Veterinary Epidemiology: Principles and Methods

Part 2: Studying Disease in Animal Populations

Chapter 6: Surveys and Analytic Observational Studies

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CHAPTER 6

Surveys and Analytic Observational Studies

All epidemiologic studies involve data collection, manipulation, and analysis. In general, the more organized these functions are, the easier the task will be. Also, appropriate data collection can improve the accuracy and precision of the data. Thus, the basic considerations necessary for the design of observational studies are described in this chapter. A discussion of the uses and limitations of animal disease surveillance is provided in Chapter 11, and applications of analytic studies are presented in Chapter 12.

6.1 Principles of Surveys and Data Collection

The nature of the study and the setting in which the data will be collected will influence the design and structure of the data recording form or questionnaire (Woodward et al. 1982). At the very least, all studies require a well-planned data collection form. Simple forms will suffice if the investigator is collecting and recording data from only a few sources (such as medical history sheets) or for recording the results of field experiments. More care and planning are required when the data to be collected are complex or the investigator is not in direct control of data (e.g., in a survey involving personal interviews or in a mailed questionnaire). For reasons described subsequently, the investigator may not wish to specify the actual objective of the study on the survey form; nonetheless objectives should be stated explicitly as part of the investigator's plan of research.

6.1.1 Title of the Study

Appearing at the top of the survey form, the title should be clear and sufficiently detailed to inform collaborators of the general purpose of the survey. Consider the following two titles as examples: "Sow Survey" versus "Diseases of Sows During Pregnancy." In most cases, the latter title would be preferred. It is not necessary however to provide specific details in the

title. In fact, sometimes it is desirable to keep the collaborators blind as to the exact purpose of the survey in order to prevent biased answers. For example, questions in the survey might relate to a number of diseases as well as management or housing factors, although one syndrome (say metritis, mastitis, or agalactia) is the primary objective of the study. If the survey form is mailed to collaborators, a brief cover letter should be included.

6.1.2 Questions

Frequently the most important step in solving a problem is knowing what question(s) to ask. Questions should be clearly worded, straightforward, and necessary (Woodward and Chambers 1980). Initially, it is useful to list all of the factors about which information is required; then structure the questions so that the answer(s) to each question provides the appropriate data. If ventilation is of interest, the investigator must consider what specific information about ventilation is required. The presence or absence of fans would provide some information, the number and sizes of fans other information, and the method of controlling the fans still other information. At least one question would be required to obtain data on each of these dimensions that describe the ventilation.

Another useful approach to identify needed questions is to construct in advance the tables necessary to meet the study objectives, then cross-check these with the data that will be obtained from the recording form. This will help ensure that the appropriate questions are asked, and that all questions asked are required.

Often in preliminary studies where questions concern a broad range of factors (so-called "data snooping surveys") it is useful to record in advance the interpretation to be placed on all of the associations that may be observed. That is, should the number of fans be positively or negatively correlated with the rate of disease? Why? Should the rate of disease differ depending on whether automatic or manual switches are used to control the fans? If so, how should it differ? The rationale behind this exercise is the more questions asked the greater the likelihood of finding at least one factor significantly associated with the disease. Most associations between unrefined factors and disease are explainable after the fact; yet there must be some explanations that are, a priori, more sensible than others. For example, one might initially hypothesize an inverse relationship (a negative correlation or an odds ratio of less than one) between the presence of fans and the level of respiratory disease. Presumably such a hypothesis relates to the maintenance of acceptable temperature and humidity levels, as well as the removal of dust and microorganisms from the air. However, suppose a positive association is observed. How does one interpret it? In general, it is preferable not to ignore observed associations, but associations running

counter to the initial explanation should be viewed with some skepticism until they are validated.

6.1.3 Sequence of Questions

Questions should be grouped according to subject matter or another logical basis such as the temporal relationship of events. This will help orient the collaborator's mind to the task at hand. General surveys might be structured on the basis of major factor categories such as housing, ration, management, etc. On the other hand, if the survey is concerned with events related to the neonatal period or to the period after arrival in the herd, flock, or feedlot, sequencing the questions on a temporal basis might be more useful.

6.1.4 Format of the Record Form

The layout of the recording form should assist the analysis and/or computer entry of data. Excess transcription of data should be avoided; each time a number is written down the probability of introducing an error increases. A useful format guideline is to keep the answers in an obvious column, usually at the extreme right side of the page. Also, to ease data entry it is useful to record the column number from the computer file next to the datum when using fixed field data entry. In other cases, the question number can specify the column where the datum is to be located in the file. If a recording form contains a lot of data that will not be analyzed (at least initially), the data to be entered may be highlighted with special colored pens. Although recent advances in interactive computer programs reduce data entry problems, these suggestions will be useful nonetheless.

6.1.5 Framing the Questions

Asking questions correctly is as much an art as it is a science (Woodward and Chambers 1980). Nonetheless, certain principles should be followed. Avoid asking leading questions; the question should begin with "Do you," not "You do." Make sure there is an obvious answer to each question, usually by providing a list of acceptable answers. In general, open-ended questions should be avoided. For example, the question "Ventilation system?" is too vague. It could be interpreted as requiring a yes-no answer for the presence or absence of a ventilating system, a judgment of the system's adequacy, a description of the fans, inlets, etc., or a host of other interpretations.

The terminology used in the question should be appropriate for the collaborators. For example, one probably should not ask a dairy farmer, Did the cow abort? but rather, Was the calf born dead? and How many months was the cow pregnant? Besides providing more detailed information, these two questions avoid confusion about the meaning of the term

abortion. Usually, animal owners may be questioned about clinical entities (such as scours or coughing) but not about entities classified on the basis of pathologic criteria (such as enteritis or pneumonia).

Some questions will have a set of mutually exclusive and exhaustive categories of answers (i.e., there is only one acceptable answer to a question and all possible answers are included). For example, in specifying "breed," each animal must fit into one and only one category. Thus all possible breeds should be specified, or the more common breeds might be listed with a final category of "other breed." If more than one answer is acceptable (e.g., an Angus-Hereford cross), nonexclusive categories are required. Other examples of nonexclusive categories relate to questions about ration content or the signs of disease. These are nonexclusive because the ration usually has more than one component, and there is usually more than one sign of disease. Although nonexclusive categories simplify the design of the recording form, they present problems in the analytic phase because of the potentially large number of combinations of answers.

