**Observational study design in equine research**

**Abstract**

When planning to conduct research, thorough consideration of the study design is essential to enable valid results and purposeful conclusions. A good study design will provide a strong basis for robust conclusions that can contribute to the evidence base. Conversely, a poor study design may unintentionally lead to invalid conclusions with inappropriate claims of the clinical importance. The purpose of this article is to review important aspects of observational study design, with an emphasis on observational clinical research. The value of an observational study can be manifold and the benefit of studying clinical cases can add substantial value to the evidence base and equine health and welfare, however this value will be diminished if study designs are flawed, or are inadequately reported. It is essential that clinicians have the skills to critically appraise observational studies, to determine strengths, limitations and the applicability to their clinical practice.

**Keywords:** Observational study designs, cross-sectional; cohort; case-control; case recruitment

**1 | INTRODUCTION**

In epidemiology, clinical study designs can be broadly categorised into observational or experimental. Observational studies can be broken down into descriptive and analytic observational studies.1 Descriptive study designs include case reports, case series and cross-sectional (surveys) (Figure 1). These studies only describe the exposures and/or outcomes measured and have no comparator group. Analytic observational studies encompass cross-sectional, cohort and case-control studies. These studies can assess associations between the exposures and the outcomes measured. Unlike experimental study designs, such as randomised controlled trials where randomisation and blinding are key to internal validity, the investigator cannot control the circumstances or allocation of the exposure in observational studies. Thus, observational studies must rely on robust and careful design, selection of appropriate study subjects to minimise bias and optimise internal validity. This article focuses on observational study designs and will not discuss experimental study designs further. The Oxford Centre for Evidence-Based Medicine has set out hierarchy of evidence based on the study design.2 This allows studies to be graded up or down the level of evidence based on the quality and details of the study design.

The choice of study design depends on many considerations including the purpose of the study, the objectives/hypothesis, the disease or condition of interest (is it a rare or common disease) and hypothesised risk factors or exposures of the disease or condition. Depending on the study design, differing levels of time, investment and resources will be required. Realistic considerations need to be made towards the likely patient compliance and enrolment onto the research project, in conjunction with sample size calculations to determine the number of individuals required to reach an acceptable level of power. Attention to ethical research conduct is a necessity, please refer to Marr (2015) for further information on Ethical Animal Research and guidelines for when ethical approval is required.3

**2 | HOW TO IMPROVE YOUR OBSERVATIONAL STUDY?**

Common pitfalls for observational research include inadequate considerations for the study validity, imprecise or incorrect case definition and inadequate recruitment. Furthermore, inadequate reporting often leads to a lack of transparency of the research undertaken.

**2.1 | Study validity**

Considerations should be made for both the internal and external study validity, however, the difference between the two may be misunderstood. Internal validity reflects when the study results provide unbiased information about the animals included in the study.4 Many considerations of study design are focused on minimising bias and maximising internal validity. External validity is the ability to extrapolate from the results in the study to the wider population. For example, results from a study on 2-year-old Thoroughbred horses may not be generalisable to the UK pony population or vice versa. There are often tensions between maximising both internal validity and external validity, but internal validity should be prioritised as biased (or incorrect results) are not of value to any population.

**2.2 | Case definitions**

Accurate, explicit and complete case definitions and well-defined outcomes need to be determined at the earliest stages of any design along with clear policies on inclusion and exclusion of individuals or cases. This may be easier to achieve for objective classifications (clear clinical case definition or where there are validated, sensitive/specific diagnostic tests available) compared to subjective assessment (e.g., pain or lameness assessments) or when defining a cut-off for severe / mild forms of a disease (e.g., osteoarthritis). Any subjective scoring system should be valid (and referenced where possible) and the assessors need careful consideration. To formulate these case or outcome definitions guidance should be obtained from previous studies, systematic reviews and expert opinion.

**2.3 | Reporting** **and flow diagrams**

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) is an initiative to increase transparency for observational studies with guidelines set out on how they should be reported.5 Checklists can be downloaded on the STROBE website6 and they include information on the material that should be reported in observational studies. This emphasises that inadequate reporting for these studies obstructs the readers’ ability to assess the studies strengths and weaknesses. EVJ encourages authors to adhere to the STROBE research reporting standards.7,8

It is becoming common practice and frequently required by journals to provide a flow chart that visually depicts individual or case recruitment. These can provide a visual guide to the study design and animal inclusion and exclusion, and crucially report on individuals at each stage of the study e.g., numbers potentially or examined for eligibility, included in the study, completing the follow-up, and finally included in analysis. Reasons for non-participation should be outlined at each stage.5 They are useful for both observational9-12 and experimental studies13 and are strongly recommended in reporting of randomised control trials according to the CONSORT14 (Consolidated Standards of Reporting Trials) statement. Simple examples of flow diagrams for cross-sectional, case-control and cohort studies are outlined under the relevant headings to give an example of the types of information that may be included in a flow diagram. More complex flow diagrams may be needed for more complex study designs.

