Bovine Abortion: The Case-Control Study as a Different Approach to Diagnosis

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Case History

A farm in the Midwest is experiencing problems with abortion. This farm is milking around 80 cows, raises its own heifers at another place, and has a rolling herd average of 16,000 pounds. Heifers are bred with a bull at the other farm and brought to the milking farm 4 - 5 weeks before freshening. These springers are housed in the same area as the dry cows until approximately one week before freshening. All cows are vaccinated with a multivalent vaccine for Leptospirosis, Infectious Bovine Rhinotracheitis, and P13 at the time of dry off. Milking cows are fed a total mixed ration of corn silage, haylage, and high moisture corn. Dry cows and springers are fed corn silage with hay or green chop, depending on the time of year.

On May 1 an aborted calf approximately 120 days gestational age was found in the freestall area. On June 1, one heifer aborted 2 weeks before her calving date. On June 10 another heifer aborted. The veterinarian was called out and he submitted serum samples from both heifers to the diagnostic lab, results were negative. A dry cow aborted on June 15. The fresh fetus and placenta were carried, by the producer, to the diagnostic lab. It will be 2 weeks before results are available. Another heifer and a cow had late term abortions on June 17 and 18. Another cow in the low group aborted twins on June 21. Immediate action is called for but no diagnosis can be made from the laboratory findings.

Clinical findings

All 7 of the animals appear in fair to good physical condition, no loss of appetite was reported. Except for the May abortion, all aborted calves were 1 to 3 weeks preterm, but were dead upon expulsion, with no autolysis. No other physical abnormalities are noted in cows or heifers.

Implementing a case-control study

In order to promptly halt the progression of the disease, by eliminating possible causal factors, a case-control study is initiated. Cases are defined as all animals having an abortion in the preceding 2 months. Potential controls are defined as any confirmed pregnant animal in the herd during this same time period that delivered a live calf or has not aborted as yet. Controls are selected, by a convenience sample, from other animals in the herd. For efficiency sake and because the animals are available, 3 controls are selected for each case. Therefore, there are 7 cases and 21 controls. The resulting data, for the cases, are shown in Table 1. Of the controls selected, 7 are heifers, 14 are cows, 4 of the controls are kept in the dry lot, 17 are not (table 2).

Table 1. Characteristics of Abortion cases (example data)

Abortion date	Production group	Due Date
5/1	high	10/1
6/1	heifer	6/14
6/10	heifer	6/24
6/15	dry	6/30
6/17	heifer	7/10
6/18	dry	7/2
6/21	low	9/1

Table 2. Characteristics of selected controls (example data)

Group	Location	Due Date
High	freestall	11/2
High	freestall	10/16
Heifer	other farm	10/1
High	freestall	9/25
High	freestall	9/3
Heifer	drylot	8/14
Low	freestall	7/28
Low	freestall	7/15
Dry	drylot	7/5
Dry	drylot	6/26
Heifer	freestall	6/20
Heifer	other farm	11/1
High	freestall	10/15
High	freestall	9/28
Heifer	other farm	9/12
High	freestall	8/30
Low	freestall	8/3
Low	freestall	7/25
Dry	drylot	7/10
Heifer	freestall	6/30
Heifer	freestall	6/25

In a case-control study the objective is to determine if there is some animal characteristic or environmental factor that is more commonly found in the diseased animals (cases) than in the nondiseased (controls). Since the heifers received no vaccinations for common infectious abortifacients, it seems reasonable to investigate if being a heifer is a risk factor for abortion. This can be accomplished with a 2 x 2 contingency table, as shown in Figure 1. Factor positive (F+) animals are heifers, factor negative (F-) are dry or milking cows. This analysis indicates that if an animal is a heifer, she is slightly more likely to have an abortion than if she were a cow, as shown by the odds ration (OR) calculation. It is assumed that if the OR = 1 then there is no effect from the risk factor. If the OR is less than 2, the effect is considered to be minimal. The calculation of the OR is discussed below.

Figure 1. Analysis of the effect of being a heifer on the risk of abortion (D+)

		Outcome D+	Outcome D-	
				OR = (3★14)/(7★4)
Heifer	F+	3	7	OR = 1.5
Cow	F-	4	14	
		7	21	

Since heifers are only slightly more likely to abort than cows, other factors must be investigated. Housing and feeding of animals in the dry lot area is substantially different than the milking cows, for example these animals are receiving green chop. The effect of this factor is analyzed in Figure 2. According to this analysis, animals in the dry lot are much more likely to have an abortion. Or, put more accurately, the odds of D+ animals having been in the dry lot are 10.6 times greater than the odds of D- animals having been in the dry lot.

