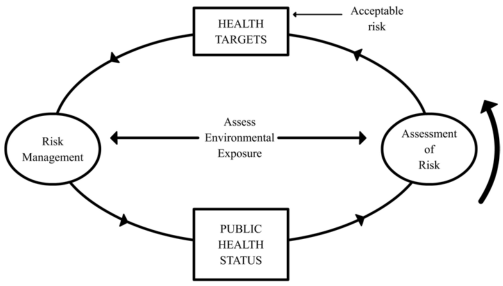

7

Epidemiology: a tool for the assessment of risk

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The purpose of this chapter is to introduce and demonstrate the use of a key tool for the assessment of risk. The word epidemiology is derived from Greek and its literal interpretation is 'studies upon people'. A more usual definition, however, is the scientific study of disease patterns among populations in time and space. This chapter introduces some of the techniques used in epidemiological studies and illustrates their uses in the evaluation or setting of microbiological guidelines for recreational water, wastewater reuse and drinking water.

7.1 INTRODUCTION

Modern epidemiological techniques developed largely as a result of outbreak investigations of infectious disease during the nineteenth century.

Environmental epidemiology, however, has a long history dating back to Roman and Greek times when early physicians perceived links between certain environmental features and ill health.

John Snow's study of cholera in London and its relationship to water supply (Snow 1855) is widely considered to be the first epidemiological study (Baker *et al*. 1999). Mapping cases of cholera, Snow was able to establish that cases of illness were clustered in the streets close to the Broad Street pump, with comparatively few cases occurring in the vicinity of other local pumps.

Epidemiological investigations can provide strong evidence linking exposure to the incidence of infection or disease in a population. They can provide estimates of the magnitude of risk related to a particular level of exposure or *dose* and so can be used in the evaluation of appropriate microbiological quality guideline levels or standards. Epidemiological methods can quantify the probability that observed relationships occurred by *chance* factors and they also have the potential to control for other risk factors and/or confounders of the outcome illness being studied. Epidemiological studies used for the evaluation or setting of guidelines must be of high quality, so that there is confidence in the validity of the results.

The following sections outline the basic elements of epidemiological studies (including comments on features that are important for high quality studies), the different types of epidemiological study, and the use of epidemiology in guideline setting, with case studies of the use of epidemiology in recreational water, drinking water and wastewater reuse settings.

7.2 BASIC ELEMENTS OF EPIDEMIOLOGICAL STUDIES

The basic elements of an epidemiological study can be characterised as follows:

- formulation of the study question or hypothesis
- selection of study populations and study samples
- selection of indicators of exposure
- measurement of exposure and disease
- analysis of the relationship between exposure and disease
- evaluation of the role of bias
- evaluation of the role of chance.

These elements will be considered here in a simplified format. Readers are referred to epidemiology textbooks for consideration of the factors in more detail (Beaglehole *et al*. 1993; Friis and Sellers 1996; Hennekens and Buring

1987; Rothman and Greenland 1998). The case studies include examples of the elements described here.

7.2.1 Formulation of the study question or hypothesis

The study question must be formulated so that it can be tested using statistical methods. For example:

- Exposure to wastewater (meeting the WHO guidelines) compared with no exposure to wastewater does not increase the rate of *Ascaris* infection.

The *null hypothesis* (which implies there is no relationship between postulated cause and effect) states that observed differences are due to sampling errors (i.e. to chance). Stated in the null form, the propositions are refutable and can be assessed using statistical tests (see section 7.2.6).

7.2.2 Selection of study populations

A study population exposed (to the factor of interest) and a control population (not exposed to the factor of interest) need to be selected (except in a prospective cohort study where a single cohort is studied and analysis is on exposure status). A sample from the exposed and control populations needs to be selected to be as similar as possible in all factors other than the factor of interest e.g. socio-economic status, and other risk factors for the disease outcome of interest. Since samples are never totally similar, we need to record possible confounding factors and control for them in the analysis (see below). For enteric infections arising from exposure to contaminated water, such factors would include sanitation, personal hygiene, drinking-water supply, food hygiene, and travel. It is important that both exposure and disease can be measured as accurately as possible in the chosen populations. For example, in studies on drinking water, the drinking water source (and therefore the quality) for each household needs to be known accurately. In most studies, a sample will be selected from a larger population exposed to the factor of interest, using a *sampling frame*. This needs to be done so that it is representative of the larger population – difficulties here can arise due to *selection bias* and inadequate sample size (see also sections 7.2.6. and 7.2.7). The choices of study population will depend on the type of epidemiological study selected (see section 7.3).

7.2.3 Selection of indicators of exposure

The quality of the water to which the population is exposed needs to be measured. The use of indicators of contamination are preferred to measurements of pathogenic organisms in the water due to the low numbers of pathogenic organisms present, the difficulties in detecting them and the expense involved (see Chapter 13). Indicators should be selected that are appropriate to the water being studied e.g. thermotolerant coliforms or *E.coli* are used in assessing the quality of drinking water whereas these are less suitable for assessing the quality of coastal recreational waters where enterococci and faecal streptococci are generally preferred. Where the density of an indicator does not accurately reflect the relative density of the underlying pathogenic organism, then it is not a valid indicator organism. This is a particular concern when bacterial indicators are used to indicate the presence of both bacterial and viral pathogens, as treatment methods are often less effective against viruses. This has led to concern about the adequacy of the zero faecal coliform guideline for drinking water quality (Payment *et al*. 1991).