A partial solution to these problems is that it may be sufficient to collect data only on the major ration component(s) or the major presenting sign(s). In other instances, a set of nonexclusive answers can be made exclusive (e.g., by asking Is the animal coughing? or Is the animal eating normally?). Another way of circumventing this is to list all possible combinations of categories (although this is usually not advisable because the list becomes too long). In the latter instance one can assign a numeric code to each possible single answer in such a manner that the sum of all possible answers produces a unique number, representing each particular combination of individual factors. For example, if there are five possible breeds, they could be coded 1, 2, 4, 8, and 16. Crossbred animals may be identified by using the sum of the numbers denoting the appropriate breeds. If an animal is a cross between the first and third breed listed, it would be coded as 5. The latter is more useful when cross-tabulation procedures will be used for analysis than when other methods such as linear regression are planned. Thus, each situation should be assessed individually.

When possible, it is desirable to record the answer as a continuous variable (e.g., the actual age, weight, titer). Grouping can be used if necessary later on. Most computer programs allow the specification of category limits, allowing a more powerful and flexible approach to the analysis than initially using categories such as $2 < 4$, $4 < 6$, and $6 < 8$. Unless it is desired to use a free-field format, when continuous variables are recorded they should be right justified. In a two column answer for age, a 9-year-old should be recorded as –9, or 09, not 9–, since the latter may be read as 90 years old. (A decimal placing could be specified, but this gives an upper limit of 9.9 years on the age if the field has only two columns.)

With numeric codes or answers, missing data must be differentiated

from no answer or "unknown." If there can be no answer, the column may be left blank, but if an answer should be given and is not available, a missing value code that will not be confused with valid answers should be used (e.g., 99 or −9 might be used to code for missing age values).

When a long list of possible answers is available, studies have shown a tendency for collaborators to select answers placed early in the list. Two solutions are offered. First, keep the list of answers short. Second, one may use two or three forms of the same questionnaire, and the order of the possible answers can be randomized within each form.

6.1.6 Editing the Data

All recording forms should be edited manually before and/or during computer data entry or manual analyses. Initially, make sure that all required questions are answered and that no inappropriate answers are recorded. This procedure is often necessary when a hierarchy of questions is used. For example, "if the answer is 'yes,' answer the specified subquestions; if the answer is 'no,' proceed to the next major question." (The question number may be specified.) Thus, manual editing should ensure that all appropriate subquestions are answered, and it should also detect any inappropriate answers (e.g., the number of fans may have been recorded although the farmer had stated that none of them was operative).

In large surveys, computer assisted editing can enhance data validity. For example, programs can be devised to check that a cow name and number are valid, that the animal's reported age is consistent with the recorded birth date, that the event specified is biologically feasible, etc. That is, if the cow is recorded pregnant, a diagnosis of metritis is not feasible unless the event "abortion" or "calving" was specified. Computer editing can be expensive however, and judgment is required in the extent of its use. It should not be performed automatically in all cases. The setting in which data entry will occur and the likelihood of entering incorrect data should be considered prior to instituting computer assisted editing. In many cases no computerized editing is necessary; in others, it should be an essential component of data entry.

6.1.7 Pretesting the Survey

Few people can design a perfect survey form in one attempt. Rather, iterative restructuring and rethinking of the questions and layout are required. A guideline about the time required to produce a useful survey is to make an initial careful estimate and then multiply by four or five.

Although framing the questions is an art, the evaluation of the survey during the pretest should be as scientifically rigorous as possible. Initially, one should check to see if the survey is too long, too detailed, or unclear. Then, some attempt should be made to establish the precision (reproduci-

bility) of the survey. This may be done by asking the same question twice during the same interview, or at a different interview. In a mail survey, attempts to elicit the same answer with two different but similar questions may provide evidence on reproducibility and validity of responses.

Note also that each question has its own sensitivity, specificity, and predictive value. Suppose the factor one wishes to obtain information on is the use of a specific vaccine. The sensitivity of the question, Do you use the vaccine? is the proportion of those who actually use the vaccine that answer affirmatively. The specificity is the proportion of those who don't use the vaccine that answer negatively. The predictive value, on the other hand, is the proportion of those who answer affirmatively who actually use the vaccine.

In order to assess the sensitivity and specificity of a question, an independent means of establishing the true state of nature is required. This may require investigative assessments (e.g., a search of the drug pail, inspection of the housing, or examining the feed bunks). One requires both care and tact in these assessments so as not to offend the collaborator. It is useful to remember that all memory (including our own) is often faulty, more frequently by omission than by deliberate action. Although it is best to evaluate a survey keeping the collaborators blind to the evaluation, in many instances it may be necessary to inform the respondents of the pretest.

6.1.8 Analysis

The details of the analysis will depend on the type of data collected as well as on the objectives of the survey. Nonetheless, one should not rush into detailed analyses before inspecting the data thoroughly and performing several simple summaries. This principle should be followed no matter how analytically adept the investigator.

When performing an analysis on a large data file, use only a portion of the data set initially. This will minimize costs if errors exist in the data set or in structuring the analytic program. Also at this stage, it is important to verify that the appropriate number of cases is present for each analysis or subanalysis.

6.1.9 Final Thoughts

Choose a time for data collection convenient for the collaborators. Sometimes this is not possible (e.g., if data relating to events in the period after arrival of calves in a feedlot are required, this is always a busy time). Be aware that the timing of the survey can affect the results. For example, if dairy farmers from California were asked to rank disease in order of importance, calf losses would likely be ranked as important if the interview was in the winter or summer, but less important if the interview was in the

early summer or fall. This is due to the seasonal nature of calf losses, not its overall importance.

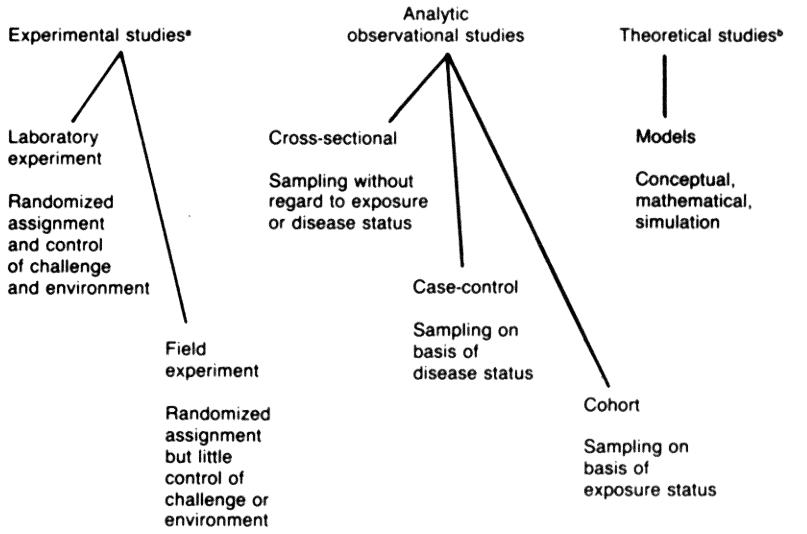
To ensure consistency, decide who should answer the questions (i.e., should it be the owner, the person who feeds the animals, or the farm manager). Make sure all personnel involved know what is expected of them. Even if only two people administer the study, regular meetings to rehearse the data collection strategies, clear up problem areas, or to reinforce procedures will prove useful.

