Dairy Split Session I

Practical Tools for Our Changing Practices

Dr. Neil Anderson, *Presiding*

Practical Field Trials within a Private Practice

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Producers expect veterinary practitioners to be able to offer sound advice on the selection of prophylactic and therapeutic products for use in their herds. However, many of these products have never been thoroughly evaluated under field conditions. Even those that have been tested have often only been compared to no treatment at all, instead of against other available products. This leaves the practitioner with little information on which to base his/her recommendations.

The solution to this dilemma is for the practitioner to carry out a randomized controlled clinical trial. While this may sound daunting, following some simple guidelines will ensure reliable, useful results. There are three key factors to be considered when designing a clinical trial.

(1) What is the question to be asked?
(2) Dealing with the role of chance.
(3) Eliminating bias.

These will each be considered in the design of a trial to compare two prostaglandins for the treatment of pyometra in dairy cows.

The guidelines outlined in this paper are not intended to replace more detailed discussions of how to carry out clinical trials (1). There are many considerations which are beyond the scope of this paper and which should be dealt with in the conduct of clinical trials required for the licensing of new products. Instead, they are intended to provide practitioners with a useful set of quidelines which can be followed in order to answer questions important to them and their clients.

What is the Question?

When trying to determine "what is the question?", there are four steps to be taken.

(1) Specify the main objective.
(2) Decide which animals or cases will be included in the trial.
(3) Decide exactly what treatments will be given. (This paper will refer to "treatments" but these can be either therapeutic or prophylactic products.)
(4) Decide what results will be measured (i.e. the outcomes).

Specify the Main Objective

It is common to start with a question in mind such as:

"Is this new prastaglandin really as good for treating pyometras as the manufacture claims?"

However, the objective of a trial must be stated in much more clear and precise terms before you can proceed. This might lead to an objective such as:

"When used for newly diagnosed pyometras (between 15 and 30 days postpartum), is this new prostaglandin better at reducing the cow's days open and chance of being culled for infertility than the one I am currently using?"

It must be noted that clarification of the objective has already identified the control group to be included. All clinical trials *must* contain a control group (2). This has been stated so often that the necessity of including controls will not be discussed further in this paper. However, consideration will be given to how the controls will be treated. In general, any new treatment should be compared to the one you are currently using. In some cases, such as a trial of a vaccine for a disease with no clearly effective vaccine currently available, the trial may compare the new product to no treatment at all.

What Animals or Cases Should be Included in the Trial?

Once the objective is clearly stated, criteria for the inclusion of animals in the study need to be developed. In addition to the postpartum time frame stated in the

objective, it is important to define what will constitute an "acceptable case" for inclusion in the trial. This may be based on a minimum uterine size as determined by rectal palpation. As a general rule, animals included in a clinical trial should also be free from other concurrent diseases.

One issue related to the decision as to which animals are included in the trial deserves special consideration. If the treatment must be administered to a group of animals (e.g. medicated feed) instead of individual animals, then the unit being studied becomes the group. The same situation may arise in vaccine trials where the problems of spread of live virus vaccines or "herd immunity" are considered to be important. ("Herd immunity" is the protection of unvaccinated animals in a herd by the vaccinated majority). The design of "group level" trials is beyond the scope of this paper. If such a trial is being considered, professional assistance should be sought.

Specify the Treatment Protocol

Obvious considerations in the specification of the treatment protocol include: dose; route and method of administration; frequency of repetition of treatments and any precautions which must be taken at the time of administration. Before the trial begins, it is important to decide who will administer all of the treatments and to prepare a written set of instructions. The treatment protocol must not be changed during the trial.

What Outcomes Will Be Measured?

This is the most important question to be answered when designing a clinical trial. In general, outcomes which can be objectively measured are preferred to those of a subjective nature. If the product is to be administered to large groups of animals, it is wise to include general measures of health and productivity in addition to the specific outcomes related to the disease of interest. For the prostaglandin trial, the following outcomes might be considered in an antibiotic trial.