7.2.4 Measurements of exposure and disease status

In the study population measurements of exposure and disease status need to be made while minimising the various types of error that can occur. Where errors occur, this is called *information bias* and results in *misclassification* (see below). For *exposure* to occur, an individual must have contact with water of a given quality. It is preferable to measure exposure at an individual level, but in many studies, exposure status is measured at a group level, which can give rise to misclassification of exposure for the individual. For example, in studies of the effects of aerosol exposure from wastewater irrigation in Israel, exposure status was assigned at the kibbutz level and no differences in individual exposure status were measured. However, the effect of exposure was assessed separately for children and agricultural workers and for the general population, so allowing for some differences in exposure between sub-groups (Fattal *et al*. 1986; Shuval *et al*. 1989). Where the misclassification does not depend on disease status, then this is called *non-differential* misclassification, and the bias would be towards the null, making it more difficult to detect true associations between exposure and disease. This is important in studies assessing the validity of specific microbiological quality guideline levels, as a study may fail to show an effect of exposure to the guideline level whereas a true effect may exist. Recent studies of recreational water exposure and wastewater reuse have put a lot of effort into avoiding misclassification of exposure (see section 7.5). Differential misclassification can either overestimate or underestimate the effect of exposure on disease. One source of misclassification of exposure results from the limited precision of current techniques for the enumeration of indicator organisms (Fleisher and McFadden 1980). This has not been taken into account in most epidemiological and experimental studies of the health impact of contaminated recreational water, drinking water or treated wastewater.

7.2.5 Analysis of the relationship between exposure and disease

The basic measures of disease frequency in each population are described by using the prevalence rate (which is the proportion of the population that has the disease at a specific point in time) or the incidence rate (the number of new cases of disease per unit of person-time). Measuring the difference between disease frequencies in the exposed and control populations is usually done using a relative measure. The relative risk (RR) estimates the magnitude of an association between exposure and disease. It indicates the likelihood of developing the disease in the exposed group relative to those who are not exposed. If the disease is rare the odds ratio will approximate to the relative risk. The odds ratio (OR) is the ratio of the odds of exposure among the cases (numbers exposed divided by numbers not exposed) to the odds in favour of exposure among the controls. Where multivariate analysis is carried out (a technique that allows an assessment of the association between exposure and disease, while taking account of other risk factors that may be confounding factors) the odds ratios is the relative measure normally calculated. In many studies, the effect of different levels or *doses* of exposure will be calculated in order to see if there is a *dose–response* relationship. Response is defined as the proportion of the exposed group that develops a specific effect in comparison to the control group. Such information is very important in the setting of guideline levels where the guideline can be set at the level at which a response first occurs, or can be set at a level that is deemed ‘acceptable’ (see Chapter 10).

7.2.6 Evaluation of the role of chance

This involves two components. The first is hypothesis testing, or performing a test of statistical significance to determine the probability that chance can explain the observed results. The role of chance is assessed by calculating the P-value – if this is low, it is unlikely that the observed results would have been caused by chance alone, and if it is high, it is more likely that they are due to chance. Although arbitrary in nature, it is usual to choose either 0.05 (5%) or

0.01 (1%) as significance values for testing the null hypothesis. The P-value reflects both the size of the sample and the magnitude of the effect, e.g., P-values can be above the level of significance where the sample is too small to detect a significant effect. The second component is the estimation of the confidence interval. This indicates the range within which the true estimate of effect is likely to lie (with a certain degree of assurance) thus reflecting the precision of the point estimate of effect. This will be calculated for the chosen measure of effect, and is normally presented as, for example, the relative risk and the 95% confidence intervals.

7.2.7 Evaluation of the role of bias

Bias is any systematic error that results in an incorrect estimate of the association between exposure and disease. The main types of bias include *selection bias*, *information bias, recall bias,* and *confounding*. The case studies (outlined in Section 7.5) give examples of studies where particular attention has been paid to reducing bias.

Selection bias occurs when inclusion of study subjects on the basis of either exposure or disease is somehow related to the disease or exposure being studied. In a recent study of the risks of enteric disease from consumption of vegetables irrigated with partially treated wastewater (Blumenthal *et al*. 1996) problems were faced in determining a suitable control population. This was due to selection bias, as the other strong risk factors for enteric disease were more prevalent in the only nearby area where fresh water was used for irrigation of vegetables. In this case, the exposed population alone was studied, and individuals with low exposure (infrequent consumption of raw vegetables) compared with individual with higher exposure levels: tests were also done for a dose–response relationship.

Information bias occurs when there are systematic differences in the way data on exposure or outcome are obtained from the different study groups. Recall bias occurs when the reporting of disease status is different depending on the exposure status (or vice versa, in a case-control study). There was potential for recall bias in the cross-sectional study of the effect of wastewater reuse on diarrhoeal disease in Mexico (Blumenthal *et al*. 2001a), where individuals exposed to untreated wastewater may have recalled episodes of diarrhoea more accurately than individuals exposed to partially-treated wastewater. Interviewer bias occurs where interviewers are aware of the exposure status of individuals and may probe for answers on disease status differentially between exposure groups. In cohort studies, where individuals leave the study or are otherwise *lost to follow-up*, there can be bias if those lost are different in status to those who remain. These types of bias can generally be dealt with by careful design and conduct of a study.

Confounding occurs when the relationship between the exposure and disease is attributable (partly or wholly) to the effect of another risk factor, i.e. the confounder. It happens when the other risk factor is an independent risk factor for the disease and is also associated with the exposure. It can result in an over- or underestimate of the relationship between exposure and disease. For example, personal hygiene is a potential confounder of the association between drinking water quality and gastro-intestinal disease status. Risk factors that could potentially act as confounders must be measured during the study and controlled for using statistical analysis (e.g. logistic regression analysis can be used to adjust the measure of association between exposure and disease for the effect of the other risks factors). Many epidemiological studies of water-related infections before the mid-1980s did not adequately control for confounding.

7.3 TYPES OF STUDY

Essentially there are three broad types of epidemiological study design:

- descriptive studies
- analytical or observational studies
- experimental or intervention studies.

These will be outlined, in turn, in the following sections.

