a drawback when the water has been treated to a high standard to remove biodegradable organic carbon and where the water distribution system is well maintained.

11.3 Choice of treatment

11.3.1 Microbiological conditions

In rural areas supplying small communities, protection of the source of water may be the only "treatment" possible. Such supplies are considered in detail in Volume 3 of *Guidelines fir drinking-water quality.* In large communities, the demand for water is high and can often be met only by using sources of poor microbiological quality.

Two considerations are of paramount importance: firstly, the quality of drinking-water is totally dependent on protection of the source, treatment of the water, and maintenance of the integrity of the distribution system; and secondly, microbiological monitoring can influence water quality only if its findings, and those of the agency responsible for surveillance, are made known to the water engineer and any remedial

measures necessary are implemented.

11.3.2 Treatment of groundwater

Groundwater extracted from well protected aquifers is usually free from pathogenic microorganisms, and the distribution of such groundwater without treatment is common practice in many countries. However, the catchment area must be protected by effective regulatory measures and the distribution system adequately protected against secondary contamination of the drinking-water. If the water, in its passage from source to consumer, cannot be protected at all times, disinfection and the maintenance of adequate chlorine residuals are imperative.

11.3.3 Treatment of surface water

The extent to which faecal bacteria, viruses, and parasites are removed by properly designed and operated equipment for flocculation, coagulation, sedimentation, and rapid filtration is equivalent to that achieved by slow sand filtration.

Additional treatment, such as ozonation, will have a considerable disinfecting action besides converting part of the total organic carbon into a biodegradable form. If it is followed by activated carbon treatment or other biological filtration stage, some of the biodegradable organic carbon will be removed by microbial activity, thus reducing the potential for aftergrowth of nuisance bacteria in distribution networks.

Disinfection should be regarded as obligatory for all piped supplies of surface water, even if derived from high-quality, unpolluted sources, since there should always be more than one barrier against the transmission of infection by a water supply. In large, properly run waterworks, the criteria for the absence of *E. coli* and coliform bacteria can then be met with a very high degree of probability.

Table 11.2 shows that conventional urban water treatment relies on pretreatment and terminal disinfection to remove much of the microbial contamination. Nevertheless, conventional treatment can be effectively operated as a three-stage multiple-barrier system involving: (1) coagulation and settling or flotation; (2) rapid filtration; and (3) terminal disinfection.

Table 11.2 An example to illustrate the level of performance that can be achieved in removal of turbidity and thermotolerant coliform bacteria in conventional urban water treatment

Stage and process	Turbidity			Thermotolerant coliform bacteria		
	Removal[a] (%)	Average loading (NTU)[b]	Maximum loading (NTU)[b]	Removal[a] (h) %	Average loading (per 100 ml)	Maximum loading (per 100 ml)
Micro-straining	NA[c]	NA	NA	NA	NA	NA
Pretreatment[d]	NA	NA	NA	>99.9	1000	10 000
Coagulation/settling[e]	90	50	300	NA	NA	NA
Rapid filtration[e]	>80	5	30	80	1	10
Terminal chlorination	NA	1	5	>99.9	<1	2
Mains distribution	NA	<1	<5	NA	<1	<1

[a] Required performance.
[b] NTU, nephelometric turbidity units.
[c] NA, not applicable, Process not designed to remove turbidity and/or bacteria. Micro-straining removes

micro-algae and zooplankton

[d] Pretreatments that can result in significant reductions in thermotolerant coliform bacteria are storage in reservoirs for 3-4 weeks, and pre-disinfection.

[e] Taken together, coagulation, settling, and rapid filtration should be expected to remove 99.9% of thermotolerant coliform bacteria.

Another approach to the application of the multiple-barrier principle has been applied in urban areas for supplies derived from rivers. It involves: (1) raw water storage (or plain sedimentation); (2) rapid sand filtration; (3) slow sand filtration; and (4) terminal disinfection. Steps 1-3 remove turbidity, while 1, 3 and 4 remove microbes. Infiltration, which is highly effective in removing bacteria, viruses, and organic carbon, has been used, notably in the Netherlands, as an additional process following storage and rapid filtration.

