Measuring the Therapeutic Efficacy of Antibiotics: A Method for Practitioners

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Every year, veterinarians and beef producers administer millions of doses of antibiotics to cattle. Yet very little work has been carried out to determine the efficacy of those treatments. It is assumed that antibiotic treatment of cattle with bacterial infections is advantageous, but given the wide variety of antibiotics available, it is surprising that there is very little evidence as to which products are most effective under field conditions.

Rates of disease and even response rates to therapy likely change from year to year. Consequently, measuring the efficacy of a single antibiotic at any point in time is likely to provide very little meaningful information. In order to obtain useful estimates of antibiotic efficacy, a practitioner must carry out a randomized controlled clinical trial of several different products. While this may sound daunting, following some simple guidelines will ensure the trials produce 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 a new antibiotic for the treatment of respiratory disease in feedlot calves to a current therapy. A good example of one such trial has recently been published (1).

The guidelines outlined in this paper are not intended to replace more detailed discussions of how to carry out clinical trials (2). 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) Specify the treatment protocol.
(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:

"How well does that new antibiotic work?"

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 on new cases of respiratory disease, does the "new" antiobiotic alter the initial response or relapse rates compared to the one currently being used?"

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 (3). This has been stated so often that the necessity of including controls will not be be discussed further in this paper. However, consideration will be given to how the controls will be treated. As was stated above, any new treatment should be compared to the one you are currently using. It is more important to know if the new product is superior to ones currently in use than to simply estimate the response and relapse rates for the new product.

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 specification in the objective that only 'new' cases should be included, it is important to define what will constitute an "acceptable case" for inclusion in the trial. This may be based on a minimum rectal temperature (e.g. 40.5 C) and the absence of any detectable abnormalities in other body systems. As a general rule, animals included in a clinical trial should be free from other concurrent diseases.

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. A minimum number of days of therapy (e.g. three) must be given to effectively evaluate an antibiotic. It is likely that the feedlot operator will be administering all treatments so it is important 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. The following outcomes should be considered in an antibiotic trial.

(1) initial response based on:
  -temperature reduced to less than 39.5C
  -subjective assessment of status
(2) relapse rate based on:
  -meeting the inclusion criteria one or more days after return to pen
  -must define length of follow up period
(3) case fatality rate—% of cases of respiratory disease that die from respiratory disease
(4) 60 day weight gain (measured from time of entry into feedlot)
(5) overall mortality rate—% of cases that die from all causes

Overall mortality rate and 60 day weight gains are included to ensure that the new product does not have any unexpected adverse effects on mortality or productivity. While many outcomes can be included in a trial it is important to select one or two economically important parameters as the primary outcomes. Decisions about the superiority of the new drug should be based on these previously identified outcomes. The initial response and relapse rates along with the case fatality rate are likely the best measures for an antibiotic trial. Other outcomes, such as number of days treated or time from initial response to relapse, may shed light on why the new treatment was, or was not, superior but should not be used to assess its overall effectiveness.

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") (4). 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 response rate in animals treated with the antibiotic you are currently using)?
(b) If the results are measured on a continuous scale (i.e. 60 day weight gain) as opposed to a yes/no result (e.g. relapse rate), how variable are the results? This is expressed as the standard deviation and a technique for estimating it is presented in Appendix A.
(c) How small a difference is clinically significant? (If you think it is important to statistically confirm that the new drug results in a tiny improvement of 2% in the initial response rate, 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 specifiecd 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 (5). 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. Assessment of outcomes which are relatively rare (e.g. a case fatality rate of less than 10%) require much larger samples than outcomes which are closer to 50% (e.g. initial response rate) or which are measured on a continuous scale (e.g. 60 day weight gain).

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 (6).

The second step is to ensure that the two groups are treated and assessed equally. 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 as the animal enters the sick pen.

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, the first animal receives treatment #1, the second gets treatment #2, the third treatment #1 and so on in an alternating manner. 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 physical examination of cattle to determine if they can be returned to the feedlot. Decisions to switch an animal to another antibiotic (i.e. a treatment failure) are often based, in part, on subjective assessments. These also must be made by someone unaware of which treatment the animal received. This requires that a third antibiotic be available for use in these cases.

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.

Outcomes which are objective in nature, e.g. "60 day weight gain" 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 (3). 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, treated with a currently used antibiotic, are 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 '60 day weight gain' 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. 40 to 100 days for 60 weight gain.). This range will be approximately the average $\pm$ 2 standard deviations (e.g. 70 $\pm$ 30 - this suggests that the standard deviation is approximately15).

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. initial response rate).

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

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 (7).

References

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