7.3.1 Descriptive studies

These examine the distribution of disease and possible determinants of disease in a defined population, and can often lead to suggestions of important risk or protective factors. They aim to identify changes in morbidity and/or mortality in time or to compare the incidence or prevalence of disease in different geographical areas or between groups of individuals with different characteristics. Descriptive studies generally use routinely collected health data, such as infectious disease notifications, and are cheap and quick to carry out. A series of descriptive studies of *Ascaris lumbricoides* infection in Jerusalem have shed light on the role of wastewater irrigation of vegetable and salad crops in the transmission of *Ascaris* infection (Shuval *et al*. 1985, 1986). Analysis of stool samples taken in a hospital in western Jerusalem between 1935 and 1947 showed that 35% were positive for *Ascaris* infection, whereas analysis of samples taken between 1949 and 1960 indicated that only 1% were positive –

the decrease was related by the authors to the partitioning of the city and the cessation in the supply of wastewater irrigated vegetables from valleys to the east of Jerusalem. Further descriptive studies indicated that the prevalence of *Ascaris* increased again when the city was reunited and the supply of wastewater-irrigated vegetables reintroduced, and decreased again when wastewater irrigation of vegetables was stopped. Descriptive studies are useful in generating hypotheses about the causes of certain disease patterns, but are not useful for testing hypotheses concerning the effect of particular exposures on particular disease outcomes.

7.3.2 Analytical studies

These are planned investigations designed to test specific hypotheses, and can be categorised into four groups:

- ecological
- cross-sectional studies
- cohort studies
- case-control studies.

7.3.2.1 Ecological (or correlational) studies

These examine associations between exposures and health outcomes using groups of people, rather than individuals, and often use surrogate measures of exposure, e.g. place and time of residence. Such a study would compare an aggregate measure of exposure (such as average exposure or the proportion of the population exposed) with an aggregate measure of health outcome in the same population. They are sometimes included under descriptive studies (e.g. in the US). In Thailand, for example, the seasonal variation in the reported incidence of acute diarrhoea in selected areas was examined in relation to rainfall and temperature records for the same areas (Pinfold *et al.* 1995). The authors found that the incidence of diarrhoea appeared to be inversely related to a sharp seasonal decrease in temperature. Rainfall did not appear to have a direct effect on the relative incidence of acute diarrhoea. The lack of ability to link individual exposure to individual disease risk and to control for possible confounders are major disadvantages of this approach and severely limit its usefulness in many settings, especially where the exposure changes over time and space and where there are many risk factors for the disease outcome of interest.

7.3.2.2 *Cross-sectional studies*

In a cross-sectional study exposure and health status are ascertained simultaneously on one occasion, and prevalence rates (or incidence over a limited recent time) in groups varying in exposure are compared. Careful measurement and statistical control of confounding variables is important to assess the effect of other risk factors for the outcome on observed prevalence. This approach has been used to assess the effects of wastewater reuse for irrigation. In India, the prevalence of intestinal parasitic infections was assessed in agricultural workers working on farms which were flood-irrigated with wastewater and compared with a control population where agricultural workers practised irrigation with clean water (Krishnamoorthi *et al*. 1973 cited in Shuval *et al*. 1986). Stool samples were examined for *Ancylostoma duodenale* (hookworm), *Ascaris lumbricoides* (roundworm) and *Trichuris trichiura* (whipworm). The exposed population had at least a two-fold excess of hookworm and *Ascaris* infection as compared to the control population. The usefulness of this study and other past cross-sectional studies has been limited by its failure to control for confounding variables and to document the type and extent of exposure of potentially exposed persons (Blum and Feachem 1985). A cross-sectional study can only provide information on the association between an exposure and disease, and the temporal relationship between exposure and disease cannot be established. Other problems include the need for large sample sizes (for infections where prevalence is low), and potential bias due to exposure and disease misclassification. However, the advantages are that such studies are relatively cheap and can provide meaningful results where exposure and confounding factors are measured carefully.

7.3.2.3 *Cohort studies*

In a cohort study the population under investigation consists of individuals who are at risk of developing a specific disease or health outcome. These individuals will then be observed for a period of time in order to measure the frequency of occurrence of the disease among those exposed to the suspected causal agent as compared to those not exposed. This type of approach has been used to examine the health effects of recreational water use (Balarajan *et al*. 1991; Cabelli *et al*. 1983). Typically, individuals are recruited immediately before or after participation in some form of recreational water exposure, with controls drawn from a population at the same location not participating in the water-based activity. During the follow-up period, data are acquired on the symptoms experienced by the two cohorts using questionnaire interviews. The quality of the recreational water is defined through sampling on the day of exposure. The exposure data are often combined to produce a daily mean value for the full group of bathers using a particular water on any one day. The problem with this approach is that the aggregation of exposure and subsequent assignment of the same exposure to many people produces a large degree of non-differential misclassification bias, which biases the measure of association. Cohort studies are useful for the study of relatively common outcomes and for the study of relatively rare exposures e.g. risks from occupational exposure to wastewater (Shuval *et al*. 1989). Careful classification of exposures and outcomes is needed, as is the measurement and control for confounding factors. The disadvantages are that the studies are often complex and difficult to manage, the time span is often at least a year (to take into account seasonality of disease incidence) and the studies can therefore be expensive. A wastewater reuse cohort study is outlined in Section 7.5.2.

7.3.2.4 Case-control studies

Case-control studies examine the association between exposure and a health outcome by comparing individuals already ill with the disease of interest (i.e. cases) and a control group who are a sample of the same population from which the cases were identified. Gorter *et al*. (1991) used a case-control study design to examine the effects of water supply and sanitation on diarrhoeal disease in Nicaragua. They compared over 1200 children with diarrhoea with a similar number of controls (children of a similar age with illnesses other than diarrhoea). They found a statistically significant association between water availability and diarrhoea morbidity. Children from homes with water supplies over 500 metres from the house had incidence rates of diarrhoea 34% higher than those of children from houses with their own water supply. This relationship remained significant after controlling for confounding factors. The advantages of case-control studies are that they require smaller sample sizes, fewer resources, require less time and less money, and sometimes are the only way to study rare diseases. The difficulties are in appropriate study design to minimise bias, including the selection of appropriate controls and the control of confounding variables and minimising recall bias. Regarding wastewater reuse and recreational water reuse, the potential for misclassification of exposure is higher within a case-control design than in other types of study due to recall bias. They are therefore of less value than other designs in evaluating risks related to exposure to water of varying qualities.

