DESIGNING FIELD TRIALS TO COMPARE VACCINES OR ANTIBIOTICS

Cate Dewey
Department of Population Medicine
University of Guelph
Guelph, Ontario N1G 2W1
E-mail: cdewey@uoguelph.ca

ABSTRACT

Field trials conducted in your own barn will provide real results of the impact of a new product in your herd on your pigs. It is essential to have control pigs so that you can determine the difference in health and production between the treated pigs and the untreated pigs that are living in your barn at the same point in time. Treatment must be randomly assigned to pigs so that there is no difference between the treated and the control pigs. Ideally, the people in the barn will not know which pigs received which treatment so they will not care for the pigs differently. This is called blinding the trial. If you want to determine whether or not there is a significant difference between two groups, the number of pigs must be large enough to measure the expected difference. Sample size equations can be used to determine the number of pigs needed. If the treatment cannot be randomly assigned to an individual pig, then the calculations will be based on pens of pigs rather than individual pigs. This is true for in-feed treatments.

INTRODUCTION

Maintaining healthy pigs, improving productivity, and minimizing the effects of disease through the use of effective management procedures or biologicals (vaccines and antimicrobials) is important. Field trials are a very useful way to test the ability of a product to improve production or decrease illness and death. We obtain information about the efficacy, and/or safety, of a specific treatment from personal and collective experience, laboratory studies, observational field studies, and experimental field studies (called clinical, or field, trials). Although each source of information can provide some insight into the effectiveness of the treatment, a well designed clinical trial will provide the most valid information about how the product works under field conditions. Field trials conducted in your own barn will provide real results of the impact of a new product in your herd on your pigs.

For a field trial to be valid, the observed association between the treatment and the outcome must be due to the treatment and not the result of chance or of bias. If you give a growth promotant to only the smallest, sickest pigs and then compare their growth rate to the large, healthy pigs who did not get the medication, you will conclude that the growth promotant did not work. That is called bias. The results were 'caused' by how you assigned the treatment.

CONTROLS

Control pigs, those that do not receive the "new" treatment, are essential if we want to know the impact of the new treatment. For example, when egg yolk antibody became available to treat post weaning E. coli diarrhea in nursery pigs, many producers and veterinarians thought the product worked well. However, this clinical problem often caused severe losses in one batch of pigs and reduced losses in the next. It was not possible to distinguish between normal variation in clinical problems due to the disease and the impact of the very expensive egg yolk antibody. Extensive research work by Dr. Bob Friendship proved that the egg yolk antibody was not effective. These results were only possible because his work included CONTROLS. The pigs in the control groups, that did not receive the egg yolk antibody, performed as well as the pigs that received the egg yolk antibody. When we do not use controls, we can only compare performance to historical data. We all know that one group of pigs is not the same as the next. Only with side by side controls, pigs that are being raised in the same barn at the same time, can we honestly measure the impact of a new product.

EXPERIMENTAL UNIT

The experimental unit is the smallest independent group of pigs that can be assigned to a treatment group. Even if you want to know the average daily gain (ADG) of individual pigs, if the treatment can only be administered to pens, then pens become the experimental unit. For example, if you are testing a feed additive, then the pen is the smallest group that can be assigned to the treatment, so the statistical analysis is done by comparing productivity at the pen level. This treatment should be randomly assigned to pens of pigs and the growth rate should be compared on a per pen basis. To determine the number of pens to use in a trial you need to decide what your expected outcome will be for each of the treatment groups on a per pen basis, and the variance (variability) between pens in the trial size (there are equations shown below that can be used to calculate sample size). To reduce the total number of pigs on the trial, you can keep the number of pens as large as possible and reduce the number of pigs per pen.

When experimental unit is the pig and pigs of different treatments are housed together we must consider spill-over of treatment effects. For example, if the treatment is a vaccination or a dewormer, herd immunity could mask a true treatment effect. Herd immunity reduces the challenge to the control group and decreases the differences in outcome between the treated and control groups. Sometimes the treatment looks less effective compared to the non-vaccinated pigs because of herd immunity. Alternately, if treatment is assigned to individual pigs and then pigs of one treatment are housed in the same pen, the unit of analysis is pen and not pig because the treatment effect is confounded by the pen.

