7

INTRODUCTION TO OBSERVATIONAL STUDIES

OBJECTIVES

After reading this chapter, you should be able to:

1. Differentiate between descriptive and explanatory studies.
2. Differentiate between experimental and observational studies.
3. Describe the general strength and weaknesses of experimental versus observational study designs for the identification and evaluation of causal factors.
4. Design a cross-sectional study which takes into account the strengths and weaknesses of this study type.
5. Identify circumstances in which a cross-sectional study is the appropriate observational study design.

7.1 INTRODUCTION

A central occupation of epidemiologists is to identify causal factors that can be manipulated to prevent disease, or minimise its harmful effects. Here we ask the question 'how do we best go about the task?' The specific objectives of the research and the context in which the study will be conducted have a major impact on the choice of study type. However, when selecting the study design to pursue causal associations, we must bear in mind the advantages and limitations of each design. Hence, in this section, we provide an overview of the range of study types available for use by health researchers.

7.1.1 Descriptive versus explanatory studies

Research studies can be classified into 2 major categories: descriptive and explanatory (see Table 7.1 and Figure 7.1). **Descriptive studies** include case-reports, case-series reports and surveys. Although our classification of study types is by no means universally accepted, descriptive studies are designed solely to describe the nature and distribution of outcome events such as animal-health-related phenomena (Grimes & Schulz, 2002b). Because no comparisons are made between the characteristics of subgroups in the study (*eg* exposed versus non-exposed or treated versus not-treated), no inferences about associations between exposures and outcomes can be made. Descriptive studies are described in more detail in Section 7.3.

Table 7.1 Characteristics of various study types

Type of study	Level of difficulty	Level of investigator control	Strength of 'proof' of causal association	Relevance to 'real-world' situations
Descriptive				
Case report	very easy	very low	not applicable	low to high
Case series	easy	very low	not applicable	low to high
Survey	moderate	moderate	not applicable	high
Explanatory - experimental				
Laboratory trial	moderate	very high	very high	low
Controlled field trial	moderate	high	very high	high
Explanatory - observational				
Cross-sectional	moderate	low	low	moderate
Cohort	difficult	high	high	high
Case control	moderate	moderate	moderate	high

Explanatory studies are designed to make comparisons between subgroups of study subjects based on exposure or outcome status. This allows the investigator to identify statistical associations between exposures of interest (*eg* risk factors, treatments *etc*) and outcomes of interest (*eg* disease occurrence, productivity effects *etc*) as a first step to making inferences about causal relationships (see Chapter 1 for more details).

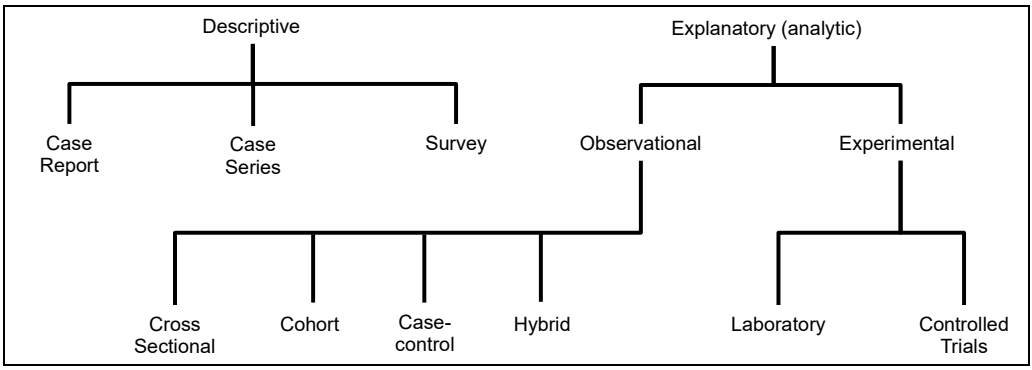

Fig. 7.1 Schematic representation of study types

7.1.2 Experimental versus observational studies

Explanatory studies can be subdivided into experimental and observational studies. Experimental studies are those in which the investigator controls (usually through randomisation) the allocation of the study subjects to the study groups (*eg* treated versus not treated, exposed to a risk factor versus non-exposed). In contrast, in observational studies, the investigators try not to influence the natural course of events for the study subjects. They confine their activities to making careful observations (which might include collection of data and a variety of biological samples) about the study subjects with particular attention paid to the exposures and outcomes of interest. In essence, experimental studies try to reduce variation from all sources through selection of study subjects and control of the experimental setting; whereas observational studies tend to embrace the presence of variation in order to identify important interactions among key variables and the exposure. The price paid by observational studies is that considerable efforts are required to prevent confounding of the exposure-disease association (see Chapter 13 for a discussion of confounding).