7.3.3 Experimental or intervention studies

These differ from the observational techniques outlined above in that the investigators determine who will be exposed. A key part of the experimental design consists of randomising a single cohort into two groups. The process of randomisation attempts to ensure the same distribution of various intra-individual traits and potential confounders between study groups so that they are as comparable as possible. One group is then assigned to exposure to the factor under study; the other group is the control and the health outcomes for the groups are compared. Randomisation of subjects is important to minimise the potential for confounding or selection bias. In terms of determining causality this type of study is generally considered to be the most powerful. It is equivalent to the randomised controlled trial used in testing the impact of drugs and other medical interventions. Its use in examining environmental exposures has been limited because of ethical concerns, since many exposures of interest are potentially detrimental. A notable exception is provided by the first case study in this chapter (section 7.5.1), which presents the study design and results of four randomised trials assessing the risk of bathing in marine waters contaminated with domestic sewage (Fleisher *et al*. 1996; Kay *et al*. 1994). In the third case study (in section 7.5.3), intervention trials are described which have recently been used in evaluating the current guidelines for drinking water quality. These have compared persons drinking ordinary tap water with those drinking water that has been 'treated' in the home, using reverse-osmosis filters or UV light (Hellard *et al*. 2000; Payment *et al*. 1991). This type of design is not applicable in the study of wastewater treatment and reuse where the intervention is at a community not an individual level, and it is not possible to assign wastewater treatment plants randomly to a number of different communities (due to costs and practical issues).

7.4 USES OF EPIDEMIOLOGY IN THE SETTING OR EVALUATING OF MICROBIOLOGICAL GUIDELINES

There are several different approaches that can be taken to the use of epidemiological studies in the setting or evaluation of microbiological guidelines for drinking water, recreational water or wastewater:

- Measure the relationship between exposure and disease for a range of levels of indicator organisms to get a dose–response curve. Set an acceptable level of risk and then find the microbiological level related to that level of risk (using the dose–response curve). This method has been used for proposing recreational water guidelines (see section 7.5.1 and Chapter 2).

- Measure the relationship between exposure and disease for water at the current guideline level, and possibly for water above or below the guideline level. Examples of this approach can be provided by both drinking water and wastewater reuse studies. The studies in the drinking-water case study (section 7.5.3) assessed the relationship between exposure and disease for water that met the current drinking-water guideline limits. The studies outlined in the wastewater case study section (section 7.5.2) assessed the relationship between exposure and disease for wastewater meeting the WHO guideline levels (WHO 1989).
- Use the results of several studies where the relationship between exposure and disease has been assessed for water of different qualities, and estimate the level at which no effect would be found. This method was used informally to propose a new faecal coliform guideline to protect agricultural workers involved in wastewater reuse (Blumenthal *et al*. 2000b). Ideally a meta-analysis, such as that conducted by Esrey *et al*. (1985, 1991) would be conducted to combine the results of several studies.

7.5 CASE STUDIES

Three case studies, using different approaches and epidemiological methods, are outlined in the following sections. The recreational water studies have been used to inform standards development, while the wastewater reuse and drinking-water studies are likely to inform future development.

7.5.1 Recreational water case study

Four separate study locations around England and Wales (UK) were used (Fleisher *et al*. 1996; Kay *et al*. 1994). The study locations were sufficiently distant from one another so that site-specific differences in the risk of bathing-associated illness could be assessed. All the study locations met European Community (EC) mandatory bacteriological marine bathing-water quality criteria as well as US EPA bathing-water criteria for marine waters. A randomised controlled trial design was used in order to minimise selection bias and control for intra-individual differences in susceptibility, immune status and so on between study groups. Equally importantly, the risk of non-differential misclassification of exposure was minimised by assigning precise measures of exposure to each individual bather (studies by Cabelli *et al.* (1993) were seriously affected by bias of this type). Healthy volunteers aged 18 or over were randomised into two groups:

- an exposed group where volunteers actually entered the water, and
- an unexposed group where volunteers spent an equal amount of time on the beach but did not enter the water.

All volunteers were blinded to the specific outcome illnesses being studied in order to control for or minimise bias in the reporting of symptoms. Volunteers also did not know which group they would be assigned to until the day of the trial.

Since the mix of underlying pathogens that could possibly be present in the bathing waters remained unknown, five indicator organisms or groups of organisms were used to assess exposure among the bather group:

- total coliforms
- faecal coliforms
- faecal streptococci
- total staphylococci
- *Pseudomonas aeruginosa*

This was done to maximise the chance of finding an indicator organism that directly correlated with the underlying pathogen or pathogens, thus reducing misclassification of exposure.

Duration and precise location of individual bather exposure was rigorously controlled. This is important because of the large spatial and temporal variations in concentration of indicator organisms that are seen at bathing water locations caused by environmental factors. Indicator organism concentration was measured every 30 minutes. Exposure was assigned to each individual bather within 15 minutes of the actual exposure and within a maximum of 10 metres of the actual point of exposure. These measures minimised misclassification of exposure among bathers.

All five indicator organisms used were assessed using the Membrane Filtration method of enumeration. In addition, three replicate determinations were made on each sample taken. Using the most precise method of indicator organism enumeration, coupled with taking three replicate determinations per sample, maximised the precision of each estimate and minimised the bias due to lack of precision in estimation.

In order to control for competing risk factors and/or confounders for the outcome illnesses under study, four separate interviews were held with each study participant. These interviews were conducted two to three days prior to each trial, on the trial day, at seven days post-trial, and at three weeks post-trial. In this manner, information about exposure to competing non-water-related risk factors and/or confounders was recorded for each participant prior to the trial, at the time of the trial, and upon completion of the trial (allowing for a suitable incubation period). These exposures to non-water-related risk factors were then controlled for in the analysis.

