Animals must be allocated to study groups in a way that is fair (i.e. no study group has an advantage)

Research studies that investigate comparisons of two or more diagnostic tests, treatment interventions, or prognostic indicators provide the evidence that veterinary practitioners need to deliver optimized care to their clients and patients. But in order for a reported result to be considered valid, research consumers must be satisfied that study animals were allocated to the treatment groups fairly. If animals in one treatment group have important differences from animals in other treatment groups, even before the test, intervention, or prognostic factor are considered – then any conclusions about differences found by analyzing the study data cannot be trusted.

Randomly assigning animals to each treatment group in a study minimizes selection bias by using a chance-governed mechanism (such as a random number generator or coin flip) so that every animal in the study has the same chance of receiving any treatment. In a study where animals are fairly allocated, each treatment group is an equivalent and representative sample of the larger population. Deterministic allocation methods such as alternate animal identification numbers, days of the week, date of birth, and gate-cutting are not random. When these methods are used, they should not be described using the term 'random' or any variation of it. When authors do not use random methods to allocate study units to treatment groups, the method of allocation should be described in a manner that would allow the reader to determine if bias was likely to be introduced.

Evaluation of appropriate allocation is best done by reviewing the materials and methods by looking for a specific description of the allocation process. Veterinary practitioners can detect either a lack of information about the allocation process or indications that animals were allocated in a manner that could lead to important differences between study groups. In addition, often one of the first tables or figures in a manuscript describes the animal population in each treatment group at the time the study is initiated which allows the reader to evaluate if the treatment groups seem similar based on important characteristics. If randomization was properly implemented and the sample size is adequate, no biologically meaningful differences in treatment groups at the start of the study should be identified.

Confounding is a special case of bias when a factor other than the treatment influences the study outcome but this factor is more commonly found in animals in one treatment group than in animals in other treatment groups. Common examples of potential confounders include the distribution of sex, breed, prior housing or environment, and age (or weight) at study onset. Randomization with a chance-governed method will eliminate inadvertent confounding when the study sample size if fairly large; however, sample size in many research projects is limited by budgetary constraints and confounding could still be present. If potential confounders are known by the investigators, treatments can be blocked by the confounders as a part of the treatment allocation strategy. For example, if a treatment is known to be modified by sex, the researcher may want to block by sex and randomize within each block to ensure the sex-ratio for each treatment group is equivalent.

In addition to blocking, another method of allocation that is not completely random, but that may be appropriate is ‘matching’. An example is to group patients into pairs who are similar in many important aspects and doing a coin flip to assign one to treatment and the remaining patient to control and then repeating a coin flip for each pair of matched patients to determine which receives each treatment. The advantage of such a system is that it can be almost as good as randomization for controlling bias, and in some situations, it is actually simpler to implement that true randomization.

The disadvantage to non-random assignment is that it allows more potential for even well-meaning individuals to introduce selection bias. In addition, matching only ensures comparability on characteristics that you matched. And, even though matching can be pretty good at ensuring the validity of allocation of subjects to study groups (and potentially superior to random allocation of a small study population) - it is never quite as good as randomization of a large study population for controlling confounding.

For experimental studies, the proper allocation of study subjects to treatment group is a critical feature in the design and conduct of the experiment. And, because of the critical nature of this study characteristic, the report must clearly inform the reader of the exact method of subject randomization. Notice that by nature, retrospective observational studies (such as cohort and case-control studies) do not include randomized allocation and the risk of bias is greater for these types of study than an experimental study with appropriate random allocation.