Finally, every effort should be made to obtain a high level of cooperation. Mail surveys often produce only a 40–50% response rate, whereas more than 80% cooperation is often obtained in personal interview surveys. Unfortunately, the results of a survey with a return rate of less than 70 or 80% are suspect. The reason is that the collaborators are self-selected volunteers and could very well have different opinions, management styles, and levels of disease than those who refuse to collaborate. Thus the general strategy is to select a practical number of individuals for the study and attempt to obtain a high rate of collaboration, rather than selecting two or three times as many potential collaborators and using the results of the 30–40% who choose to volunteer. Strong associations are unlikely to be reversed if the cooperation rate is high; this may be shown by assuming the opposite association exists among all nonrespondents. All associations are suspect if the cooperation rate is low; hence, it should be noted that it is the proportion of prospective collaborators who cooperate, not the absolute number of cooperators, that is important in terms of obtaining valid data.

An excellent critique of the methods used in national surveys of disease occurrence in animals is available (Leech 1971). The use of questionnaire data in smaller scale field studies has also been described (Selby et al. 1973, 1976; Ruppaner 1972; Ruppaner and Goodger 1979). It is particularly interesting to note that observers from different sectors of the industry may rank diseases quite differently in terms of their importance (Ruppaner 1972). An example of the use of mail questionnaire data and its validation are provided by Hutchings and Martin (1983).

6.2 Analytic Observational Studies

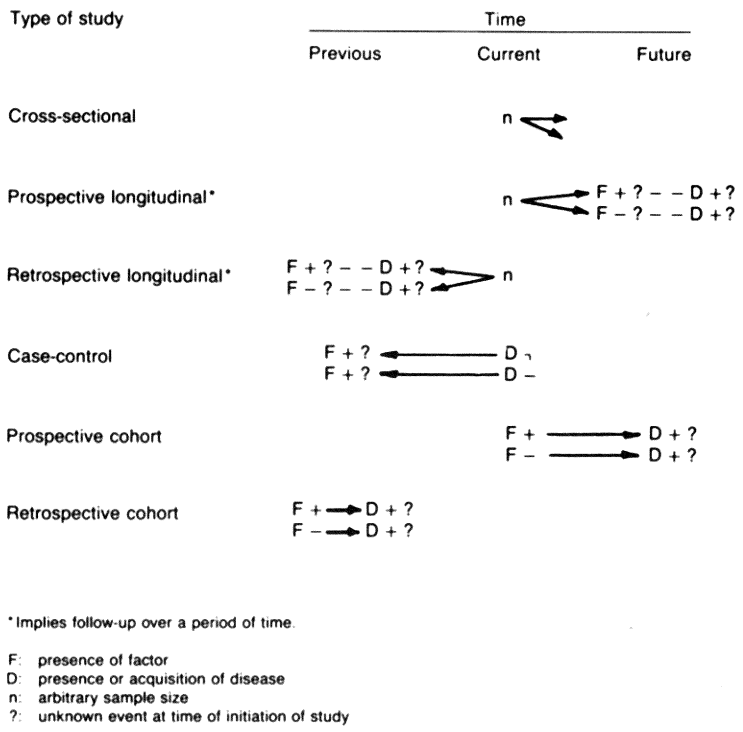
A general classification of the types of studies used to test hypotheses are shown in Figure 6.1. A more detailed description of analytic observational study methods is contained in Figure 6.2. General considerations regarding the selection of study type and the sampling schemes appropriate to each study type were explained in Chapter 2. The remainder of this chapter provides an outline of key items to be considered in the design and performance of each type of analytic observational study.



6.1. Types of studies to test hypotheses.

A chief advantage of analytic studies is that they are directed toward the species of concern in its natural environment. This greatly reduces the problems associated with extrapolating results from a particular study to the target population. It also allows the investigator to test a much broader range of hypotheses than would be possible under controlled experimental conditions. However, it is often necessary to place restrictions on the source and selection of animals, for practical limitations and in order to make the groups to be contrasted comparable, although these restrictions may reduce any investigator's ability to extrapolate results beyond the sample. As a specific example, it is important to concisely and clearly describe the criteria used to define the status of the sampled units with respect to the independent and dependent variables. Although the specific criteria might lead to the exclusion of a few sampling units, without them there would be an increased probability of misclassification of the sampling units, and the validity of the results might be questioned.

For the observational studies discussed here, it is assumed that exposure and disease status are expressed as dichotomous or binary variables. Hence the chi-square test may be used to analyze the relationship between the putative causal factor and disease. In veterinary medicine, since it is also extremely important to quantify the effect of disease on the level of



6.2. Types of analytic observational studies, according to sampling strategy and temporal events related to the factor and/or disease. F = presence of factor; D = presence or acquisition of disease; n = arbitrary sample size; and ? = unknown event at time of initiation of study.

production, many studies have disease status as the independent variable and level of production as the outcome or dependent variable. In this instance the outcome variable is continuous and the chi-square test is inappropriate (unless one divides production into categories). If the impact of production level on disease occurrence was being investigated, level of production would be the exposure variable and disease occurrence the outcome of interest. Here the independent variable is continuous, and again the chi-square test is inappropriate. Nonetheless, the general methodology of observational studies is easily transposed to the latter studies and the t-test (described in Chapter 5) is suitable for the preliminary analysis of data from studies of this type.

Throughout this chapter the term sampling unit is used rather than individual, because in many epidemiologic studies a group of animals (e.g., a herd or flock) is the sampling unit rather than the individual. Although this makes the grammar somewhat formal, the distinction between individ-

uals versus aggregates as sampling units is very important to note. Many reports fail to make this distinction, rendering their results of little or no value.

Finally, a current biologic problem will be used to give substance to the discussion of study types. Suppose the objective is to study the association between the presence of ureaplasma in the vagina and infertility in dairy cows. (It is assumed that ureaplasma can cause infertility; the objective here is to determine the extent to which ureaplasma and infertility are associated under field conditions.) Further assume that individual cows will be the sampling units, and that only 2 cows per farm are included in a study; this will prevent bias from farm-size related effects. Prior to performing the study, the method(s) and timing of culturing cows for ureaplasma would need to be decided and standardized, and infertility would need to be defined in a workable, concise manner. The actual definitions and procedures could differ depending on the type of analytic study selected, but these differences will be ignored for illustrative purposes.