The decision about whether to use an experimental or an observational approach might be evident early on in the planning process. However, it is often valuable to consider the full range of study designs available rather than fixing on a particular study design too early and then trying to fit the investigation of the problem within the chosen design. Experiments often are the preferred choice if the treatment (or exposure) is straightforward and easily manipulated, such as a vaccine, or a specific therapeutic agent such as a hormone or antibiotic. The major advantage of the experimental approach is the ability to control potential confounders, both measured and unmeasured, through the process of randomisation. Observational studies usually are the preferred study design if the exposure(s) is more complex (*eg* if multiple exposures are of interest), or if exposure is not easily manipulated by the researcher either for practical, ethical, or economic reasons. They have the advantages that a much wider array of hypotheses can be tested. In many instances the study subjects will be exposed to the risk factor(s) whether the study is done or not, and thus, observational studies can capitalise on these 'natural events' to investigate possible causal associations. Nonetheless, if a controlled trial (experimental) of a specified intervention is 'do-able' then, this is the approach of choice.

Experimental studies can be broadly classified as laboratory-based or field-based trials. Laboratory trials are carried out under strictly controlled 'in-house' conditions. These have the advantage that the investigator has almost complete control over the experimental conditions

(*eg* type of animal used, environmental conditions, timing, level and route of exposure, method of outcome assessment *etc*). Evidence of an association between an exposure and a factor obtained from this type of study provides the best evidence of causation. However, given the very artificial environment in which laboratory trials are conducted, the relevance of the results to 'real-world' conditions is often somewhat in doubt. Because epidemiologists are interested in health events in populations, laboratory-based trials are not major components of our work and will not be discussed further in this text. Nonetheless, epidemiologists frequently use field trials for the investigation of health problems. In these, the investigator 'controls' the allocation of the subjects to the study groups (*ie* through randomisation), and the study is performed under natural 'real-world' conditions (hence, these studies are often referred to as **controlled field trials, randomised controlled trials**, or just **controlled trials**). The design and implementation of field trials is discussed in Chapter 11.

Observational studies make up a substantial portion of the research carried out by veterinary epidemiologists, and can be broadly classified as **cross-sectional** (Section 7.5), **cohort** (Chapter 8), **case-control** (Chapter 9) and **hybrid** (Chapter 10) studies. As noted, observational studies often can take advantage of the fact that exposed subjects already exist and therefore, with an appropriate design, the impact of the exposure can be investigated without having to manipulate the exposure status of the selected study subjects. It would be a stretch to imply that these are 'natural' experiments, but the fact that subjects are being exposed, and the outcomes are happening regardless of the presence or absence of the study, begs the question 'why not seize the opportunity to capture data that can help assess possible associations between the exposure and the outcome'? Kalsbeek and Heiss (2000) have noted that most empirical knowledge has been based on observations of incomplete samples (*ie* selected subgroups) of human (subject) experience. Often, it is impractical to study the entire population and thus sampling strategies must be considered; indeed, the sampling strategy is the basis for the classification of the observational approaches introduced here and discussed in detail in Chapters 8-10.