**3 | OBSERVATIONAL STUDY DESIGNS**

**3.1 | Case reports and case series**

A case report describes a single case, and case series are a description of a number (often a small number but can be large for example a whole register) of animals. The latter are more commonly retrospective but may be designed prospectively. They provide useful information especially when a condition is not well understood (e.g., novel cases or new diseases) and may be useful in the formulation of a hypothesis. However, case series do not have a comparator groups and hence these types of studies are unable to determine causality nor efficacy of treatment. The priority areas for case reports and series will vary on the journal and for many journals there may be limited space for inclusion of such studies.8,15

**3.2 | Cross-sectional study**

Cross-sectional studies are frequently used to determine prevalence of disease or condition and may establish an association between an exposure and the outcome at one time point (Figure 1). Depending on the research question being asked a cross-sectional study could be either descriptive16 (determining the prevalence) or analytical (an association with exposure/ risk factors is assessed). The former is often in the form of a survey.

Cross-sectional studies can be utilised in certain surveillance systems17 reporting prevalence data. The studies can be repeated to monitor trends and changes in prevalence over time at the aggregate level.

Common pitfalls to consider include obtaining a truly representative sample of the intended target population, if this is not achieved it can contribute to selection bias and invalid estimates.18 The methods of sample selection, which should be a random sample, should be clearly defined and the resulting study sample available for analysis will determine how valid the results are. The final sample included in the study may be affected by the response rates (e.g., respondents of a survey) or the sampling frames used or the number of animals that complete data are available for (Figure 2). Considerations should be made towards those that are lost or excluded at each stage of sampling. Flow diagrams which depict enrolment on to the study and sampling of the population can be used to outline where animals may be excluded (and why?) as well as displaying the number of individuals overall (Figure 3). An equine example can be found in Pollard *et al.* (2017),10 this paper assesses horse owners’ ability to recognise laminitis10 and further examples from human medicine19,20 can be found in the literature.

Analytic cross-sectional studies only provide relatively weak evidence of causality because the temporal relationship between the exposure and outcome cannot be determined. When assessing exposures and outcome, a consideration for disease process should be made. For example, as cross-sectional studies rely on existing cases the prevalence is related to both the occurrence of disease (the incidence) and the disease duration. Exposures may therefore be associated with disease occurrence or disease duration contributing to prevalence-incidence bias.1

**3.3 | Case-control study**

In case-control studies, animals are initially classified by disease status (i.e., case or control) and then past exposures are measured comparing for each group (Figure 1). Case-control studies are especially useful if the disease is rare (e.g., an uncommon genetic disorder21). Unlike cohort studies, they can be useful if there is a prolonged time between the exposure and disease, or disease and the outcome of interest. They can provide valuable information on risk factors, e.g., assessing the risk factors for Equine Grass Sickness22,23 and epiploic foramen entrapments9. Case-control studies cannot provide estimates of prevalence or incidence because only a fraction of the non-diseased animals are sampled.

They can be prospective (wait for the cases to occur and enrolled based on outcome) or retrospective (look back at cases with a desired outcome). Case and control selection are very important considerations. An accurate case definition needs to be identified with clear inclusion and exclusion criteria. Ideally studies should include only incident (new) cases as opposed to prevalent cases (where exposures could be related survival of the disease). The controls need to be representative of the population that the ‘diseased’ cases arose from thus the source population (or study base) of a case-control study needs to be well defined, including whether this is a primary or secondary study base. The exposures or confounders should be represented and measurable in the same manner to the cases. Controls may be selected in a number of ways e.g., from non-diseased animals at the end of the study period, sampled from the population‐at‐risk at the time the case was diagnosed (and thus matched on time), or sampled from all individuals in population throughout the study.

Non-representative selection of controls is one of the most common errors in case control studies leading to selection bias and reducing the ability for robust conclusions to be formulated. Hospital or practice-based case-control studies are a common approach, but care must be taken when selecting hospitals controls because animals presented to hospitals have other conditions which may be related to various other factors, for example, age, breed and use, leading to Berkson’s bias.4,18

Case-control matching on the basis of one or more variables is also possible in circumstances where a confounder is associated with disease and exposure. However, this should be carefully considered because it requires analysis to take this into account and has other disadvantages such as inability to identify the contribution of the matched factor to the outcome. Matching can sometimes create additional cost and difficultly identifying suitable controls.