6.3 Cross-Sectional Study Design

In the example, a cross-sectional study would require that a random sample of dairy cows be made (the sampling frame would need to be defined and a sampling method, probably multistage, selected), accompanied by an assessment of the current ureaplasma and infertility status of each cow. Subsequent to this, comparisons could be made between the prevalence of existing infertility in cows currently infected with ureaplasma and the prevalence of infertility in noninfected cows.

Technically, cross-sectional studies provide a snapshot of events at a particular time. The point of time may range from an instant ("at the time of sampling") to longer periods (such as "during the past year"), although all are treated as static, point-in-time events. For purposes of causal interpretations, cross-sectional studies are best suited to studying permanent factors (such as breed, sex, or blood-type), since such factors can not be altered by the passage of time or by the presence or absence of disease. When the independent variable is a nonpermanent factor (as in the ureaplasma example), one can never be sure whether the factor status is influencing disease occurrence or vice versa. That is, perhaps infertility allows ureaplasma to colonize and multiply in the vagina.

If random selection of sampling units is used and applied with adequate rigor, the key features relating to validity of cross-sectional study results are the accuracy of the data regarding the factor and disease status. Thus, criteria for classifying the sampling units as exposed and/or diseased should be clearly stated. In particular, one usually attempts to exclude potential false-positives when specifying these criteria. That is, if misclassi-

fication of sampling units may occur, it is better to have a few exposed (diseased) units classified as nonexposed (nondiseased) than to have nonexposed (nondiseased) units classified as exposed (diseased). This makes the study results more conservative, but gives credence to any observed differences in rates of disease according to exposure status.

If the information about the factor and disease status may be biased by knowledge of the reason for the study, collaborators need not be informed of the major objective of the study. For example, in a study to identify ration factors associated with the occurrence of left displaced abomasum in dairy cows, questions were asked relating to nonration factors as well as the occurrence of diseases other than displaced abomasum (Pearson 1978). It was hoped that this prevented the farmers from keying on the ration-displaced abomasum relationship and perhaps biasing the answers depending on their beliefs about the subject. Also, useful data to answer secondary objectives were obtained.

Sometimes the original sample is obtained by cross-sectional methods; then the sampling units are observed over a period of time, and changes in exposure and/or disease status are noted. These studies are known as longitudinal studies, combining the benefits of cohort study methods (the ability to determine the factor status prior to disease occurrence and thus obtain incidence data) with the benefits of cross-sectional sampling (the knowledge of the frequency of the factor and/or disease in the source population). Thus, the distinction between study types becomes blurred, particularly since longitudinal studies may be performed in a prospective or retrospective manner as described in Figure 6.2. Many studies reported in the veterinary literature are longitudinal in type, although most have used purposive or convenience samples rather than a true probability sample, reducing the ability to generalize beyond the sample data.

Questionnaire-based surveys, studies relating ancillary data to the results of immunologic, microbiologic, or toxicologic testing, and slaughterhouse surveys are common examples of cross-sectional studies. Examples of longitudinal studies include a California survey investigating pulmonary emphysema in cattle (see Table 2.6), a mail survey on factors associated with morbidity and mortality in feedlot calves (see Table 6.1), a retrospective study of diseases and productivity in dairy cattle (see Table 6.2), a prospective study of diseases and productivity in dairy cattle (see Table 6.3), and a study of respiratory disease in racing standardbred horses (see Table 6.4). (See 12.4.3 for other examples.)

6.4 Case-Control Study Design

In case-control studies, separate samples of units with (cases) and without (controls) the specified disease are selected. Then the relative fre-

Table 6.1. Summary of the effects and importance of CALFNO, ANTIMICROBIAL, and RUFCHANGE on mortality rates in feedlot calves

Factor(s)						
CALFNO	ANTI-MICROBIAL	RUFCHANGE	$P(F_i)$[a]	Mortality rate[b]	Relative[c] risk (RR)	PAR%[d]
+	+	+	.133	1.185	3.5	12.0
+	+	−	.031	2.039	6.0	5.6
+	−	+	.251	1.230	3.6	23.7
−	+	+	.046	1.990	5.9	8.1
+	−	−	.097	1.198	3.5	8.9
−	+	−	.046	0.751	2.2	2.0
−	−	+	.195	0.791	2.3	9.4
−	−	−	.200	0.330	1.0	0.0

[a]Proportion of farms treated in this manner.

[b]Mortality rates derived from the mean of $\log_{10}$ transformed rates.

[c]Mean mortality rates in each grouping divided by the rate of mortality in "small farm, no antimicrobial added to water on arrival and major roughage source not changed within four weeks" group. The relative risk of the latter group is arbitrarily set to "1."

[d]Population attributable risk % describes the percentage of all deaths that is attributable to each of the CALFNO-ANTIMICROBIAL-RUFCHANGE groups. This is calculated by the formula:

$$\frac{100p(F_i) \times [p(D+/F_i) - p(D+/F_0)]}{p(D+)}$$

Source: Hutchings and Martin 1983, with permission.

Notes: Where $p(D+)$ is the overall mortality rate and $p(D+/F_i)$ is the mortality rate in a specific grouping of factors; CALFNO was dichotomized into >155 per farm and ≤ 155 per farm; ANTIMICROBIAL indicated whether prophylactic antimicrobials were added to the water supply; and RUFCHANGE indicated whether or not the type of roughage was changed within one month of arrival.

Note the general increase in RR as the number of risk factors present increased. Also note that the importance (PAR%) is affected by the RR and the prevalence of the factors; hence two factor groupings can have nearly similar RRs, but quite different PAR%. Note that PAR% is called population attributable fraction (PAF) in this text.

quency of the factor in each of these groups is compared using the odds ratio. Often all units with the disease and an equal number of controls are selected. In the present example, all infertile cows in a defined area might be used as cases, and an equal number of fertile cows selected as controls. (Matching for herd, age, and level of production might be used to increase the comparability of these groups.) Each cow's current ureaplasma infection status would be determined, and the proportion of infertile cows infected with ureaplasma would be compared to the proportion of fertile cows infected with ureaplasma. If the rate of infection were higher in infertile cows, this would support but not prove the hypothesis of ureaplasma producing infertility.