RANDOM ASSIGNMENT

The pigs should be allocated to treatment groups using a formal random process. This means that each pig in the trial has the same chance of being selected to receive the treatment or

control. There should be a clearly outlined treatment protocol prior to the beginning of the trial. This should include how the drugs will be prepared, stored, and delivered; where the injections will be given; how often the feed should be delivered; when and how to remove fines from the feeders. The control can either be no treatment or the standard treatment. For example you might compare a new vaccine to the vaccine your herd typically uses.

BLINDING

Blinding is what we do to make sure that people involved in the trial do not know which pig is in which treatment group. This reduces bias. Blinding ensures that pigs in the two treatment groups are treated in the same manner and prevents one treatment from falsely appearing better than the other treatment. For example, if a farm worker knows that the pigs with yellow ear tags were given the new vaccine, he might be less likely to treat that pig for sickness than the pig with the red ear tag that is part of the control group.

SAMPLE SIZE

We need to determine the appropriate sample size to use. A sample that is too small will result in finding no difference between the groups when a difference does exist. A sample that is too large is expensive. The sample size is determined by how variable the outcome (ADG or death rate) is expected to be, the expected difference between treatment and control groups, and how sure you want to be of the results (usually set at 95%).

Chi-square tests determine the difference between independent rates or proportions. The death rate for unvaccinated pigs is compared to the death rate of pigs that were vaccinated using a chi-square test. Sum the number of unvaccinated pigs that died and the number of vaccinated pigs that died and record the number of pigs in each group that lived. We had 500 pigs in the study and each pig was randomly assigned to receive the vaccine or not receive the vaccine.

	Died	Lived
Not vaccinated	22	228
Vaccinated	9	241

The p-value is 0.01 and the odds ratio is 2.5. We conclude that pigs were 2.5 times more likely to die if they were not vaccinated than if they were vaccinated. This difference would occur by chance alone 1% of the time.

ADJUSTING RATES OF GAIN FOR DEATH LOSS

A feeding trial was conducted on 24 pens of pigs (3 replicates of 8 pens). The pigs were grouped as 30 pigs per pen. Alternate pens were given either feed A or feed B. The unit of concern is the smallest level at which the factor of interest changes. Because we assign feed type by the pen, the unit of concern is the pen, not the pig. Hence the unit of concern was the pen, and the sample size was 24. The target population refers to the pigs about which the decision is to be made. In this example, the target population is the population of nursery pigs on the farm.

All statistical tests and sample size equations assume that the treatments were randomly assigned. In our nursery feed example, if 250 pigs were to be fed in 8 pens, the pigs should be randomly assigned to pens. The numbers from 1 - 8 are put in a hat. As each pig runs down the hall, a number is drawn from a hat and the pig is marked according to his pen number. Alternatively, each pig can be given an ear tag and a random number table can be used to determine which pigs go in each pen. If two feeds are to be used, flip a coin to determine which feed pen #1 is assigned, then alternate feeds for every other pen.

BIAS is a systematic error in measurement or a systematic difference between groups. Selection bias occurs when a group of pigs is assembled incorrectly. Random assignment of pigs to pens, and feed to pen should remove bias. It is assumed then that the weight of the pigs will be randomly distributed by pen and thus there will be no association between the weight of the pigs and any other factor. Even if the producer normally sorts pigs by weight at weaning, it was inappropriate for a feed trial. Controls, pigs fed the usual diet, are important as a comparison group. In a trial dealing with an injection, the controls should receive an injection of sterile water. The person(s) measuring the outcome should be blinded to the trial. The diets should be labelled A and B in similar bags. Often preconceived ideas of the results of the trial will bias the outcome. All pigs weaned on the farm during the trial should be included in the trial so that the results are representative for the herd. The trial should be repeated over time to ensure that the results are consistent.

For each pen we had the weight of pigs starting and finishing the trial. We plotted the distribution of average daily gain by pig per pen by feed and the mean, median, and standard deviation of growth rate. We used a Student's t-test to determine if there was a difference in growth rate by feed. Although, on average, these pigs were fed for 20 days, this varied by pen. Typically the crude daily gain per pig for feed A will be compared to the crude daily gain per pig for feed B. But, for a more accurate comparison between the feeds we should use weight gains adjusted for dead and culled pigs.

PIG-DAYS are calculated as the (number of pigs at the end of the trial * the number of days those pigs were on the trial) + (the number of pigs that were removed * the days they were in the trial).