7.2 A UNIFIED APPROACH TO STUDY DESIGN

Hernan (2005) has stressed that, when we attempt to find a causal association between an exposure and an outcome, we should think about the design of a field experimental to accomplish that. This approach is reinforced by Rubin (2007) who stresses that 'design trumps analysis' and all elements of the design are completed before seeing any outcome data. We concur with these views. There are a number of reasons to think about the details of a field experiment even if it is clear that such an experiment cannot be implemented. In any event, the 'thought experiment' can be accomplished and should specify the key elements of study group selection, assignment to exposure, procedures for follow-up, and detecting the outcome. The important part of the 'thought experiment' is the fact that formal randomisation would ensure 'exchangeability' which, for our purposes here, can be taken to mean that the groups being compared are so similar that it does not matter which group was assigned to exposure and which group to non-exposure. Confounding would not be an issue. However, if the causal association must be pursued through an observational study, the exposed and non-exposed groups might differ in ways that could bias the outcome (*ie* confounding would be present—see Chapter 13). Hence, the design challenge through subject exclusion, selection criteria and control of confounding is to prevent that bias. Rubin (2007) provides examples of how a group of experts met and discussed these issues until all parties were in agreement that the process would achieve balance in the covariates between the exposed and non-exposed groups. And, the

key was that all of this was accomplished before anyone had seen the outcome data. Rubin formalises the process through propensity scores (these are the probability of exposure given the covariates available) in the exposed and non-exposed groups. Unless these are virtually equal in the 2 groups, then some degree of confounding is possible. We believe a third component of good study design is to use 'forward projection' (*ie* critical appraisal techniques) (Elwood, 2002). In this process, after completing the initial design, we project ourselves forward to the presentation of our study results under 3 different scenarios—namely, a positive finding, a negative finding, and a no-effect finding. For each one we must then defend the proposed design and through this process help identify potential weaknesses in the design. The main features of this process are shown in Table 7.2. The formal implementation of these 3 strategies, in conjunction with the key elements of study design that should be reported (Section 7.4.2 and Table 7.3), should help ensure an efficient and appropriate study design for uncovering causal associations.

Table 7.2 A scheme for critical assessment of study design

Critical appraisal of causation	Application to study design
A. Description of the evidence	
1. What was the exposure or intervention?	1. Clear definitions required in study design
2. What was the outcome?	2. Define key outcome and document it
3. What was the study design?	3. Consider alternatives; justify choice
4. What was the study population?	4. Define carefully; consider alternatives; justify choice
5. What was the main result?	5. Consider the interpretation of positive, negative, and neutral results
B. Internal validity—consideration of non-causal explanations	
6. Are the results likely to be affected by observation bias?	6. Consider methods to avoid bias,
7. Are the results likely to be affected by confounding?	7. Identify potential confounders and decide on ways of controlling
8. Are the results likely to be affected by chance variation?	8. Assess power and sample size
C. Internal validity—consideration of positive features of causation	
9. Is there a correct time relationship?	9. When is start of exposure and of outcome? Consider latent effects.
10. Is the relationship strong?	10. What strength is likely or important?
11. Is there a dose-response relationship?	11. How will dose-response be shown?
12. Are the results consistent within the study?	12. What consistencies or specificity would be useful to test or amplify the hypothesis?
13. Can the study results be applied to the source population?	13. Consider response rates and how to assess representativeness, as well as eligibility and exclusion criteria?
D. External validity	
15. Can the study results be applied to the target or results be applicable to them?	15. What groups are relevant, and will the other relevant populations?

From the perspective of drawing causal inferences, experimental studies usually are referred to as the gold standard with observational studies being of somewhat lower validity (you might refer again to the section on causality in Chapter 1). Non-randomised intervention studies (*ie* where the researcher decides, without randomisation, which study subjects become 'exposed' or 'treated'—these are sometimes called quasi-experiments) are ranked above most observational study designs for causal inference purposes (Grimes & Schulz, 2002a). The issue of random allocation of subjects to interventions is discussed in Section 4 of Chapter 11. Within observational studies, cross-sectional studies are of lower 'causal inference' rank than other study designs because they measure prevalence not incidence, and because of the inability to refute reverse-causation (*ie* determine which came first, the exposure or the outcome—see Section 7.5.2) for non-permanent exposures. Hence, when possible, other study designs should be used. Case-control and cohort studies are better for making valid causal inferences than cross-sectional studies because of the longitudinal nature of their designs and their use of incidence data, both of which should allow refutation of reverse-causation. Cohort studies are generally considered superior to case-control studies in this regard.

7.3 DESCRIPTIVE STUDIES

Although descriptive studies are not designed to evaluate any associations between exposures and outcomes of interest, the observations made in a descriptive study can form the basis of hypotheses which then are further investigated in analytic studies (Grimes & Schulz, 2002b). Three forms of descriptive studies are case reports, case series reports and surveys.