The outcome illnesses used were gastroenteritis, acute febrile respiratory illness, and skin, ear, and eye infection. All study participants reporting symptoms of any of these five outcome illnesses during the pre-trial interview or at the interview conducted on the actual trial day were excluded from the study. The same interview was used 7 and 21 days post-trial. Since gastroenteritis is often used as the 'index' illness for assessing waterborne illness, the results presented here are for gastroenteritis. Table 7.1 shows a partial list of the confounders or competing risk factors that were recorded.

Table 7.1. Non-exposure-related risk factors for gastroenteritis

Non-exposure related risk factor
Age – grouped by 10-year intervals
Gender
History of migraine headaches
History of stress or anxiety
Frequency of diarrhoea (often, sometimes, rarely or never)
Current use of prescription drugs
Illness within 4 weeks prior to the trial day (lasting more than 24 hours)
Use of prescription drugs within 4 weeks prior to the trial day
Consumption of the following foods in the period from 3 days prior to 7 days after the trial day:
mayonnaise
purchased sandwiches
chicken
eggs
hamburgers
hot dogs
raw milk
cold meat pies
seafood
Illness in the household within 3 weeks after the trial day
Alcohol consumption within the 7 day period after the trial
Frequency of usual alcohol consumption
Taking of laxatives within 4 weeks of the trial day
Taking of other stomach remedies within 4 weeks of the trial day
Additional bathing within 3 days prior and 3 weeks after the trial day (this was included in order to control for possible confounding due to multiple exposures among bathers and exposure among non-bathers prior to or after the trial day)

Faecal streptococci (FS) was the only indicator organism that predicted gastroenteritis among bathers. Crude rates of illness among bathers versus non-bathers were 14.8% versus 9.7% (P = 0.01). Crude rates do not, however, reflect the effects of variation in exposure to differences in indicator organism densities among individual bathers, and should be viewed with caution. Faecal streptococci densities ranged from 0–158 per 100 ml of water. Therefore, the crude difference in rates dampens out this variability in exposure of individual bathers to differing levels of sewage (and thus risk). However, the rates of illness among those exposed to the highest quartile of exposure (50–158 FS) shows the rates of illness to be 24.6% among bathers versus 9.7% for non-bathers. The stratification of rates of illness over increasing levels of indicator organism exposure is an important feature of the analysis. This becomes especially important in the construction of mathematical models used to quantify microbial risk. Using crude rates of illness would invariably lead to an underestimate of risk produced by the model, and possibly question the validity of the model itself.

Using multiple logistic regression modelling, a dose–response curve was produced relating the probability of a bather acquiring gastroenteritis relative to individual bather faecal streptococci exposure while adjusting for the non-water-related risk factors and/or confounders. Using this technique, the probability of competing risk factors for the same illness can be quantified. Such information on competing risk factors can be important in setting water quality criteria.

The results of the randomised trials discussed above are based on a total sample size of only 1216 participants. This illustrates that the use of an appropriate epidemiological study design (randomised trial) can yield extremely informative and precise information regarding quantitative microbiological risk assessment without the need for large sample sizes. In addition, randomised trials can be conducted at multiple sites over wide geographical areas within a region while assessing for any site-specific differences. Such an epidemiological design contains no assumptions, relies solely on data collected during the study, and yields more valid and precise estimates of risk than mathematical risk assessment models.

The implications of the studies for the setting of microbiological guidelines for recreational water are discussed in Chapter 2.

7.5.2 Wastewater reuse case study

A series of epidemiological studies were conducted in Mexico to assess the occupational and recreational risks associated with exposure to wastewater of different microbiological qualities. Observational study methods were used to assess the risks associated with existing practices, as there was no possibility of introducing a wastewater treatment facility and assessing its impact on health through an intervention study or randomised trial. Infections (from helminths, protozoa and diarrhoeal disease) in persons from farming families in direct contact with effluent from storage reservoirs or raw wastewater were compared with infections in a control group of farming families engaged in rain-fed agriculture (Blumenthal *et al*. 1996; Blumenthal *et al*. 2001a; Cifuentes 1998; Peasey 2000). The storage reservoirs fulfilled a 'partial treatment' function and produced water of differing microbiological qualities. The effects of wastewater exposure were assessed after adjustment for many other potential confounding factors (including socio-economic factors, water supply, sanitation and hygiene practices).

Raw wastewater coming from Mexico City to the Mezquital valley, Hidalgo, is used to irrigate a restricted range of crops, mainly cereal and fodder crops, through flood irrigation techniques. Some of the wastewater passes through storage reservoirs and the quality of the wastewater is improved before use. The effluent from the first reservoir (retention time 1–7 months, depending on the time of year) met the WHO guidelines for restricted irrigation (Category B, ≤1 nematode eggs/litre), even though a small amount of raw wastewater enters the effluent prior to irrigation. Some effluent from the first reservoir passes into a second reservoir where it is retained for an additional 2–6 months, and the quality improved further. Local farming populations are exposed to the wastewater and effluent through activities associated with irrigation, domestic use (for cleaning, not for drinking) and play.

The untreated wastewater contained a high concentration of faecal coliforms (10^6–10^8/100ml) and nematode eggs (90–135 eggs/l). Retention in a single reservoir reduced the number of nematode eggs substantially, to a mean of <1 eggs/l whereas faecal coliform levels were reduced to 10^5/100 ml (average over the irrigation period) or 10^4/100ml, with annual variations depending on factors such as rainfall. The concentration of nematode eggs remained below 1 egg/l (monthly monitoring) even after a small amount of raw wastewater entered the effluent downstream of the reservoir. Retention in the second reservoir further reduced the faecal coliform concentration (mean 4×10^3/100ml) and no nematode eggs were detected. Faecal coliform levels varied over the year depending on the retention time in each reservoir, which varied according to demand for irrigation water. Three studies were carried out in this study area. The first used a cross-sectional methodology to study the prevalence of a range of parasitic infections and diarrhoeal disease (and included two surveys); the second used a prospective cohort methodology to study the intensity of *Ascaris lumbricoides* infection; and the third used a cross-sectional methodology to study prevalence of diarrhoeal disease. Use of a cross-sectional methodology was recommended by Blum and Feachem (1985) as a cost-effective way to study the association between wastewater exposure and a range of infections.