Since a number of biases can affect the results of case-control studies, key items are the criteria and methods used in the selection of cases and

Table 6.2. Decomposition of estimated bivariate associations into direct, indirect, and common cause components (810 Holstein lactations; 20 ROP-herd health herds; 1970-1975)

Variables		Association				
		Causal			Correlation	
Independent	Dependent	Direct	Indirect	Spurious	Estimated	Observed
Retained placenta	Metritis	.47	.00	.00	.47	.47
Retained placenta	Cystic follicle	.00	.06	.02	.08	.09
Retained placenta	Luteal cyst	.11	.01	.00	.12	.12
Retained placenta	Calving interval	.00	.06	.04	.10	.13
Retained placenta	BCM	.00	.01	.01	.02	.02
Retained placenta	Days in milk	.00	.04	.05	.09	.14
Metritis	Cystic follicle	.12	.00	.01	.13	.13
Metritis	Luteal cyst	.00	.01	.06	.07	.03
Metritis	Calving interval	.12	.02	.01	.15	.15
Metritis	BCM	.00	.02	.01	.03	.03
Metritis	Days in milk	.00	.10	.02	.12	.15
Cystic follicle	Luteal cyst	.12	.00	.01	.13	.14
Cystic follicle	Calving interval	.14	.01	.03	.18	.18
Cystic follicle	BCM	.09	.02	.00	.11	.14
Cystic follicle	Days in milk	.00	.13	.04	.17	.16
Luteal cyst	Calving interval	.08	.00	.03	.11	.11
Luteal cyst	BCM	.00	.01	.01	.02	.08
Luteal cyst	Days in milk	.00	.06	.02	.08	.09

Source: Erb et al. 1981, with permission.

Note: Using path analysis (see 5.6.3), the observed correlations between two variables (left hand columns) were decomposed into direct and indirect causal effects and spurious (the result of confounding variables) effects.

controls, the comparability of cases and controls, and an accurate unbiased history of exposure to the factor of interest.

6.4.1 Selection of Cases and Controls

In addition to being clear and concise, the criteria required to be a case should be highly specific in order to exclude false-positive units. If by design, certain types of sampling units are to be excluded from the case group (e.g., cases with known causes other than the factor of interest), these units should be excluded from the study; they should not be included in the control group even if not diseased.

In most studies, lists of cases are obtained from one or more clinics or

Table 6.3. Average probabilities of a cow being culled in the first 150 days of lactation (without and with selected diseases) and the relative risk (RR) and population attributable fraction (PAF) associated with those diseases

Disease	Average probability of being culled	Estimated RR	Estimated PAF (%)
None	.012	1.0	0.0
Severe mastitis	.956	79.7	53.2
Milk fever–stage 3	.350	29.2	64.7
Foot-leg disease	.373	31.1	38.7
Teat injury	.425	35.4	19.4
Mild mastitis	.045	3.8	19.2
Respiratory disease	.107	8.9	8.0

Source: Dohoo and Martin 1984, with permission.

Note: The presence of each of the above diseases increased the risk (RR) of a cow being culled. The importance (PAF) of a disease in terms of its effect on the risk of culling is influenced by its RR and prevalence. More than 100% of culling is explained because the diseases were components of the same sufficient cause (see 5.6.2).

Table 6.4. Relationship between upper respiratory tract disease and anti-influenza (E1) titers in Standardbred horses

	Probability of disease	
Antibody titer	1974	1975
640	.003	.092
320	.009	.124
160	.027	.124
80	.108	.232
40	.265	.295
20	.519	.366
10	.694	.704

Source: Sherman et al. 1979, with permission.

Note: Anti-influenza titers appear to be protective since there is an indirect relationship between titer and the probability of disease. However, even horses with high titers acquire upper respiratory tract disease, probably because other factors in addition to antibody presence are required for protection, and/or other agents may have been the proximate cause of disease in these horses.

diagnostic laboratories. Except for specified exclusions, all cases first diagnosed in a specified time period can be included in the study. Usually there is a very large number of potential controls. If little or no effort is required to obtain the history of exposure to the factor(s) of interest, then all noncases or all noncases with specified other diseases may be used as controls. Whether explicit sampling of noncases is used depends on the time and expense required to obtain the factor status for each unit selected. When sampling from a large number of potential controls, random or random systematic selection is preferred, provided no matching of cases and controls is to be used.

When both of the study groups are obtained by purposive selection from laboratory or clinic records, the cases and/or controls may not be representative of all cases and noncases in the source population. In particular, the prevalence of the factor(s) of interest in the available controls may not reflect its prevalence in the source population as it ought to, particularly if valid estimates of the importance of the association are desired. If there is doubt about the representativeness of the cases and/or controls, additional data should be obtained to help evaluate the situation. Unfortunately, in practice only qualitative data are readily available to test how representative the groups are, and these deficits should be borne in mind when interpreting and extrapolating the results.

A particular form of unrepresentative sample that gives rise to biased estimates of association arises when the rate of admission to the laboratory or clinic is associated with both the factor(s) of interest and the disease status. When these records are used in a subsequent study, the differential admission rate acts as a confounding variable and can bias the true association between the factor(s) and disease. This phenomenon is often called Berkson's fallacy after the person initially describing it. A classic example of Berkson's fallacy occurred in a study of the association between cancer and tuberculosis based on human autopsies (Pearl 1929). The initial study results indicated less tuberculosis in autopsied cancer victims than in autopsied people dying from diseases other than cancer; thus suggesting a sparing effect of tuberculosis on cancer. It was later found that the autopsy series contained a disproportionately large number of tuberculosis cases because the latter were more likely to be autopsied, and when this was taken into account the association between tuberculosis and cancer disappeared.

Documented instances of Berkson's fallacy in veterinary medicine are rare; however, the effects of differential admission rates may have been observed, using hospital records, in a case-control study of the relationship between clinical mastitis and age of dairy cows. No association between age and mastitis was found in the case-control study; yet in a subsequent longitudinal study in the population of cows giving rise to the data for the case-control study, the rate of mastitis was found to increase significantly with the cow's age. The difference in results was due to the fact that many diseases of dairy cows increase in frequency with age, and thus the population of cows with diseases (the hospital population) was older than the average age in the source population. Hence, only diseases whose frequency increased with age more rapidly than the average of other diseases were observed to have a significant association with age in the case-control study. Thus in this example, submission rate for diagnosis was related to both age and diagnosis and biased the association between these two variables (Erb and Martin 1978; 1980).

The likelihood of admission rate bias can be assessed by comparing the

characteristics of the control group(s) to independent samples from the source population; if the control group and population appear to have similar distributions with respect to a number of factors, admission rate bias is unlikely. Also, the probability of admission bias occurring may be reduced by selecting controls from all available noncases. It may be slight comfort that the majority of case-control study results apparently have not been unduly affected by this phenomenon. In some studies (e.g., the association between lung cancer and smoking based on hospitalized patients), when the effects of admission rate are removed, the association between smoking and lung cancer becomes stronger because smokers are more likely to be hospitalized than nonsmokers, and lung cancer patients are more likely to be hospitalized than non–lung cancer patients. Thus the observed association based on hospital data is weaker than the association in the source population. Further, admission rate biases are unlikely to explain strong associations (relative risk > 3) and are unlikely to explain a gradient of risk with different levels of exposure. This is an additional reason for inclusion of these two items when considering the likelihood that an observed association is causal.