Case reports generally describe a rare condition or an unusual manifestation of a more common disease. Often, case reports are based on only one or a very few cases, and the fact that they are based on unusual cases might limit their relevance to typical 'real-world' conditions. However, these unusual observations also can help researchers generate useful hypotheses to be investigated in future observational studies. For example, Schmidt *et al* (2008), published a case report on a African Grey Parrot that was presented to a veterinary clinic in Germany. The parrot, originally captured in Zaire, in 1996, had sublingual nodules which were limiting its ability to eat. In addition, lesions on the bird's legs had a 'yellow cheesy content'. The parrot's owner had been feeding the bird with pre-chewed food for some years. It later turned out that the owner had been treated for tuberculosis. Post-mortem findings on the parrot established that it also had *M. tuberculosis* of the same strain as the owner. This case is a reminder, that any parrot with ocular, sinus, oral, or cutaneous nodular lesions should be considered as suspect for tuberculosis. In some case reports, the author(s) attempts to draw conclusions about the cause, the outcome or the relative merit of a therapy. However, these hypotheses are purely the author's conjecture as no data to support such a conclusion are available directly from a case report.

A case series report generally presents a description of the occurrence of, or usual clinical course of, the condition of interest in a group of subjects. Typically, the case series should document the who (*ie* the affected subjects), the when (*ie* the temporal aspects of the disease occurrence) and the where (*ie* the geographic aspects of disease occurrence). Case series reports also might provide valuable information about the prognosis of the condition, provided the cases described are representative of most cases in the population. For example, Bidwell *et al* (2007), reported on peri-operative fatalities associated with general anaesthesia at a private equine practise. Their records based on over 17,000 horses during a 4-year period, indicated that their mortality rate was lower than in many other clinics. Having a 'simple standard

anaesthetic protocol' was deemed to be a major reason for this. The deaths of greatest concern occurred in healthy young athletic horses, and elevated vagal tone was postulated as a risk factor. As this example shows, the features of the series might help a researcher posit hypotheses about causal or prognostic factors for the outcome in question, but the case series usually has limited data on these factors since only the characteristics of the cases are included and no explicit comparison group (*ie* a group of suitable non-cases) is present.

Descriptive surveys are proactive activities to estimate, with some specified precision, the frequency and distribution of selected outcomes in a defined population. In many cases, their principal objective is to provide data about the frequency and distribution of a disease in a specific population. For example, Karama *et al* (2008), reported on a survey of slaughter cattle in Ontario for verotoxin-producing *Escherichia coli* (VTEC). Cattle slaughtered at a large federal plant in Canada represented the source population. Rectal fecal samples from 25 animals per visit (every 25th animal was sampled) over a 20-week period from April to October 2004 were obtained for culture. A VTEC-prevalence of 10.2% was found; no 0157 isolates were detected. This study exemplifies the 2 main design issues which need to be considered in designing a survey; namely, the sampling protocol (see Chapter 2) and the design of the data-collection instrument (see Chapter 3). Speybroeck *et al* (2003) have described the appropriate analysis of surveys bearing in mind the study design. If the survey is designed to collect information about both an outcome of interest and potential exposures of interest, it then becomes a cross-sectional analytic study (Section 7.5) and as such can be used to evaluate associations between exposures and outcomes.

7.4 OBSERVATIONAL STUDIES

Observational studies (a subgroup of analytic or explanatory studies) have an explicit formal contrast as part of their design. They differ from descriptive studies in that the comparison of 2 (or more) groups is the central foundation of their design. As noted above, observational studies differ from experiments (*eg* controlled trials) in that the researcher has no control over the allocation of the study subjects to the groups being compared.

7.4.1 Prospective versus retrospective designs

Observational studies can also be classified as prospective or retrospective. Although the usage of these terms in the epidemiological literature is not consistent, in prospective studies, the disease or other outcome of interest has not occurred at the time the study starts. The design of prospective studies needs to include information-gathering techniques so that all the necessary data are recorded as part of the study itself, or the study could build on existing data sources, supplementing these data as necessary. In retrospective studies, both the exposure and the outcome have occurred when the study begins. Typically retrospective studies rely on pre-recorded data from one or more secondary sources. Although the availability of pre-recorded data is a major advantage, often the quality and scope of these data may be limitations of this approach. Here again, selecting a suitable study design can maximise the information gained from the data available.