In the first study (Blumenthal *et al*. 2001a; Cifuentes 1995, 1998) a census was conducted to locate households where one or more persons were actively involved in agriculture. Exposure groups included agricultural households using untreated wastewater for irrigation, households using effluent from a reservoir and households practising rain-fed agriculture (control group). In the first cross-sectional study (rainy season), the reservoir group was exposed to wastewater retained in two reservoirs in series and in the second survey, the reservoir group was exposed to wastewater retained in the single reservoir. Measures were taken to reduce the misclassification of exposure. Data on the siting of agricultural plot(s) worked by the farming families, the irrigation canals feeding them and the source (and therefore quality) of water in the canals was used in an algorithm to define the exposure status of the farming family (Cifuentes 1995). Inclusion criteria for households were: location in an agricultural community, one or more adults with tenure of a farm plot and occupational contact with wastewater of a defined quality (raw wastewater, effluent from the reservoir) or farming of a rain-fed plot (control group). Farmers were excluded if they had contact with an unknown or unclassified source of irrigation water, if they had plots in more than one area or contact with more than one type of water, and if they lived in the control area but had contact with wastewater. Members of every household were assigned to the same exposure category as the members working on the land, to allow for intra-familial transmission of infection. Information was collected on the agricultural profile of every household (i.e. location of farming plot, type of irrigation water used, cultivated crops), whether and when the person had contact with wastewater, and on other risk factors that were potential confounders. Socio-economic variables collected included land tenure, maternal literacy, house roof material, number of bedrooms and number of chickens eaten per week. Hygiene- and sanitation-related characteristics included excreta disposal facility, source of drinking water, storage and boiling of drinking water, hand washing, hygienic appearance of respondent, rubbish disposal facilities, animal excreta in the yard and local source of vegetables. Exposure to wastewater was defined as having direct contact ('getting wet') with wastewater (or reservoir water) in a particular time period. Recent exposure (in the last month) was related to diarrhoeal disease and past exposure (from 1–12 months previously) was related to A*scaris* infection. A diarrhoeal disease episode was defined as the occurrence of three or more loose stools passed in a 24-hour period and the recall period was two weeks. The prevalence of specific intestinal parasite infections was assessed by means of microscopic identification of the presence of ova or cysts in stool samples. The results for *Giardia intestinalis* and *Entamoeba histolytica* were reported separately (Cifuentes *et al*. 1993, 2000).

In the analysis, the estimates of the effect of exposure to wastewater and reservoir water were adjusted for the effects of all other variables that were potential confounders. The main results that have implications for guidelines setting are summarised in Table 7.2. Exposure to effluent from one reservoir (meeting WHO guideline level of ≤1 nematode egg per litre) was strongly associated with an increased risk of *Ascaris* infection in young children and in those over five years of age, when compared to the control group. Exposure to effluent from two reservoirs (where the quality was further improved) was not associated with an increased risk of *Ascaris* infection in young children, whereas a small risk remained for those over five years of age. Exposure to effluent from one reservoir was associated with increased diarrhoeal disease in those over five years of age (compared to the control group), whereas exposure to effluent from two reservoirs was not. The later result is not conclusive, however, since the effect of exposure to effluent from two reservoirs was only assessed in the rainy season. In the dry season the effect may be greater, as the effect of exposure to untreated wastewater was both stronger and more significant in the dry season in both age groups (compared to the control group).

Table 7.2. Effect of exposure to untreated wastewater and degree of storage of wastewater (Cifuentes 1998; Blumenthal *et al*. 2001a)

	Ascaris infection OR* (95% CI)	Diarrhoeal disease OR* (95% CI)
Effect of exposure to untreated wastewater		
0–4 years		
Dry season	18.01 (4.10–79.16)	1.75 (1.10–2.78)
Rainy season	5.71 (2.44–13.36)	1.33 (0.96–1.85)
5+ years		
Dry season	13.49 (6.35–28.63)	1.34 (1.00–1.78)
Rainy season	13.49 (7.51–23.12)	1.10 (0.88–1.38)
Effect of exposure to stored wastewater (by degree of storage)		
0–4 years		
One reservoir, dry season	21.22 (5.06–88.93)	1.13 (0.70–1.83)
Two reservoirs, rainy season	1.29 (0.49–3.39)	1.17 (0.85–1.60)
5+ years		
One reservoir, dry season	9.42 (4.45–19.94)	1.50 (1.15–1.96)
Two reservoirs, rainy season	1.94 (1.01–3.71)	1.06(0.86–1.29)

* All ORs (Odds ratios) use the control group as the reference.

The prospective cohort study of the effect of exposure to partially-treated wastewater on *Ascaris* infection was done in the same area (Peasey 2000). The study groups were the same as for the dry season study and the sample was selected from the census as outlined above. The inclusion criteria for households were: the head of the household was a farmer, male, at least 15 years old and had contact with only one quality of irrigation water, i.e. only rain-fed or only untreated wastewater. The inclusion criteria for individuals within each selected household were: at least two years old, resident in the house at least five days a week and any wastewater contact was with the same quality of wastewater as the head of the household. A baseline survey was done where the prevalence and intensity of *Ascaris* infection (as measured by the egg count) was measured on full stool samples. Subjects with *Ascaris* infection were given chemotherapy to expel the adult worms, such that the egg counts were reduced to zero. A follow-up survey was done 12 months later, and the prevalence and the intensity of reinfection after treatment measured. This design provided a more sensitive measure of prevalence of infection than the cross-sectional surveys above, as well as a measure of intensity of reinfection over a specific time period, thus reducing any misclassification of disease. Each individual was assigned a personal exposure status according to their activities involving direct contact wastewater and the frequency of that contact. This time-method further improved the classification of exposure and infection with *Ascaris* in comparison with the cross-sectional studies, and provides a more valid measure of infection related to exposure over a specific time period. Data was collected on other risk factors for *Ascaris* infection and the estimates of the effect of exposure on infection adjusted for potential confounding factors.