When using noncase patients from a clinic as controls, it is advisable to select the controls from all noncase patients rather than a specific subset of other diagnoses. It is possible to select different sets of controls from a number of diagnostic categories—one set from all noncase patients and another from patients with diseases X, Y, or Z. When this is done, it is advisable to record biologically reasonable interpretations for all possible associations prior to conducting the study. Often, logical explanations for some possible differences in associations between different control groups are not apparent, and the investigator should reconsider the selection of controls.

The use of controls selected from the source population is another way of circumventing the problem of admission rate bias. Population-based controls are particularly useful when the list of cases represents essentially all cases in a defined population (such as all infected farms in a county) or all cases of a disease in a set of farms serviced by a veterinary practice. Within reason, when selecting controls from defined populations, attempt to maximize collaboration among potential controls or nonresponse may bias the results in a manner similar to different admission rates.

If genetic comparability between cases and controls is desirable for the study, relatives of the case may be selected as controls. However, since siblings tend to share similar environments, their selection will indirectly make the environment of cases and controls more comparable, and this is not always desirable. In selecting siblings as controls it is important to select a fixed number of controls per case and to exclude those cases where this ratio can not be obtained. Otherwise large sibling groups may bias the

results. Usually one would not select relatives of cases if the factor of interest is related to genotype (e.g., if the factor was phenotype).

If environmental comparability is required, controls may be selected from the same original source as the cases (i.e., from the same farm or kennel). Again, cases and controls should be selected in a fixed ratio to ensure that larger farms or kennels do not bias the results. (This was also noted when the example of ureaplasma and infertility was introduced.)

6.4.2 Comparability of Cases and Controls

Theoretically, the cases and controls should be similar in all respects except for the disease (dependent variable) being investigated. Of course, they would also differ with respect to the exposure factor if it were associated with the disease. One indication of comparability of groups is a similar response (collaboration) rate in both groups. Very different response rates should lead to skepticism about the validity of results, particularly if the overall response rate is low (less than 75–80%). In practice, the cases and controls may differ in many ways as described in 5.4, and two commonly used methods to increase the comparability of groups are analytic control and matching. Restricted selection (e.g., only selecting cows between 4 and 7 years of age) also tends to make the groups more similar, since the restriction applies to both the cases and controls.

In analytic control, data on ancillary factors are obtained and appropriate statistical methods (such as the Mantel-Haenszel technique) are used to prevent distortion of results from extraneous factors. Host factors are frequently confounding variables and should be included in the list of ancillary factors if it is known that the risk of disease is influenced by them. If the list of ancillary factors is long, complex analytic methods beyond the scope of this text (such as logistic regression) may be required for analysis of the data.

Matching may be used to increase the similarity of cases and controls. The characteristics of each case with regard to potential confounding factors are noted, and a control is sought with the same characteristics. In most studies the number of factors that can be matched is small (perhaps two or three); otherwise it becomes difficult to identify controls with the required characteristics. In case-control studies, only factors known to be associated with the risk of disease should be included as matching factors. It is a peculiarity of case-control studies that overmatching (matching for noncausal factors) may reduce the ability (power) to detect true associations between the factor and disease. If one wishes to study the effect of an extraneous factor, it is necessary to use analytic control rather than matching, since the effects of matched factors cannot be studied.

As an example, matching was used in a study of factors related to mycoplasma mastitis in dairy herds. Two sources of control herds were

used, one matching on size of herd, the other on level of milk production (Thomas et al. 1981). See Table 6.5.

6.4.3 Obtaining Information about Factor of Interest

A major objective in case-control studies is to collect accurate, unbiased information about the factor of interest. To assist in this, data should be obtained in the same manner and with the same rigor from both cases and controls. One way of ensuring equal rigor is to keep the investigator blind to the disease status and/or to keep the respondent unaware of the exact reason for the study. To test its validity, the information collected may be compared with the data in other records or the results of selected tests. As was previously mentioned, this is very similar to evaluating a screening test. If the sensitivity and specificity of the question are equal in both cases and controls, although errors may reduce the apparent strength of the association, they will not falsely inflate it.

Table 6.5. Means for selected production variables for mycoplasma case-herd and control-herd groups in California dairy herds

Herd group	Herd size	Percentage dry	Percentage culled	Milk (kg/yr)
Case				
1–49 colonies[a]	598	14	32	7470
50+ colonies	661	15	35	7607
Control				
Production matched	316	15	26	7535
Herd size matched	615	14	29	7746

Source: Thomas et al. 1981, with permission from Am. J. Vet. Res.

[a]Number of pathogenic mycoplasma colonies per ml of bulk-tank milk.

Note: Control herds with the same production as case herds are smaller and cull a smaller percentage of cows. Control herds of the same size as the case herds have higher production and lower culling rates. These suggest that infection is more common in larger herds, that milk production is lowered, and that culling is increased by mycoplasma infection.

6.4.4 Analysis

The proportions being compared (the proportion of cases that are exposed and the proportion of noncases that are exposed) in the case-control study should be calculated and displayed together with the results of statistical analysis and the appropriate epidemiologic measures of association (see Table 5.6 and Table 6.6).

If the factor has more than two levels on the nominal scale (e.g., breeds), the level of factor that makes the most biologic or practical sense should be chosen as the reference group. If the factor is ordinal in type, the nonexposed or least exposed group may be used as the reference group

Table 6.6. The relationship between level of crude fiber in the ration and the occurrence of left displaced abomasum in dairy herds

Crude fiber <16%	Case herds	Control herds	Chi-square	Odds-ratio
Yes	20	6	5.13	10
No	2	6		1
	22	12		
Proportion <16%	0.91	0.50		

Source: Grymer et al. 1981, with permission.

(odds ratio = 1). A series of 2 × 2 tables each containing the referent group is constructed, and the strength of association assessed in the usual manner. As an example, the referent group in a study of the association between breed and hip dysplasia in dogs was "other breeds." This group consisted of a number of crossbred dogs and a number of breeds having only a few dogs each (see Table 6.7) (Martin et al. 1980).

Table 6.7. Rate of canine hip dysplasia (CHD) for breeds represented by twenty or more dogs radiographed at OVC, 1970-1978

Breed	No. of dogs	Percent of CHD	Risk[a]	Significance of risk[b]
Afghan hound	46	10.9	0.49	NS
Alaskan malamute	66	37.9	1.38	NS
Bouvier des Flandres	55	36.4	1.21	NS
German shepherd	402	46.8	1.85	S
Great Dane	118	16.1	0.48	S
Great Pyrenees	29	20.7	0.76	NS
Irish wolfhound	36	22.2	0.77	NS
Newfoundland	116	63.8	3.66	S
Norwegian elkhound	29	34.5	1.42	NS
Old English sheepdog	119	47.1	1.88	S
Miniature poodle	48	25.0	0.94	NS
Standard poodle	33	30.3	1.10	NS
Golden retriever	140	55.7	2.75	S
Labrador retriever	211	37.4	1.27	NS
Rottweiler	26	30.8	1.10	NS
Saint Bernard	131	73.3	5.14	S
Samoyed	64	34.4	1.12	NS
English setter	38	39.5	1.53	NS
Irish setter	77	33.8	1.28	NS
Siberian husky	151	5.3	0.25	S
"Other breeds"	354	30.7	1.00	...

