An Epidemiologic Approach to Investigating Abortion Problems in Dairy Herds

A thorough investigation, including appropriate laboratory samples, may greatly increase the probability of an agent diagnosis in an abortion case.

The evaluation of bulk tank milk shipment weights over time may provide clues as to when an infectious agent entered a dairy herd.

The scan statistic offers a method for evaluating the likelihood of the start of an abortion outbreak.

ABSTRACT: A standardized approach to investigating abortion problems in dairy herds can greatly increase the chance of making a positive diagnosis. This article discusses the definition of an abortion, expected abortion frequency, data that should be collected during an abortion investigation, methods of processing the data, and interpretation of the results. The epidemiologic approach is not intended to replace the submission of tissue samples to the diagnostic laboratory but rather to supplement that process. This article provides dairy practitioners with a set of tools that will maximize the likelihood of successfully identifying and correcting the underlying cause of dairy herd abortions.

Abortion problems in dairy herds often pose a frustrating challenge for herd managers and veterinarians trying to solve these problems. The difficulties arise for several reasons: 1) there are no well-established guidelines on what constitutes an abortion problem, 2) the magnitude of the problem is often difficult to estimate because most abortions are not observed, 3) the inciting event for an abortion may occur months prior to the abortion, 4) known abortifacient agents are identified in only about one-quarter of the cases submitted to diagnostic laboratories, and 5) if given an agent diagnosis, intervention strategies are often limited to herd vaccination or feed changes. To alleviate some of the frustration, an epidemiologic approach to investigating abortion problems was developed. The investigation protocol consists of seven steps (see box on opposite page) that are aimed at identifying key determinants (i.e., risk factors under management control) as potential points of intervention and is intended as a supplemental procedure to standard diagnostic methods.
CONFIRMING THE PROBLEM

The first step in investigating the reported abortion problem is to confirm that a problem exists and to determine to what degree. There are two scenarios in which abortion becomes a herd problem. The first scenario is a herd in which there is a significantly increased incidence of abortion over a long period. The second is when a significant clustering of abortions (an abortion outbreak) occurs within a short time. Determining when a herd has had a significant number of abortions is both difficult and controversial and requires knowledge of what normal abortion frequency is and what level above normal is acceptable for that particular dairy herd. The box on the right gives standard definitions of pregnancy loss.

Estimates of the normal annual abortion frequency for dairy herds range from 0.4% to 10.6%. The following three procedural differences may account for the wide variation in these frequency estimates:

- The gestational age that is considered an abortion if the conceptus is lost.
- Determination of whether unobserved abortions were included as cases. Cows are defined to have an unobserved abortion if they fail to calve or are determined to be open after being diagnosed as pregnant. An assumption in determining the number of unobserved abortions is that there are no false-positive pregnancy diagnoses by rectal palpation. It is important to remember that about 3% to 10% of pregnant cows will show signs of estrus and that estrus behavior alone is not a reliable indicator of an unobserved abortion.
- Determination of whether adjustments were made for changes in the population at risk over time (i.e., calculating the abortion rate). Changes in the population at risk over time are a common problem in the analysis of biologic data because events tend to be clustered in space and time. In studies of abortion, changes in the population at risk occur over time because cows that are pregnant may leave the herd prior to delivery of the calf, such that their subsequent abortion status is unknown. Thus the number of pregnant cows in the denominator of the rate equation is overestimated, and the proportion aborting is falsely low.

Taking these procedural differences into account, normal annual abortion frequency appears to be about 2% to 5% for observed abortions, 5% to 8% for both unobserved and observed abortions, and about 10% for both unobserved and observed abortions, including adjusting for changes in the population at risk. Therefore, annual abortion frequencies in excess of 8% should be considered significantly increased.

Determining what constitutes a significant clustering of abortions is a difficult task. One approach to help determine the magnitude of an outbreak has been the use of the scan statistic, which operates on the basis that biologic events are not uniformly spaced but are randomly distributed on a time line. If the number of events expected for a given time interval (e.g., a year) can be estimated from previous experience, then the probability for a given number of events to occur in a smaller time interval (e.g., 30 days) can be calculated based on random distribution theory. The drawbacks of this approach are that it assumes that conceptions are

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**Abortion Investigation Protocol**

1. Confirm the extent of the problem.
2. Collect core individual cow data concerning both aborted (case) and nonaborted (control) cows.
3. Complete a management questionnaire.
4. Generate a list of events that have occurred at the farm level.
5. Create temporal plots of relationships between events on the farm and the start of the problem.
6. Conduct a case–control comparison.
7. Collect risk group–based samples.