Figure 2. Analysis of the effect of being in the Dry lot on the risk of abortion (D+)

		Outcome D+	Outcome D-	
				OR = (5★17)/(4★2)
Dry lot	F+	5	4	OR = 10.6
Cow lot	F-	2	17	
		7	21	

Further questioning reveals that the producer had just purchased 2 new dry cows. These cows were from a farm that was going out of business due to an abortion storm of undetermined origin. These animals were separated from the rest of the group. No further abortions occurred.

Epidemiology has saved the day!

It is important to note that these data were hypothetical and not meant to infer that feeding green chop will cause abortion.

Conducting a case-control study

The essential goal of a case-control study is to determine if the frequency of occurrence of a particular hypothesized exposure is higher in the diseased (case) group than the non-diseased (control) group. It is a way to quantify our intuition about the relationships between risk factors and disease. This is in contrast to other experimental studies that begin with exposed (treated) and non-exposed groups then compare the number of cases in the two groups. In many situations, such as the above example, it is not clear what has caused the exposure so experimental analysis is not possible. Also, the immediacy of the situation precludes planning and conducting an experimental study.

Although, the discovery of an exposure occurring in higher frequency in the diseased versus non-diseased group does not necessarily prove causation, it does suggest some association. This should be combined with clinical experience, prior hypothesis, and reasonable biology to provide a rational basis for action.

Selection of cases and controls

The first step in implementing a case-control study is the definition and selection of cases and controls. The signs of disease that are of particular interest should be carefully noted, and only animals meeting those criteria should be considered as cases. Certain exclusion criteria may also be defined. For example, recently purchased animals might be excluded if the focus was on farm factors only. The selection of controls can be confusing unless one remembers this simple rule. **A control is an animal that would be counted as a case if it had exhibited the signs of disease.** This assures that cases and controls will come from the same population of interest. It is not necessary that controls be randomly chosen, nor that they accurately represent the overall animal population. If enough controls are available, as in the above example, more than one control can be included for each case. This increases the sample size and improves the statistical power. Generally, more than 3 or 4 controls per case are inefficient.

When selecting controls, care must be taken that the hypothesized exposure is not somehow tied to the controls. For example, if in the above case, heifers had been selected as controls so as to match the proportion of heifers in the case group, the results would have been biased. This is due to the fact that being a heifer virtually assures an animal of being in the dry lot and getting exposed to the green chop. This is separate from the issue of matching in case-control studies. Matching does indeed bias results and must be adjusted for in the analysis. Matching will not be discussed in the paper.

Analysis

The odds ratio is a fairly simple statistic that compares the odds or probability of an event in one group with the odds of the event in another group. The comparison, in this situation, being the occurrence of an exposure in the diseased group compared to the occurrence of that same exposure in the non-diseased group (figure 3). The odds of being exposed and diseased (F+, D+) equals a/c. The odds of being exposed and non-diseased (F+,D-) equals b/d. The ratio of these two odds converts to the classic "cross products ratio", (a★d)/(b★c).

Figure 3. Example 2 x 2 table for odds ratio (OR) Calculation

		Cases D+	Control D-
Exposed	F+	a	b
Nonexposed	F-	c	d
		a+c	b+d

OR = (a★d)/(b★c)

The magnitude of the odds ratio reflects the strength of association between an exposure and disease. The value of 1 for an odds ratio infers no association. Generally, the OR must be greater than 2 in order to be considered as a likely cause for disease. It should be noted that a high value in the odds ratio is not the same as statistical significance. One can test if an odds ration is "statistically significant" by a simple Chi-square analysis on the above table (Martin *et al.*,1987). Statistical significance does not prove causation, nor does that the lack of it disprove causation. The computation of a confidence interval on the odds ratio will provide more information than a simple test for significance. In the above example, the 95% confidence interval on heifer (F+) as a risk factor was .19 -11.8. That this interval includes 1.0 indicates that an association is unlikely. The interval on the dry lot exposure was 1.12 - 129. This interval does not include 1.0 and can be considered statistically significant. Inexpensive computer programs are available to calculate Chi-square statistics and confidence intervals on the odds ratio (Epistat, 1987; Epiinfo, 1988)

Advantages of case-control study

Case-control studies have several advantages, the formost being their simplicity. As in the above example, only a few animals are needed to begin to make inferences. The calculation of an odds ratio can be implemented on the farm. These studies are retrospective, thereby allowing a clinician the luxury of looking back into history once a problem begins to emerge versus planning and implementing an experimental trial. It is even possible to use clinical records that were collected over a long period of time for a case-control study. Another significant advantage is their applicability to rare diseases such as the above example. An advantage of case-control studies, similar to other epidemiologic studies, is that they take place in the "real world" instead of a controlled experimental setting.

References

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