(1) days to first breeding;
(2) first service conception rate;
(3) days from calving to conception;
(4) rate of culling due to infertility;
(5) rate of subsequent treatment for reproductive diseases such as chronic metritis or cystic ovaries.

While several outcomes can be considered, it is important to select one or two economically important parameters as the primary outcomes for the trial. For the prostaglandin trial these may be days from calving to conception (days open) and rate of culling for infertility. Other outcomes, if measured, may shed light on why the new treatment was, or was not, superior.

Dealing with the Role of Chance

The role that chance plays in a clinical trial is determined by the number of animals included in the trial (in statistical terms, the "sample size") (3). A trial with too few animals will likely not be able to detect even relatively large, clinically important differences between products. On the other hand, a huge trial will always find something statistically significant, even if the differences between the two treatments are trivial.

In order to determine the sample size required it is necessary to answer the following questions.

(a) What results do you expect from the control drug (i.e. the average days open in cows treated with the prostaglandin you are currently using)?
(b) If the results are measured on a continuous scale (e.g. days open) as opposed to a yes/no result (e.g. culled due to infertility), how variable are the results? For the prostaglandin trial, this will be the standard deviation of the days open for cows with pyometra.
(c) How small a difference is clinically significant? (If you think it is important to statistically confirm that the new drug results in a reduction of 1 day in the days open, you will need a huge sample size.)
(d) If you find a statistically significant difference, how confident do you want to be that it was not due to chance? This is usually set to 95% (i.e. the familiar p=0.05).
(e) If a real difference of the magnitude specified in (c) is present, how certain do you want to be of detecting it? This is known as the power of the trial and is usually set to 80%.

These are difficult questions to answer, but it is better to answer them to the best of your ability and then calculate a sample size, than to ignore the issue altogether. Formulas for these calculations are given in Appendix A (4). It must be remembered that the calculated sample size is just an estimate and it is wise to include a few more animals in the trial than are called for by the calculations. In general, trials with continuous variables as outcomes will require fewer animals than trials with dichotomous (yes/no) type outcomes.

Eliminating Bias

Prevention of bias in a clinical trial is a two step process. First, in order to ensure that the animals assigned to each of the treatments are roughly comparable, there must be some form of random assignment. Any clinical trial in which the animals are not randomly assigned in some fashion is highly suspect (5).

The second step is to ensure that the two groups are treated and assessed equally, once the groups have been formed. This is accomplished through the use of "blind"

techniques. This means the person dealing with the animals (e.g. applying treatments or assessing the response) does not know which treatment group the animal is in.

Randomization

Randomization can be achieved by elaborate techniques such as using random numbers tables or computer generated random numbers. On the other hand, drawing numbers from a hat or flipping a coin (if there are only two treatment groups) is equally effective. The important criterion is that there is no "second guessing" once the randomization procedure has assigned an animal to a group. The person treating the animal must not have any latitude in selecting the treatment to be administered. Assigning animals to groups before the time of treatment requires a list of the animals to be included in the trial. This is often not available so the random assignment has to be carried out at the time of treatment.

One special form of randomization, known as systematic allocation, deserves consideration. In this case, animals are systematically assigned to the groups in rotation. For example, every second animal will be given treatment #1 and the others treatment #2. The procedure can work very well but is risky unless strict attention is paid to animal identification and record keeping. If the numbering system gets out of step by one animal, results exactly opposite to the truth will be obtained.

Blind Techniques

It is desirable to use blind techniques wherever possible. However, it is only *crucial* to use blind techniques in the assessment of subjective outcomes such as rectal palpation for cystic ovaries or classification of a culling as "due to infertility." Decisions about the administration of subsequent treatments to animals which do not appear to respond (e.g. intrauterine infusions) are generally based on subjective assessments. These too should be made by someone unaware of which treatment the cow received.

The administration of the treatments can be kept blind if the products can be made to look identical, with only a drug code to identify them. However, a pharmacist's advice should be sought before drugs are modified by the addition of colouring agents or diluents. If the volumes of the two treatments are roughly equal, a simpler approach can be taken. Syringes can be prepared ahead of time and then covered with tape to hide the contents. In small herds, it is common for both the producer and the practitioner to know cows individually and be capable of remembering which treatment they receive. In this case, it is important to use blind techniques when administering the treatments if possible.