**Definitions of Pregnancy Loss**

- **Early embryonic death** occurs from conception to the 42nd day of gestation (the end of placentation).
- **Abortions** are losses that occur from the 42nd to the 260th day of gestation (the point at which the fetus is considered capable of sustaining life outside the uterus).
- **Premature deliveries** are losses that occur from the 260th day to term.
evenly spaced throughout the year and that herd size does not change significantly. Figure 1 shows the probabilities associated with 0 to 14 cases of abortion in any 30-day period for herds with 4 to 48 cases expected annually. Based on this figure, a herd that normally expects 16 abortion cases in a 12-month period has about 0.50 or a 50% chance that four cases will occur in a 30-day period. In other words, there is a 50% chance that four cases occurring in a 30-day period is not an outbreak but would be anticipated given that 16 abortions are expected in a year. This approach should not be used as a statistical test (i.e., less than a 5% chance is considered to be significant) but is intended to give the investigator an idea of how likely this amount of clustering is.

COLLECTING THE DATA

After the extent of the problem has been determined, basic data must be gathered about each case for later use in the case–control analysis. Examples of the core data used in the diagnostic protocol include animal identification, lactation and location or string numbers, freshening and breeding dates, breeding type (artificial insemination versus natural service), pregnancy diagnosis and abortion dates, mature equivalent milk or relative value, and disease history.

Data on the management policies of the dairy should then be collected. This can be accomplished by administering a management questionnaire. The questionnaire serves three functions: It allows comparison of management influences between farms, it structures questioning such that specific topics are not overlooked, and it serves as a measure of dairy policy compliance. Factors that should be included in the survey are general information (e.g., farm identification, location), information about new animal arrivals, vaccination history, housing management/facilities, reproductive management, grouping of animals, nutritional management, and waste management/sanitation.

The third step of the investigation should focus on creating a farm event listing, which is a written record of farm-level events listed by date of occurrence. Events that should be recorded include the arrival of new animals, feed changes, whole-herd vaccination, previous episodes of illness, and any herd-level management changes. Information from at least 3 months prior to the start of the abortions should be collected.

The final data to be collected is a record of the bulk tank milk shipments. Evaluation of this information may provide evidence of a date of exposure to the inciting event, such as an infection spreading through the herd. Shipping weights alone must be carefully interpreted according to the number of cows contributing to the tank; the average production per cow is a better measure of milk production. Daily variation in milk production may be smoothed using a 10-day moving average. Data should be collected for at least 6 months prior to the first abortion case.

PROCESSING THE DATA

The first data processing step is to create temporal plots of the farm event listing and bulk tank data. These graphic depictions of events allow juxtaposition of abortion case dates with farm level events or changes in production to help provide valuable insight into events that may be associated with the onset of the abortions. Plotting of farm events is most easily performed using a horizontal bar graph with the farm events listed on the ordinate (y axis) and the time plotted on the abscissa (x axis). Events that occur on a single day are best represented by a symbol, such as an "X." The abscissa is set up with day 0 being listed as the date of the initial abortion case. Abortion cases are best plotted as the first row above the abscissa. Using this arrangement, investigators can scan for vertical associations of farm events that coincide with the start of the abortion problem. A hypothetical farm event temporal plot is shown in Figure 2. In this example, the dairy used three different forages prior to the outbreak, vaccinated the whole herd 36

![Figure 1](image-url)
days prior to the outbreak and 16 days after, purchased 10 new springers 17 days before the outbreak, and hired a new feeder 20 days prior to the outbreak. From the timing of events, it appears most likely that the new feed, the new feeder, and the purchased springers (factors most closely related to the date of the initial abortion) are possible sources of the abortion outbreak.

A temporal plot of bulk tank data can be constructed in a similar manner with moving average milk production, fat, protein, and somatic cell counts plotted against time. Figure 3 shows a bulk tank milk shipment temporal plot for two *Neospora* abortion outbreaks. Milk production has been standardized to a percentage of day 0 production to allow comparison across herds.

Further questioning of the herd managers on these dairies identified the initial drop as being associated with a change to a wet feed, suggesting the possibility that the *Neospora* outbreaks were initiated by a cofactor.

After construction of temporal plots, a matched case–control analysis should be performed. The concept of a case–control analysis is to compare the history of animals that aborted with the history of those that are pregnant and did not abort. In the protocol, cases and controls are matched by month of conception to reduce the potential bias due to uncontrollable variables, such as weather changes or contaminated batches of feed. Selecting controls requires the following three-step process:

- **Step 1**—Construct a table of all abortion cases by month of conception.
- **Step 2**—Make a similar table of all cows that did not abort by month of conception.
- **Step 3**—For each aborting cow, randomly select one control cow from the same month. Thus there should be one control cow for each abortion case and the same number of controls from each month as abortion cases. After the controls have been selected, the core information recorded earlier for each abortion case should also be recorded for each control. Data from cases and controls should then be used to create $2 \times 2$ tables (Figure 4) for analysis of the proportion of cases (and controls) found in each risk group.