Source: Martin et al. 1980, with permission.

[a]Measured by odds ratio. This statistic compares the rate of CHD in each breed to the rate in "other breeds." Odds ratios significantly greater than one imply increased rates. Odds ratios significantly less than one imply decreased rates.

[b]The significance of the odds ratio is tested with a chi-square statistic. NS = not significant. S = significant at $p < 0.05$.

6.5 Cohort Study Design

In cohort studies, separate samples of exposed and unexposed units are selected. The groups are observed for a predetermined period, and the rate of disease in each is compared. In the ureaplasma example, the investigator might obtain an arbitrary number of ureaplasma infected cows and select a similar number of noninfected cows, perhaps matching for herd and age. Any cows known to be infertile would be excluded at the start of the study. (In a practical situation, one might have to settle for excluding all cows with obvious reproductive tract abnormalities unrelated to ureaplasma, within 60 days of parturition.) All cows would be observed for a defined period of time (say 90 days after breeding commenced), and the subsequent rate of infertility in each group identified.

Although bias is less of a problem in cohort than case-control studies, key items to ensure validity are the criteria for and selection of the exposed and unexposed groups, equality of follow-up in both groups, and accurate diagnosis of disease.

6.5.1 Selection of Exposed and Unexposed Groups

In most cohort studies, special exposure groups are purposively selected for comparison. This could include comparing rates of disease(s) in different breeds; comparing rates of pneumonia in animals on different rations; comparing rates of disease in animals with and without serum antibodies to selected antigens; or comparing disease rates and production levels in herds on preventive medicine programs to similar herds not on these programs. As mentioned previously, the sampling units are frequently obtained through purposive sampling, not probability samples from a defined sampling frame. Because of this and in order to extrapolate results beyond the study groups, some indication of how representative the study groups are of exposed and unexposed segments of the population should be obtained.

A further concern in selecting the cohorts is that they should be comparable (i.e., not differ in ways other than the exposure). This may require the measurement of ancillary variables so that analytic control can be used to adjust for known differences between the groups, although matching may be used to increase the similarity of the groups as it was in case-control studies. More than one unexposed group may be selected as the referent if the information provided will be useful. For example, in a study of the effects (benefits) of preventive medicine programs, the comparison group might include two groups: the first composed of herds using veterinary service regularly, but not a formal prophylactic program; and the second composed of herds using veterinary service only irregularly. Obviously, a

clear and practical set of definitions of the different types of veterinary service would be required.

Although the exposure status of selected units may seem obvious, the probability of misclassification of exposure status can be reduced by clear, concise descriptions of what constitutes exposure (possession of the factor). Specific tests may be used to help assess exposure status in a manner similar to their usage as diagnostic aids. When feasible it is useful to classify the cohorts according to a gradient of exposure, allowing investigation of a potential dose-response relationship.

If prerecorded data on exposure history are used to define the cohorts, investigations into the meaning, validity, and completeness of the data should be performed. Certainly one should not interpret "no recorded history" of exposure as meaning no exposure, unless the records are known to be complete.

In prospective studies, the collaboration of a number of people will be required. Hence, it is important that a high percentage of selected individuals cooperate in the study, and failing this, the study design should increase the likelihood of equal cooperation rates in the exposed and nonexposed groups. If these rates are very different, lack of cooperation can distort the results of the study in the same manner as differential admission rates in case-control studies. In general, it is informative to elucidate reasons for lack of cooperation

Whenever possible, all the sampling units entering the study should be examined for the presence of the disease(s) of interest at the start of the study. By starting the study with disease-free units, the investigator can determine incidence rates, and this also establishes a clear temporal relationship between the factor and disease. Sometimes such an examination is very difficult; thus the sampling units are assumed to be disease-free at the start of the study. This is frequently true in retrospective cohort studies.

6.5.2 Follow-Up Period

The cohorts should be observed for the occurrence of disease(s) at regular periods throughout the study; both groups should be followed with equal rigor; and the withdrawal of sampling units from the study should be minimized. Withdrawals can bias the results if the losses are related to both exposure and disease status. Obviously, this problem is more severe in studies spanning many years. If a high percentage (e.g., 95%) complete the study, potential biases from withdrawal will be minimized. Care is also required when cohorts are defined retrospectively, because many withdrawals (due to culling, sale, or death) will have occurred before the study begins. For example, if the weight gain and feed efficiency of a group of

swine that received antimicrobial therapy were compared to that of an untreated group, one would have to note and adjust for death losses prior to slaughter. Such losses might not negate the results, but their potential significance should be borne in mind. Whenever possible, the reason(s) for withdrawal from a prospective cohort study should be recorded.

6.5.3 Determining Occurrence of Disease

The diagnostic criteria for the disease of interest must be clearly defined, and whenever possible those making the diagnosis should be unaware of exposure status of the units being examined. Since more than one disease may be of interest, the criteria for diagnosing a few important diseases should be specified in detail, with other diseases being diagnosed and recorded in a rigorous but ad hoc manner.

6.5.4 Analysis

If the duration of the study is relatively short, the average period of risk is equivalent in both cohorts, and the losses to follow up are minimal, the usual 2 × 2 table format may be used to display and analyze the data (see Table 6.8). The rates of disease in each cohort are calculated and compared directly, or the Mantel-Haenszel technique, or standardization of rates may be used to control the effects of extraneous qualitative variables.

Often the duration of the period at risk may differ greatly between cohorts. This is particularly likely when the cohorts are not completely formed at the start of the study. If the study is designed to last 3 years, the cohorts may be formed over this period as appropriate exposed and unexposed individuals are identified and placed under observation. A hypothetical example of this situation and the problems it creates is provided in Table 6.9.