The main results can be summarised as follows. Contact with effluent from one reservoir was associated with an increase in prevalence of *Ascaris* infection among adults and children when compared with the control group. Multivariate analysis was done using internal comparison groups and not the external control group, since numbers of positives in the external control group were very small (due to the low prevalence of infection in the external control group and the small sample size) and a multivariate model would have been very unstable if this group had been used as a baseline. Contact with effluent from one reservoir through playing was associated with an increase in prevalence of *Ascaris* infection in children under 15 years of age, compared with those who lived in a wastewater-irrigated area but did not have contact with wastewater during play (OR = 2.61, 95% CI: 1.10–6.15). Contact with effluent from one reservoir for irrigation was not associated with a significant increase in *Ascaris* infection in children under 15 years of age when compared with children from the same area who did not irrigate. For adult men, wastewater contact during work related to chilli production was associated with an increased prevalence of *Ascaris* infection in those exposed to untreated wastewater (OR = 5.37, 95% CI: 1.79–16.10) but not in those exposed to effluent from one reservoir (OR = 1.56, 95%

CI: 0.13–18.59) when compared with adult men living in wastewater-irrigated areas who did not cultivate chilli. For adult women, contact with untreated wastewater through tending livestock in wastewater-irrigated fields was associated with increased prevalence of *Ascaris* infection (OR = 4.39, 95% CI: 1.08–17.81) but contact with effluent from one reservoir was not (OR = 0.70, 95% CI: 0.06–8.33) when compared with adult women living in wastewater-irrigated areas who did not tend livestock or who had no wastewater contact while tending livestock.

The third study was carried out mainly to assess the effect of consumption of vegetables, irrigated with partially treated wastewater, on a range of enteric infections. Infections included symptomatic diarrhoeal disease, enterotoxigenic *E. coli* infection and infection with human Norwalk-like virus (Blumenthal *et al*. 2001b). However, since a section of the study population was involved in agricultural work and were in direct contact with effluent from the second reservoir it was possible to estimate the effect of direct contact (as well as to adjust the estimate of the effect of consumption for the effect of direct contact). The effect of exposure on diarrhoeal disease was assessed through two cross-sectional surveys, in the rainy and dry seasons. The design of the surveys was similar to that used in the previous cross-sectional surveys except in two aspects where the design and analysis was improved: measures of individual exposure to effluent from the second reservoir were used (instead of the exposure of the adult male farmer) and the comparison group was individuals of the same age in the same area but who did not have contact with effluent from the reservoir (whereas earlier the comparison group was a control group from a rain-fed area). When children with contact with the effluent from the second reservoir were compared to children from the same population but with no contact with the effluent, a two-fold or greater increase in diarrhoeal disease in children aged 5–14 years was found (OR = 2.34, 95% CI: 1.20–4.57 dry season). In the first study it was found that there was no excess of diarrhoeal disease related to exposure with this water compared to the level in the control group, where rain-fed agriculture was practised (Cifuentes 1998).

Taken together, the results show that contact with wastewater retained in one reservoir and meeting WHO guidelines for restricted irrigation was associated with an increased risk of *Ascaris* infection (especially in children, in contact through play), and an increased risk of diarrhoeal disease (especially in the dry season). When the quality of the water was improved through retention in two reservoirs in series (10^3–10^4 faecal coliforms/100ml and no detectable nematode eggs), the risk of *Ascaris* infection to children was decreased, but there was still an increased risk of diarrhoeal disease to exposed children compared with those not in contact with effluent. These results indicate that the nematode egg guideline of ≤1 nematode egg per litre is adequate for the protection of farm workers but inadequate where children have contact with the wastewater (especially through play). A faecal coliform guideline for the protection of farming families is also needed. The implications of these results, and those from other studies, for modification of the 1989 WHO guidelines are discussed further elsewhere (Blumenthal *et al*. 2000a,b).

7.3.3 Drinking-water case study

In studies of drinking water, randomised control trials of interventions have been used to explore whether there is a risk of gastrointestinal (GI) disease due to consumption of drinking water meeting current microbiological standards. Payment *et al*. (1991) used a randomised controlled trial to investigate whether excess gastroenteritis was being caused by potable water supplies (outlined in greater detail in Chapter 4). The suburban area of Montreal, Canada, chosen for the study, is served by a single water treatment plant, using pre-disinfection flocculation by alum, rapid sand filtration, ozonation and final disinfection by chlorine or chlorine dioxide. The raw water was drawn from a river, which was contaminated with human sewage discharges. The study design consisted of the randomised installation of reverse-osmosis filters in study participants' households. Therefore, two groups were formed: those households with filters (control group), and those households using plain tap water. GI symptomatology was evaluated by means of a family diary of symptoms. The study lasted 15 months. The results of this study estimated the annual incidence of GI illness among tap-water drinkers to be 0.76 versus 0.50 among filtered water drinkers ($P<0.01$). In addition, the results of this study estimated that 35% of the total reported gastroenteritis among tap-water drinkers was water-related, and thus preventable. Payment *et al*. (1997) conducted a second study a few years later, altering the exposed and control groups. In this second study, two groups (tap-water group and tap-valve water group) received normal tap water through kitchen taps; the only difference between these groups was that the tap-valve water group had a valve fitted to their house to control for stagnation of water in their household plumbing. Two additional groups received bottled finished water from the plant (plant water group and purified water group) that was bottled before it entered the distribution system. The water for the purified water group was passed through a reverse-osmosis filter before it was bottled. Again, illness was assessed using a household diary. Using the purified water group as the baseline, the excess of gastrointestinal illness associated with tap water was 14% higher in the tap group and 19% higher in the tap-valve group. Children ages two to five were the most affected, with an excess of 17% in the tap-water group and 40% in the tap-valve group. Payment *et al*. concluded that their data suggest that 14–40% of the observed gastrointestinal illnesses were attributable to tap water meeting current standards, and that the water distribution system appears to be partially responsible for these illnesses. However, these studies have been criticised for failing to blind study subjects to their exposure status: those with filters knew they had filters and may have been less likely to report GI symptoms than those without filters, so biasing the results. Currently, the US Centers for Disease Control and Prevention (CDC) have started two large-scale studies of illness transmission through treated tap water to address some of the criticism of the Canadian studies.