11.3.4 Small-scale treatment of surface water

The multiple-barrier concept, as applied in the treatment of surface water for urban supplies, can be adapted for use in rural and remote regions. A typical series of processes would include: (1) storage, sedimentation, or screening; (2) triple-stage gravel prefiltration; (3) slow sand filtration; and (4) disinfection. Table 11.3 lists typical performance objectives for the removal of turbidity and thermotolerant coliform bacteria in such plants.

A detailed account of water treatment and supply for small remote communities is given in Volume 3 of *Guidelines for drinking-water quality.*

Table 11.3 An example of performance objectives for removal of turbidity and thermotolerant coliform bacteria in small-scale water treatment

Stage and process	Turbidity			Thermotolerant coliform bacteria		
	Removal[a] (%)	Average loading (NTU)[b]	Maximum loading (NTU)[b]	Removal[a] (h)%	Average loading (per 100 ml)	Maximum loading (per 100 ml)
Screening	NA[c]	NA	NA	NA	NA	NA
Plain sedimentation	50	60	600	50	1000	10 000
Gravel pre-filters (3-stage)	80	30	300	90	500	5000
Slow sand filter	>90	6	60	95	50	500
Disinfection	NA	<1	<5	>99.9	<3	25
Distributed water	NA	<1	<5	NA	<1	<1

[a] Required performance.

[b] NTU, nephelometric turbidity units.

[c] NA, not applicable. Process not designed to remove turbidity and/or bacteria.

11.4 Distribution networks

A distribution network transports water from the place of treatment to the consumer. Its design and size will be governed both by the topography and the location and size of the community. The aim is always to ensure that the consumer receives a sufficient and uninterrupted supply and that contamination is not introduced in transit. The shape of the network will be influenced by the location of consumers.

Distribution systems are especially vulnerable to contamination when the pressure falls, particularly in the intermittent supplies of many cities in developing countries. Suction is often created by direct pumping

from the mains to private storage tanks, a practice which should be prohibited.

The bacteriological quality of water can deteriorate during distribution, and there are a number of places where contamination can be introduced. If the water contains significant assimilable organic carbon and adequate residual levels of disinfectant are not maintained, or if water mains are not flushed and cleaned frequently enough, growth of nuisance bacteria and other organisms can occur. Where the water contains appreciable assimilable organic carbon and where the water temperature exceeds 20 °C, a chlorine residual of 0.25 mg/litre may be required to prevent the growth of *Aeromonas* and other nuisance bacteria. Attached microorganisms may grow even in the presence of residual chlorine.

Underground storage tanks and service reservoirs must be inspected for deterioration and for infiltration of surface water and ground-water. It is desirable for the land enclosing underground storage tanks to be fenced off, both to prevent access by people and animals and to prevent damage to the structures.

Repair work on mains provides another opportunity for contamination to occur. Local loss of pressure may result in back-siphonage of contaminated water unless check valves are introduced into the consumers' water system at sensitive points, such as supplies to ornamental pools, garden irrigation, urinals, and water closets. When repairs to mains have been completed or when new mains are installed, it is essential that the pipes are cleaned, disinfected, and then emptied and refilled with mains water. The water should then be tested bacteriologically after 24 hours, and new mains should not be brought into service until the water quality is bacteriologically satisfactory. If the main has been damaged and water from a fractured sewer or drain may have entered, the situation is most serious and, in addition to chlorination of the water in the repaired main, the level of chlorination should be increased and the main not returned to service until the water quality is satisfactory. These and other actions to be taken should be specified both in national codes of practice and in local instructions to waterworks staff.

As already mentioned, microbial contamination can occur as a result of the use of unsuitable materials for items coming into contact with water; such materials include those used for washers, jointing and packing materials, pipe and tank lining compounds, and plastics used in pipes, tanks, and faucets, all of which can deteriorate to form substances that support the growth of microorganisms. Such materials should be subject to approval by the authority responsible for the water-supply system.

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