Two analytic approaches are used to adjust for the differing periods of observation. The first method is based on the calculation of true rates and the concepts of unit-time (for example animal-years) of risk as introduced in Chapter 3. Each animal or sampling unit contributes 1 year each full year

Table 6.8. Feline leukemia (FL) incidence rate in cats with and without infectious anemia (FIA): a retrospective cohort study[a]

	FL +	FL −	Total	Incidence rate (risk) (%)	Relative risk
FIA +	6	291	297	2.02	11.9
FIA −	1	593	594	0.17	1
	7	884	891		
Chi-square = 8.71					

Source: Priester and Hayes 1973, with permission.
[a]Called a prospective case-control study by the authors.

it is under observation (e.g., 1 unit observed for 3 years contributes 3 unit-years of observation, and 3 units observed for one year also contribute 3 unit-years). The total unit-time of risk in each group is used as the denominator for calculating true rates in the usual manner. Although these data may be summarized in a 2 × 2 table, the regular chi-square test should not be applied to these data. Thus, using true rates is useful for removing biases from differences in period of risk, but does not allow the evaluation of the

Table 6.9. Animal-years of observation in cohort or longitudinal studies

Suppose that in a cohort study the cohorts ($F+$ and $F-$) were not fully formed at the time the study began. In particular, assume the $F+$ group formed in the following manner. The number of cases of disease in each year of the study are shown also. (Assume also that an animal only gets the disease once.)

Calendar year of entry	Number entering	Disease incidence by year of study			
		1	2	3	Total
1	300	30	27	24	81
2	400		40	36	76
3	500			50	50
	1200				207

The $F-$ group and the number of cases formed in the following manner:

Calendar year of entry	Number entering	Disease incidence by year of study			
		1	2	3	Total
1	500	25	24	23	72
2	400		20	19	39
3	300			15	15
	1200				126

Had both groups been fully formed at the start of the study and been observed for 3 years, the usual 2 × 2 table format for calculating risk rates would be appropriate.

	$D+$	$D-$	Total animals	Rates/3 years (%)
$F+$	207	993	1200	17.3
$F-$	126	1074	1200	10.5

However, since these conditions were not met, the total period of observation for the cohorts may differ. In fact, the number of animal years of observation for the $F+$ group was

$$300 \times 3 + 400 \times 2 + 500 \times 1 = 2200$$

for a true rate of 9.41% per animal-year (207/2200).

The number of animal years of observation for the $F-$ group was

$$500 \times 3 + 400 \times 2 + 300 \times 1 = 2600.$$

for a true rate of 4.15% per animal-year (126/2600).

(These true rates are not exact because the diseased animals were not at risk after developing the disease—see Chapter 3.) After making this adjustment, the years of observation are 1969.5 and 2443 for the F+ and F− cohorts, respectively.

role of chance by standard statistical methods. (Suggestions for analyses are included in Kleinbaum et al. 1982, pp 336–8.) If the groups are very large, sampling variation is not of great importance and may be ignored.

The second analytic approach is the follow-up life table method that allows the investigator to calculate risk rates. This is accomplished by taking into account the different periods of risk, and the technique also allows formal statistical evaluation of observed differences. This method is introduced in Table 6.10 as an extension of the problem presented in Table 6.9. An example of the application of follow-up life tables is shown in Table 6.11.

6.6 Choosing the Analytic Study Method

Often the choice of study method is influenced by the structure of the files or population to be sampled. For example, if the exposure and disease status of the units to be sampled are unknown, cross-sectional methods would be used. Case-control sampling may be a natural choice if records are filed or retrievable by diagnosis.

The choice of study type may also be influenced by the objective of the study and the amount of knowledge already known about the relationship between the factor(s) and disease(s) of interest. Case-control studies allow initial screening and identification of multiple risk factors for a given disease, whereas cohort studies are suited to the screening and identification of multiple effects from a single cause. Cross-sectional and longitudinal studies allow the simultaneous study of many factors and diseases, and in addition provide direct estimates of the frequency of these events in the source population.

Finally, one must be aware of general advantages and disadvantages specific to each design. Cross-sectional studies usually only provide estimates of prevalence; thus one can not differentiate factors associated with having disease from factors causing the disease. Cross-sectional and longitudinal studies are not well suited to studying rare diseases, whereas case-control methods (requiring the smallest total sample size of any study type) are ideal in this situation. Case-control studies are relatively easy and inexpensive to conduct, but suffer from many potential biases. Cohort and longitudinal studies provide direct estimates of incidence rates and the time sequence of events is well established. These studies are, however, the most difficult and expensive to conduct.

In summary, if the objective of the study is to screen for risk factors, use either cross-sectional or case-control studies, whereas if testing specific hypotheses use longitudinal or cohort methods. In some instances, field experiments are required as the ultimate evaluation of associations found in observational studies.

Table 6.10. Follow-up life table for analysis of data from cohort and longitudinal studies (data from Table 6.9)

Years under observation	Exposed				Unexposed			
	Number initially at risk	Number new cases	Number withdrawals	Probability of new case (P)	Number initially at risk	Number new cases	Number withdrawals	Probability of new case (P)
$0 < 1$	1200	120	450	.12	1200	60	428	.06
$1 < 2$	630[a]	63	324	.13	712	43	361	.08
$2 < 3$	243	24	219	.18	308	23	285	.14

This is a risk rate, and hence if animals are withdrawn or lost during the period, one half of the number withdrawn is subtracted from the number initially at risk before calculating the probability of becoming a case. Animals whose observation period is terminated by the end of the study are also considered as withdrawals.

The probability of not becoming a case during each period is found by subtracting P from 1. The cumulative probability of not becoming a case during the study period is the product of these probabilities. For the $F+$ cohort this is $.88 \times .87 \times .82 = .63$. Hence the probability of developing the disease in a three year period is $1 - 0.63 = 0.37$ or 37%. For the $F-$ cohort this is $.94 \times .92 \times .86 = .74$. Hence the probability of developing disease in the three year period for $F-$ animals is 0.26 or 26%.

[a]This is derived from the 700 animals starting the second year of observation, but subtracting the $30 + 40$ cases that had already occurred in the first year of observation.

Table 6.11. Survivorship of infant *Macaca mulatta* according to place of birth at the University of California Primate Center, 1968-1972

Age (days)	Cumulative probability (P) of surviving to specified age			
	Born inside		Born outside	
	P (%)	SE(P)	P (%)	SE(P)
< 8	97.8	0.91	91.5	2.71
8 < 15	95.9	1.22	83.9	3.58
15 < 22	94.1	1.19	82.9	3.66
22 < 29	93.7	1.24	80.1	3.89
29 < 60	91.5	1.50	76.2	4.15
60 < 91	89.6	1.68	74.3	4.27
91 < 122	89.3	1.71	72.4	4.49
122 < 153	88.5	1.78	70.4	4.47
153 < 184	86.7	1.93	67.4	4.60

Source: Hird 1975, with permission.

Note: The cumulative probability of surviving to a specified age for infants born inside can be compared to that for infants born outside; statistically, $P \pm 2\text{SE}(P)$ are approximate 95% confidence intervals. If the intervals do not overlap, the survivorship may be deemed different in the two groups (SE = standard error).

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