A recent study conducted in Melbourne, Australia, is also contributing to the debate on the validity of current microbiological standards for drinking water (Hellard *et al*. 2000). The study was set up to explore whether tap water in Melbourne that was chlorinated but not filtered was associated with an increase in community gastroenteritis. Melbourne's raw water comes from large reservoirs in an unpopulated forested catchment area (markedly different from that used in the Canadian studies). A randomised double-blind controlled trial was set up. Participants in one group were given a functioning water treatment unit in the home (consisting of a filter to remove protozoa and an ultraviolet (UV) light unit to kill viruses and bacteria) while the 'tap water' group were given a mock water treatment unit, which looked identical to the functioning water treatment unit but did not alter the water. The participants were therefore 'blinded' to their exposure status. The characteristics of the two groups were the same at randomisation. Families in the study completed weekly health diaries and faecal specimens were taken when an episode of diarrhoeal disease was reported. Gastroenteritis was defined by a combination of symptoms similar to the Canadian studies, and the subject had to be symptom-free for six days before a new episode was registered. Loss to follow-up (41/600 families) was lower than in the Canadian studies. The results showed that the rate of gastroenteritis was almost the same in both groups (0.79 versus 0.82 episodes/person/year; RR = 0.99, 95% CI: 0.85–1.15). This was the case even though the tap water failed to meet the 1996 Australian Drinking Water Guidelines for water quality in terms of total coliform detection (total coliforms were present in 19% of samples, rather than <5% samples as recommended in the guidelines). The lack of an effect on community gastroenteritis of drinking this water may have been due to the cleaner catchment and better source water protection. However, it may be related to the superior epidemiological study design, using a randomised double-blinded design (with real and mock water treatment units), which may have eliminated any reporting bias present in earlier studies.

7.4 DISCUSSION

Epidemiological methods have the ability to estimate risk with a good degree of precision, but also, and perhaps just as important, have the ability to control for other risk factors and/or confounders of the outcome illness being studied. As outlined in Chapter 5, most gastrointestinal illnesses such as those related to drinking water, recreational water and wastewater reuse can be spread by more than one route. Epidemiological study is the only method that can utilise real data to separate the risk of the illness caused by the contaminated water from other risk factors for the outcome illness. Without such control, risk can be substantially overestimated.

Well designed and conducted epidemiological studies can also minimise the many biases that may occur. Experimental or intervention studies can provide the most accurate results, having minimised the potential for selection bias and confounding, but may not be suitable in some cases due to ethical or cost considerations and where subjects cannot be blinded to exposure/intervention status. Prospective cohort studies are the next best option, where the exposure precedes the disease outcome and attention is paid to selection bias and potential confounders are measured and controlled for in the analysis. Where cost, logistical or other considerations preclude the use of such studies, cross-sectional studies can provide useful results where attention is paid to measuring exposure and disease accurately and allowing for potential confounding factors (Blum and Feachem 1985). Case-control studies are not so useful in evaluating microbiological guidelines, due to recall bias in the measurement of exposure, and retrospective cohort studies are not recommended where there is bias in the measurement of exposure or disease. In the selected study types, where adequate sample sizes are used, the risk of illness related to a specific exposure can be calculated with a good degree of precision. It is clearly important that the highest quality studies are used for the setting of water-related guidelines as these can result in considerable outlay by governments and water industry.

The limitations of epidemiological studies have been thought to lie in the need for unrealistically large sample sizes to uncover very small increases in risk, and in the costs incurred and expertise needed to mount a good study. However, the case study examples show that epidemiological studies can be designed and carried out in such a way as to provide very valuable information on the validity of current guidelines and for recommending new guidelines. The sample size requirements are not unreasonable, especially if cohort studies or experimental studies are carried out. Given the cost of complying with more restrictive standards, a case can anyway be made for significant expenditure on an epidemiological study, especially if there is the chance that this will indicate that more restrictive standards are not needed.

Epidemiological studies can assess the effect of 'real' exposures and can measure the effect on more vulnerable groups (e.g. young children) as well as adults. The effect of related exposures can also be taken into account, for example children playing with wastewater as well as being exposed to it through agricultural work.

7.5 IMPLICATIONS FOR INTERNATIONAL GUIDELINES AND NATIONAL REGULATIONS

Epidemiological studies have been used in setting the guidelines for wastewater reuse (WHO 1989), and in proposing the draft guidelines for safe recreational water environments (WHO 1998) as outlined in Chapter 2. However, different approaches have been taken both in the use made of the epidemiological studies (as outlined above) and in the level of risk that was considered acceptable. In the case of wastewater reuse, evidence from a range of studies was taken into account and a guideline level proposed that was estimated to result in no measurable excess infection in the exposed population. In the case of recreational water use, an acceptable level of risk was set, and the microbiological level related to that level of risk was found, using the dose–response curve produced by the best epidemiological study available linking microbial concentrations with gastroenteritis. It seems possible, therefore, that the wastewater guidelines protect against a lower level of risk than the proposed recreational water guidelines. In contrast, the drinking water guidelines are based on '*tried and tested principles of prevention of faecal pollution and good engineering practice*' (Chapter 2). Now that more epidemiological studies of drinking water are available (see Chapter 4), it is essential that all available epidemiological evidence is taken into account in the setting of future guidelines.

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