The choices of observational analytic study design have traditionally been among 3 main approaches which we will now introduce. In a cross-sectional study (Section 7.5) a sample of study subjects is obtained from the study population and the prevalence of both disease and

exposure are determined at the time of study subject selection. Cross-sectional studies have been described as non-directional, however we prefer to denote them as retrospective. In a cohort study (Chapter 8), a single sample of study subjects from a source population with heterogeneous exposure, or a sample of 2 or more groups of study subjects defined by known exposure status, is obtained, and the incidence of the outcome in the follow-up study period determined. While these are often prospective in nature, in select cases, with sufficient information recorded in routine data banks, they might be carried out retrospectively. In a case-control study (Chapter 9), subjects with the outcome of interest (usually a disease) are identified and the exposure history of these case subjects is contrasted with the exposure history of a sample (often randomly selected from a defined source) of non-case subjects (also called the control subjects). These studies usually are carried out retrospectively using a data bank of cases that have already occurred. A case-control study can be performed prospectively, by enrolling cases in the study as they occur after the study begins. Because subjects are selected based on their outcome status, they differ from cohort studies, in which subjects are selected based on exposure status. Variations on these themes are described under the heading of hybrid study designs in Chapter 10.

7.4.2 Reporting of observational studies

von Elm *et al* (2007), note that 'research should be reported transparently so that readers can follow what was planned, what was done, what was found, and what conclusions were drawn. The credibility of research depends on a critical assessment of the strengths and weaknesses in study design, conduct, and analysis.' They also note that in published observational research important information is often missing or unclear. Thus, in 2004, a network of methodologists, researchers, and journal editors was established to develop recommendations to develop the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. Here, we reproduce the checklist of 22 items (see Appendix 1) they consider to be essential for good reporting of observational studies. We shall refer to these often throughout this and chapters following on study design. You are also referred to the STROBE website under www.epidem.com for details and ongoing discussion on this topic.

7.5 CROSS-SECTIONAL STUDIES

The defining feature of a cross-sectional study is that it is an observational study whose outcome frequency measure is prevalence (*ie* it is based on the number of cases that already exist in the source population when the study begins). The basis of the cross-sectional design is that a sample, or census, of subjects is obtained from the target population and the presence or absence of the outcome is ascertained at that point. Usually, the current or pre-existing exposure status is noted at the first contact with the study subjects after their selection. Despite their disadvantages for supporting causal inferences, cross-sectional studies are one of the most frequently chosen study designs in veterinary epidemiology. Perhaps because the basic structure is straightforward, there is very little written concerning details of design, at least relative to what is written regarding other designs such as cohort and case-control studies.

7.5.1 Obtaining the study group

If the researcher wants to make inferences about the frequency of the outcome or the prevalence

of exposure in a **target population**, then the study subjects should be obtained by a formal random sampling procedure (see Example 7.1). The **source population** is that listing (real or implied) of potential study subjects from which the members of the study group were obtained. Often, not all members of the target population are contained within the source population as the latter may have constraints such as 'having computerised records of health and production' or the animals of interest are 'clients of selected veterinary practises or teaching hospitals'. Often, judgement is required to assess if such constraints will seriously limit causal inferences. The **study group** is that set of subjects who agree to take part in the study. The exact random process used to select study subjects can vary but could include stratified, cluster or multistage sampling approaches as discussed in Chapter 2. For example, Guerin *et al* (2007), investigated potential risk factors for the presence of *Campylobacter* in batches of broilers at, or just prior to, their time of slaughter in Iceland. Almost all broiler farms in Iceland were included in the study and sampling was not used. In contrast, Schouten *et al* (2005), provide an example of a herd (flock) level cross-sectional study based on the Dutch national monitoring program for *E. coli* O157, in veal, poultry and swine farms. This was a one-time sample of randomly selected farms in The Netherlands to determine the herd prevalence and herd risk factors for *E. coli* O157. Nasinyama *et al* (2000) describe the use of both purposive and random selection of study subjects to identify risk factors for acute diarrhoea in humans in Uganda.