Loss of eligible cases and controls through non-response or missing data, for example in clinical notes can lead to recall bias, and this may be different in cases compared to controls (possibly leading to differential bias).

Case-control studies should clearly report the eligibility criteria, and the sources and methods of case and control selection6 and results should include the numbers of cases and controls at each stage and report on non-response or non-participation. Flow diagrams for case recruitment and progression can be utilised for case-control studies clearly indicating the case and the control groups (Figure 4). For example, Archer *et al.* (2008) assessing the risk factors for epiploic foramen entrapment9 and there are further examples in the human literature24.

**3.4 | Cohort study**

Cohort studies are highest in the hierarchy of evidence of the observational study designs.2 Cohort studies are longitudinal analytical studies in which animals in the study population are either exposed or unexposed to the risk factors being studied and then followed over time to determine the measured outcome(s) (Figure 1). More than one outcome can be measured but they should be clear and unambiguous, and criteria applied equally to the exposed and unexposed groups. They can help to hypothesise a causal relationship between the exposure and outcome. This type of study design can be retrospective25,26 or prospective27,28. Prospective studies can be time consuming depending on how rapidly the development of the outcome/ disease occurs. A retrospective study of this type would require thorough and accurate record keeping and is less commonly performed. Cohorts can follow a fixed population or be dynamic to allow animals to enter and leave the study as they progress. Often animals in a cohort are selected from the population under study and exposures are then measured at the start or throughout the cohort.27,28 However separate exposure cohorts can be selected at the start, especially if there is a specific hypothesis and the exposure or risk factor is known but not a frequent occurrence. Many exposures of interest in cohort studies may vary over time (time-varying exposures) and this adds to the complexity of both measurement and analysis. There should also be careful consideration of potential confounders and their measurement prior to commencing a cohort study.1,29

The duration of follow-up can be short30 but many are several month or years long27 which may have costs and logistical implications. Loss to follow-up is a major consideration in animal studies as owners may choose to sell or euthanase animals or they may not be contactable. This may contribute to bias if this is related to the risk factors and/or outcomes. Other considerations include selection bias arising from biased selection of the cohort and ensuring adequate enrolment/consent for participation.

Reports on cohort studies should have clearly defined eligibility criteria and the sources and methods of participant selection. Methods of follow-up should be described and the numbers of individuals at each stage of study (e.g., eligible, recruited, included, completing follow up and analysed) should be reported along with reasons for non-participation at each stage6 (Figure 5). Examples of flow diagrams depicting recruitment and progression for equine cohort studies can be found in the literature.11,12

**4 | CONCLUSIONS**

Careful consideration for the study design is needed in light of the purpose and hypothesis of the study, the expected incidence and prevalence or the disease under study and known risk factors or exposures for the disease or condition of interest. Depending on the study design, this will alter the approach taken to case recruitment but all require thorough deliberation to reduce bias. For further discussion on bias, refer to Fosgate (2020).18

Observational epidemiological studies will continue to have a substantial contribution to clinical practice and to equine research and evidence base. To maximise the value of these studies it is of critical importance that the study design is accurate, valid and appropriately reported in the literature. For further reading in detailed textbooks please refer to Dohoo *et al.* (2009)31, and Thrusfield and Christley (2018)32.

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**FURTHER READING**

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**Figure legends**

**Figure 1:** Schematic representation of the processes of selection and measurement in cohort, case-control and cross-sectional studies. Figure and figure legend from Christley and French (2018)1 –Pending Copyright permission (figure 16.1).

**Figure 2:** Schematic representation of the process of selection of the sample available to the analyst from the target. At each stage only a sub-set may proceed to the subsequent stage. The aim of the selection process should be to ensure that all study units present at higher levels have equal probability of selection into the subsequent level, and failure to achieve this may result in selection bias.Figure and figure legend from Christley and French (2018)4 – Pending Copyright permission.

**Figure 3:** Exampleflow diagram for an analytic cross-sectional study design depicting the sample recruitment and outcome results. More complex designs such as clustered, stratified or multistage will require more complex flow diagrams. *(n= ) denotes the number of cases at each phase in the study.* Diagram generated using Microsoft Visio Professional 2013 (Microsoft Corporation).

**Figure 4:** Exampleflow diagrams of a case-control study design depicting the case and control recruitment and progression. In a matched study, details of matching should also be shown. *(n= ) denotes the number of cases at each phase in the study.* Diagram generated using Microsoft Visio Professional 2013 (Microsoft Corporation).

**Figure 5:** Exampleflow diagram of a cohort study design depicting the cohort recruitment and progression. Where repeated follow-up and assessment is performed losses at each stage should be shown. *(n= ) denotes the number of cases at each phase in the study.* Diagram generated using Microsoft Visio Professional 2013 (Microsoft Corporation).