For example, a pen that started with 30 pigs and finished with 26 pigs would be as follows:

26 pigs on a 20 day trial	=	520 pig-days
1 pig died on day 5	=	5 pig-days
1 pig died on day 10	=	10 pig-days
2 pigs died on day 15	=	30 pig-days
TOTAL	=	565 pig-days

Similarly, to calculate the **WEIGHT GAIN** of the **PEN** we weigh the pigs that die during the trial and include their weights in the total gain of the pen. We also need to determine if significantly more pigs die on feed A than on feed B (or vice-versa). After adjusting for the number of pig-days and the actual weight gain of the pen, we compare the adjusted daily gain on feed A to the adjusted daily gain on feed B. The Student's t-test is used for this statistical comparison.

If you do not know on which day the pig died or the weight of the dead pig then use the midpoint of the trial as the day of death and the average weight of the pigs at the midpoint of the trial as the weight of the dead pig. Alternatively, if we know that all of the pigs died within one week of the trial then the assumptions can be changed.

SAMPLE SIZE ESTIMATION

Sample Size Estimation for Two Means (Averages)

$n = 2[Z_a - Z_b) S / (X_e - X_c)]^2$

n = number of sows required in each group

$Z_a = 1.96$ (for a 95% confidence interval)

$Z_b = - 0.84$ (for a power of 80%)

X_c = the average litter size in naturally bred sows

X_e = the average litter size in artificially bred sows

S = the standard deviation of litter size

$n = 2[Z_a - Z_b) S / (X_e - X_c)]^2$

$n = 2 * [((1.96 - (-0.84)) * 2.6) / (9.7 - 9.2)]^2$

$n = 2 * [(2.8 * 2.6) / (9.2 - 9.7)]^2$

n = 424

To find a difference of 0.5 pigs born alive, there needs to be 424 sows randomly assigned to the treatment group and 424 sows randomly assigned to the control group.

Sample Size Estimation for Proportions

$n = [Z_a (2PQ)^{1/2} - Z_b (P_eQ_e + P_cQ_c)^{1/2}]^2 / (P_e - P_c)^2$

n = number of sows required in each group

Z_a = 1.96 (for a 95% confidence interval)

Z_b = - 0.84 (for a power of 80%)

P_c = the average farrowing rate in vaccinated sows (0.85)

$Q_c = 1 - P_c$
= 1 - 0.85
= 0.15
= the average (return + open) rate in vaccinated sows

P_e = the average farrowing rate in unvaccinated sows (0.80)

$Q_e = 1 - P_e$
= 1 - 0.80
= 0.20
= the average (return + open) rate in unvaccinated sows

P = overall average farrowing rate = [(0.85 + 0.80)/2] = 0.825

Q = the average of (1 - P)

= the average (return + open) rate for both groups

= [(0.15 + 0.20)/2] = 0.175

$n = [Z_a (2PQ)^{1/2} - Z_b (P_eQ_e + P_cQ_c)^{1/2}]^2 / (P_e - P_c)^2$

$$n = \frac{1.96 (2*0.825*0.175)^{1/2} + 0.84 (0.85*0.15 + 0.80*0.20)^{1/2}]^2}{(0.85 - 0.80)^2}$$

n = 45

To determine if farrowing rate differs in vaccinated and unvaccinated sows the vaccine needs to be randomly assigned to 45 sows and 45 sows need to be left unvaccinated.

EXERCISE

Assume you are working with a herd that has high mortality in the finisher barn due to Actinobacillus pleuropneumonia (APP, formerly called Haemophilus pneumonia). The herd is positive for Porcine Circovirus Type II and the herd is not currently vaccinating the nursing or nursery pigs. The death loss in the finisher barn is likely caused by both Circovirus and APP.

You wish to vaccinate weaned pigs against Porcine Circovirus Type II but you don't know which commercial product will work the best in your herd.

How are you going to randomly vaccinate pigs with vaccine A and vaccine B?

What do you want to consider as you decide how to randomly assign pigs to treatment?

What factors do you think might influence the response to the vaccine?

I expect the litter will have an effect. Pigs in one litter will have similar levels of maternal immunity and therefore will be more alike than pigs in different litters.

If we randomly assign the vaccine to one litter or another, then our unit of analysis is litter because that is the smallest group at which we randomly assigned the vaccine. If we randomly assign the vaccine to pen in the nursery, then nursery pen is the unit of analysis. If however, we randomly assign vaccine to piglet, then the piglet is our unit of analysis. This will result in the smallest required sample size.

How are you going to randomly vaccinate pigs with vaccine A and vaccine B?

How are you going to follow the pigs through the barn?

What measures will you take to decide which vaccine works best in your barn?