If the primary objective does not entail estimating population parameters, but is limited to the evaluation of associations between the exposure(s) and outcome(s) of interest, a non-random sample of study subjects is often obtained purposively. For example, Williams *et al* (2004) provide an example of an individual-level cross-sectional study. They examined 2,000 dogs from a number of purposively selected 'populations' for the presence of cataract and related this to the age and breeds of dog at the time of examination. Some researchers decry this non-random approach because the design is open to considerable selection bias. Although this selection bias limits the external validity of the study (ability to extrapolate results beyond the source population) the design of the study should ensure that the internal validity (ability to extrapolate results from the study group to the source population) remains good.

As noted, studies using a one-time sampling of study subjects for the presence of a disease, microorganism, or level of toxin are using 'prevalence' as an outcome measure (*eg* (Arthur *et al*, 2007; Minihan *et al*, 2004; Woerner *et al*, 2006)) although with repeated samplings of the same subjects over time, the incidence of new infections can be established. Sometimes the prevalence of the outcome might change with the passage of time (*eg* with age or season).

Example 7.1 A cross-sectional survey of humans to assess the role of pets as risk factors for respiratory disease

During August 1 to 20, 2002, Suzuki *et al* (2005) conducted a cross-sectional survey in Saitama Prefecture, Japan, which has a total population of approximately 7 million. One hundred areas were selected from 5 administration districts in proportion to the population size. From each area, 30 households were chosen: 15 in detached houses and 15 in other types of dwelling, such as apartment houses. A questionnaire dealing with household conditions, including pet keeping was used to obtain information on potential risk factors for respiratory disease. The health questionnaire asked "whether the respondents had experienced respiratory symptoms (wheezing and/or breathlessness and/or bad cough) in the last 12 months." The response rate was 78.9%. There was no association between dog or cat ownership and respiratory symptoms, but keeping hamsters was positively associated with respiratory symptoms

Typically these studies model the prevalence at each sampling as the outcome (Berge *et al*, 2006; Trotz-Williams *et al*, 2007).

7.5.2 Assessing exposure

Usually, the exposure status is obtained at the time of study subject selection; however, not infrequently, an additional and more detailed history of prior exposure may be sought after the subjects are selected. Because the outcome measure is prevalence, it is sometimes difficult to know the appropriate time frame in which the exposure might cause the outcome. For example, the time at which poultry became infected with *Campylobacter* in the study by Guerin *et al* (2007) was unknown, but assumed to be close to the time of slaughter; thus, exposures in the last few weeks of life received the major attention as possible risk factors. When researchers try to reconstruct the exposure history of the selected study subjects, for time-varying exposures, the approach has been denoted as a cross-sectional cohort study and the strengths and weaknesses of this approach have been discussed by Hudson *et al* (2005). Some researchers (Backer *et al*, 2001) have sampled the study population based on the known exposure status of the study subjects (*ie* living, or not living, near contaminated toxic sites) and then measured prevalence of the 'disease' outcome(s). Because the sampling is based on exposure status and the outcome is a prevalence measure, we might be tempted to refer to this as a prevalence-based cohort study. However, given that the outcome is measured by prevalence we would prefer to denote this as a cross-sectional study.

Although the cross-sectional study design can support the investigation of a variety of potential causal factors and a number of outcomes, usually one primary outcome of interest is chosen and a set of potential causal factors are selected for investigation of their association with that outcome. A potential drawback to this study design is that the search for potential causes may not be very focused and thus a lot of data-mining for statistically significant associations may result.

7.5.3 Sample-size aspects

The risk-based approach to sample size estimation shown in Chapter 2 is often sufficient for planning purposes when one major outcome is of interest. If the association between exposure and outcome in specific subsets of the source population is the principal goal then the researcher should ensure that adequate numbers of study subjects are available within these subgroups to provide reasonable power for assessing the hypotheses.