This odds ratio represents the risk of disease for animals in the top row of the table (animals with the risk factor) relative to the risk of disease for animals in the bottom row (animals without the risk factor). For example, if the top row refers to animals older than 3 years of age and the bottom row refers to animals 3 years of age or younger, an odds ratio of 2.0 means that the older animals are twice as likely to get the disease than the younger animals. Note that the odds ratio by itself does not provide any indication of the actual risk; it would be the same if 50% of the old cows and 25% of the young cows were diseased or if 5% of the old and 2.5% of the young were diseased.
What does it mean if the odds ratio is less than 1? When setting up the table, it may not be known whether the factor that is placed on the top row is related to an increase in disease risk. In a case in which the odds ratio is less than 1, the risk factor chosen for the top row is actually protective (i.e., animals with this characteristic are at less risk). In this case, the odds ratio can be inverted and the risk factor can be listed as the bottom row. For example, suppose the table is constructed with old cows on the top row and young cows on the bottom, and the calculated odds ratio is 0.5. This finding can be interpreted as old cows are 0.5 times as likely to develop this disease or that young cows are twice as likely (1 ÷ 0.5 = 2.0) to develop the disease.

In general, an odds ratio greater than 2.0 (or less than 0.5) is considered to be fairly large. A statistical testing approach is not being used but rather the strongest relationships are being identified. For example, if investigating an abortion outbreak in cattle and the odds ratio for old versus young cattle is 1.9, the odds ratio for fat versus thin cattle is 2.3, and the odds ratio for pasture one versus pasture two is 14.0, then the investigator should be curious about how pasture one differs from pasture two. Using this approach, investigators must always be cognizant of the biological plausibility of these relationships. A large odds ratio does not necessarily indicate that the relationship is important in the disease progression.

**RISK GROUP–BASED SAMPLING**

The last step in investigating abortion problems in dairy herds is to collect laboratory samples based on risk groups. Risk group–based sampling has two benefits. First, it allows comparison of case animals with animals in other risk groups for changes in laboratory values. Second, this process increases the total number of samples submitted to the laboratory, which improves the likelihood of identifying the source of the problem. Common risk group categories include age, location, level of production, reproductive status, and treatment and feed groups. An example of a risk group sampling protocol for an abortion investigation would be to bleed animals for serology in the following groups: cows that have aborted (at risk, affected), cows that are pregnant but did not abort (at risk, unaffected), and cows that are not pregnant (not at risk). At least 10 samples should be collected from each risk group, if possible, to ensure adequate sample size for comparison across risk groups. If possible, samples should also be collected from matched pairs (i.e., both a case and control that calved in the same month).

Interpreting the results of this analysis is based on comparing findings from abortion cases to those from controls in another case–control analysis. Using the serology example, a cutoff titer value must be established and the number of cases and controls above and below this value should be compared. For example, if investigators were looking at leptospirosis titers and had determined that a value of 100 was significant, they could then compare the number of cases above and below 100 with the number of controls above and below 100. Using a 2 x 2 table (Figure 4), investigators would conclude that cows with leptospirosis titers over 100 were four times more likely to abort, suggesting a leptospirosis problem.

**CONCLUSION**

This epidemiologic approach to abortion investigation should supplement rather than replace laboratory diagnosis. The probability of an agent diagnosis is highly influenced by the samples submitted. One study found that the probability of getting an agent diagnosis from the state diagnostic laboratory was increased more than four times by submitting a complete kit instead of serology alone. For that study, a complete kit was defined as paired sera, placenta, and an intact fetus. Investigating abortion problems is a frustrating task. Using a standard investigational approach, however, will maximize the likelihood of successfully identifying and correcting the underlying cause.

**REFERENCES**


ARTICLE #5 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. Choose the best answer to each of the following questions; then mark your answers on the postage-paid envelope inserted in Compendium.

1. The minimum gestational age in which pregnancy loss should be considered an abortion is ____ days.
   a. 20
   b. 31
   c. 42
   d. 60

2. The maximum gestational age in which pregnancy loss should be considered an abortion is ____ days.
   a. 230
   b. 240
   c. 250
   d. 260

3. The maximum gestational age at which pregnancy loss is considered an abortion corresponds to the time a cow enters the dry pen.
   a. at which the fetus is capable of life outside the uterus.
   b. when the fetus may be expelled by prostaglandin use.
   c. none of the above

4. What is a normal annualized abortion rate for a dairy when considering observed and unobserved abortions?
   a. 1% to 2%
   b. 2% to 5%
   c. 5% to 8%
   d. greater than 10%

5. The client should fill out a management questionnaire because it
   a. enables a comparison of management influences between farms.
   b. structures questioning so that specific topics are not overlooked.
   c. serves as a measure of dairy policy compliance.
   d. all of the above

6. Data for a farm event listing should span at least
   a. 1 month.
   b. 3 months.
   c. 6 months
   d. 1 year.

7. From which group of cows should controls for the case–control analysis be selected?
   a. all cows that did not abort
   b. cows in the same pen as those that aborted
   c. open cows
   d. pregnant cows that did not abort

8. Matching in the case–control analysis should be based on
   a. month of conception.
   b. month of calving.
   c. milk production.
   d. lactation number.

9. Submitting a full abortion kit (paired sera, placenta, and an intact fetus) rather than serology alone increases the odds of a positive diagnosis by ____ times.
   a. two
   b. three
   c. four
   d. more than four

10. The minimum number of samples that should be taken for risk group–based sampling is
    a. 3.
    b. 5.
    c. 10.
    d. 20.