Outcomes which are objective in nature, e.g. "days open" can be obtained from the herd's records, although care must be taken in ensuring animals have been accurately identified and records diligently kept. As mentioned above, subjective assessments *must* be made by individuals unaware of which treatment group the animal is in. This is best accomplished by keeping all records on sheets in which the identity of the drug used is not recorded or is only present in a coded form.

Other Considerations

Before starting the trial, it is important to ensure that you have the consent of the producers involved (2). In addition to obtaining their approval, you should discuss the issue of compensation. It is conceivable that the new product may be significantly inferior to your current standard and its use during the trial may cost the producer money. You should come to a clear understanding as to who is responsible for such losses before carrying out the trial.

Once the trial is completed, you need to analyze the data. When doing this, it is tempting to keep "digging" until something statistically significant is found. However, if the trial has been properly designed and carried out, decisions about the superiority of a new product should be based largely on the results dealing with the outcome(s) identified as most important.

Finally, if there are any aspect of the trial design which concern you, consider seeking professional assistance. Most schools of Veterinary Medicine now have qualified analytical epidemiologists and/or biostatisticians who will be willing to provide assistance. If consulted during the design phase of the trial, they may be able to assist with, or carry out, the subsequent analysis of the data.

Conclusion

This paper has addressed the three key steps to be considered when designing a clinical trial. It assumed that control animals, preferably treated with a currently used product, will be included in the trial. Failure to include appropriate controls will make the results totally meaningless.

Attention to "specifying the question to be answered," "dealing with the role of chance" and "preventing bias" will ensure that a clinical trial generates useful and reliable results. The conduct of randomized controlled clinical trials can be within the realm of all bovine practitioners. Providing producers with reliable results, based on locally conducted trials will not only generate client support, but may greatly enhance a practitioner's level of professional satisfaction.

Appendix A

Formulas for Calculating Sample Sizes for Clinical Trials.

Continuous Variables

These are variables such as days from calving to conception, which are measured on a continous scale (i.e. they can have a wide range of values.

$$n = 2\left(\frac{(Z_{\alpha} - Z_{\beta})s}{(X_1 - X_2)}\right)^2$$

Where: n = the number of animals in *each* treatment group

Z_{α} = value of Z for desired confidence level
for = 0.05 Z = 1.96
for = 0.1 Z = 1.65

Z_{β} = the value of Z for the desired power of the trial (the power is 1-β)
for β = .2 (power = .8) Z = -0.84

X_1 = the expected result in treatment group #1

X_2 = the expected result in treatment group #2 (Note the difference between X_1 and X_2 represents the minimum difference that you want to be able to detect).

s = the estimate of the variability (standard deviation) of the result. The easiest way to estimate this is to choose a range of values which will encompass 95% of cows (e.g. 100 to 200 days for calving to conception interval). This range will be approximately the average $\pm$ 2 stardard deviations (e.g. 150 $\pm$ 50 – this suggests that the standard deviation is approximately 25).

Proportions

These are variables in which the result for an individual cow is recorded as a yes/no result and the proportion of cows with a "yes" result calculated (e.g. proportion culled for infertility).

$$n = \frac{(Z_{\alpha}\sqrt{2PQ} - Z_{\beta}\sqrt{P1Q1 + P2Q2})^2}{(P1 - P2)^2}$$

Where: n = the number of animals in *each* treatment group

Z_{α} = value of Z for desired confidence level
for = 0.05 Z = 1.96
for = 0.1 Z = 1.65

Z_{β} = the value of Z for the desired power of the trial (the power is 1-β)
for β = .2 (power = .8) Z = -0.84

P_1 = the expected proportion in treatment group #1

P_2 = the expected proportion in treatment group #2.
(Note the difference between P1 and P2 represents the minimum difference that you want to be able to detect).

P = The average of P1 and P2
Q = 1 - P (similarly for Q1 and Q2)

A more detailed discussion of sample size estimation procedures has been published (6).

References

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