7.5.4 Ensuring that the exposed and non-exposed study subjects are comparable

The 2 main approaches used to prevent bias, from factors associated with the outcome and whose distribution differs between the exposure groups (*ie* confounders), are restricted sampling and analytic (statistical) control. As an example, through the use of restricted sampling, if all study subjects are of the same age and sex (*eg* 2-year-old cows), then age and sex cannot bias the observed association between exposure and the outcome. (Dhand *et al*, 2007) (Example 7.2) used a multivariable regression technique (see Chapters 13 and 16) to control for the age and sex of the study group of sheep on each farm before looking at other potential risk factors for the outcome.

Example 7.2 A cross-sectional study of the extent of infection with *Mycobacterium avium tuberculosis* in sheep flocks

Dhand *et al* (2007) described the source population and study group, the latter being a subset of *Mycobacterium avium tuberculosis (Map*) infected flocks in Australia. The flock was the unit of concern, but only a subset of all sheep in the flock were included in the study. Thus, a sample of 210 sheep, of a specific age and sex, was enrolled at the time of fecal sample collection, and culture results for 7 pools of 30 sheep were used to classify each group as a low (<2%), medium (2–10%) or high (>10%) prevalence group. A questionnaire was administered in a face-to-face interview with the owner or manager about potential risk factors for each enrolled flock.

7.5.5 Analysis

The main comparison in a cross-sectional study is between the prevalence of the outcome in the exposed subjects and the prevalence in the non-exposed subjects and the natural measure of association is the prevalence risk ratio (see Chapter 4). However, since many researchers use logistic regression for multivariable modelling of their cross-sectional data, odds ratios are the usual measure of association. As noted in the subsequent section on cohort studies (see Chapter 8), a Poisson model with robust variance, or a log binomial model could be used to obtain direct estimates of the prevalence risk ratio (Barros & Hirakata, 2003; Schiaffino *et al*, 2003).

7.5.6 Inferential limitations of cross-sectional studies

By its nature, a cross-sectional study design measures prevalence which is a function of both incidence and duration of the disease. Consequently, it is often difficult to disentangle factors associated with persistence of the outcome (or survival of study subjects with the outcome) from factors associated with developing the outcome in the first instance (*ie* becoming a new case). Animals with a factor which contributes to their survival once they have the disease of interest will be included in a cross-sectional study more frequently than animals without the factor (by virtue of the fact that the factor keeps them alive longer). Thus, the factor may be associated with the disease and the investigators might conclude that it is a cause of the disease, when in reality the factor affects the duration but not the occurrence of the disease.

Cross-sectional studies are best suited for time-invariant exposures such as breed, sex, or permanent management factors. In these instances, the investigator can be certain that the exposure preceded the outcome (one of the fundamental criteria for establishing causation) although it does not circumvent the problem of the factor affecting only the duration of the outcome. When the exposure factors are not time-invariant, it is often very difficult to differentiate cause and effect because of the so-called reverse-causation problem. For example, if one is studying the relationship between a management factor (*eg* hoof-trimming) and the frequency of hoof disorders, if the association is positive, one cannot differentiate between herds that initiated hoof-trimming in response to a problem with hoof disorders from those that developed the disease (*ie* had foot disorders diagnosed) because of the management factor (hoof-trimming) (Cramer *et al*, 2008). This issue arose in a recent publication on a study designed to identify risk factors for mastitis; udder health programs were deemed to be risk factors (Sampimon *et al*, 2009). The same difficulty arose in studies of pet ownership and existing blood pressure in the pet's owner (see Example 7.3); which is the cause and which the effect? The more changeable the exposure, the worse this issue becomes. If the factor truly is

Example 7.3 Two cross-sectional studies of pet ownership and the owners' blood pressure

Both studies used a continuous measure of outcome (blood pressure) rather than a dichotomous (diseased/non-diseased response). Wright *et al* (2007), followed 1,137 people who had answered a questionnaire on pet ownership in 1991-92 and determined their blood pressure (elevated blood pressure was the outcome) at some point prior to 1997. Unconditional analyses indicated an association of pet ownership with blood pressure, but after adjustment for covariates (chiefly age, but also a number of other factors some of which could be intervening variables) no association was observed.

Parslow & Jorm (2003) conducted a cross-sectional study of over 5,000 randomly selected people from 2 areas in New South Wales, Australia. The history of pet ownership was obtained by interview and 2 readings of blood pressure were taken, their average being the outcome measure of interest. Age, sex and education were controlled when assessing the association of pet ownership and blood pressure; interactions between these covariates and pet ownership were also evaluated. Those with pets had significantly higher diastolic blood pressure than those who did not own pets.

Both studies are limited by the potential reverse causation bias (*eg* that people with higher blood pressure got pets to help them lower their blood pressure).

preventive and often implemented when the disease has occurred, or after it has reached a threshold frequency, the positive and negative associations could cancel each other leaving the factor appearing to be independent of the outcome. Many researchers attempt to circumvent this problem by trying to ascertain when the exposure to the potential causal factors occurred; however, unless the timing of disease occurrence is known, with some certainty, the problem is not fully resolvable.

7.6 REPEATED CROSS-SECTIONAL VERSUS COHORT STUDIES

Sometimes it is desirable to follow a population over time and here one must consider whether to use repeated cross-sectional samplings of the population or a longitudinal study of the initial study subjects (this is called a cohort approach but note that in this instance, we are following a study group some of which have the outcome event at the start of the follow-up) (Diehr *et al*, 1995). The time period between samplings can be short (*eg* as in following a group of cattle from the feedlot to the slaughter house to ascertain contamination levels with micro-organisms such as *E. coli*) or longer, such as annual surveys. Briefly, if the objective is to follow specific individuals over time then following the same cohort of study subjects (see Chapter 8) is preferable. Because of the repeated observations on the same individuals, the analysis may need to adjust for the within-subject correlation (see Chapters 20-24). With this approach, as the length of the study period increases, the individuals remaining in the study will become increasingly different from the existing population at that time (*eg* they will be much older and in many instances represent a highly selected surviving subgroup of those originally studied). If the research objective relates more to the events and associations within the full population at different periods of time, then a series of repeated cross-sectional studies might be the preferred approach. In this design, depending on the sampling fraction, most of the study subjects selected at different points in time will not have been included in prior samples. However, with larger sampling fractions, sufficient subjects might be selected in 2 or more samplings to allow within study-subject comparisons (*eg* occurrence of new disease) over time.

APPENDIX 1

Table 7.3 The STROBE—Checklist of items that should be addressed in reports of observational studies

TITLE and ABSTRACT
1 (a) Indicate the study's design with a commonly used term in the title or the abstract
(b) Provide in the abstract an informative and balanced summary of what was done and what was found
INTRODUCTION
2 Explain the scientific background and rationale for the investigation being reported
3 State specific objectives, including any pre-specified hypotheses
METHODS
4 Present key elements of study design early in the paper
5 Describe the setting, locations, and dates, including periods of recruitment, exposure, follow-up, and data collection
6 (a) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
(b) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed
Case-control study—For matched studies, give matching criteria and the number of controls per case
7 Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
8 For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
9 Describe any efforts to address potential sources of bias
10 Explain how the study size was arrived at
11 Explain how quantitative variables were handled in the analyses. Describe which groupings were chosen, and why
12 (a) Describe all statistical methods, including those used to control for confounding
(b) Describe any methods used to examine subgroups and interactions
(c) Explain how missing data were addressed
(d) *Cohort study*—If applicable, explain how loss to follow-up was addressed
Case-control study—If applicable, explain how matching of cases and controls was addressed
Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
(e) Describe any sensitivity analyses

(continued next page)

Table 7.3 (continued)

RESULTS
13 (a) Report the numbers of individuals at each stage of the study—eg, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
(b) Give reasons for non-participation at each stage
(c) Consider use of a flow diagram
14 (a) Give characteristics of study participants (eg, demographic, clinical, social) and information on exposures and potential confounders
(b) Indicate the number of participants with missing data for each variable of interest
(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
15 *Cohort study*—Report numbers of outcome events or summary measures over time
Case-control study—Report numbers in each exposure category, or summary measures of exposure
Cross-sectional study—Report numbers of outcome events or summary measures
16 (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence intervals). Make clear which confounders were adjusted for and why they were included
(b) Report category boundaries when continuous variables were categorised
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
17 Report other analyses done—eg, analyses of subgroups and interactions, and sensitivity analyses
DISCUSSION
18 Summarise key results with reference to study objectives
19 Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
20 Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
21 Discuss the generalisability (external validity) of the study results
22 Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

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