An Epidemiologic Approach to Investigating Abortion Problems in Dairy Herds

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Introduction

Abortion problems in dairy herds often pose a frustrating challenge for herd managers and veterinarians trying to solve them. Reasons for this difficulty arise because: 1) there are not well established guidelines on what constitutes an abortion problem, 2) the magnitude of the problem is often difficult to estimate as most abortions are not observed, 3) the inciting event for an abortion may occur months prior to the abortion, 4) known abortificaient agents are identified in only about one fourth of cases submitted to diagnostic laboratories, and 5) given an agent diagnosis, intervention strategies are often limited to herd vaccination or feed changes. As a result of this frustration, an epidemiologic approach to investigating abortion problems was initiated at Washington State University. The investigation protocol consists of seven steps: confirmation of cases, a management questionnaire, development of a farm event listing, collection of bulk tank milk shipment and milk quality reports, construction of temporal plots, a matched case-control analysis, and risk group based sampling. The goal of this protocol is to identify key determinants (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 what degree. There are two scenarios in which abortion becomes a herd problem. The first is a herd in which there is an "significantly" increased incidence of abortion over a long time frame. The second is when a "significant" clustering of abortions occurs in a short time frame (an abortion outbreak). Determining when a herd has had a significant number of abortions is a difficult and controversial question and requires a knowledge of what is a "normal" abortion rate and what level above the normal rate is acceptable to that particular dairy.

Estimates of the normal annual abortion rate for dairies range from 0.4% to 10.6% (Holt, 1952; Kirkbride *et al.*, 1973; Kirkbride, 1979; Klingborg, 1987; Norton *et al.*, 1990; Thurmond *et al.*, 1990). There are three procedural differences that may account for the wide variation in these rate estimates. The first difference is the gestational age that is considered an abortion if the conceptus is lost. The standard definitions for pregnancy loss in use today are: losses from conception to the 42nd day of gestation (the end of placentation) are early embryonic death, losses from the 42nd to the 260th day of gestation (point when fetus is considered capable of life outside the uterus) are abortions, and losses from the 260th day to term are premature deliveries (Thurmond & Picanso, 1990). One practical advantage to this set of definitions is that cows are not considered at risk of aborting until they are known to be pregnant and pregnancy diagnosis is usually performed near the 42nd day of gestation.

The second procedural difference is whether or not 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 pregnant. An assumption in determining the number of unobserved abortions is that pregnancy diagnosis by palpation is 100% accurate. It is important to remember that about 5-10% of pregnant cows will show signs of heat and that estrus behavior alone is not a reliable indicator of an unobserved abortion.

The last procedural difference in previous estimates of abortion is whether adjustments were made for changes in population at risk. Changes in population at risk are a common problem in the analysis of biological data as events tend to be clustered in space and time. In studies of abortion, changes in population at risk occur because cows that are pregnant may leave the herd prior to delivery of the calf such that their subsequent abortion status is not known. 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 abortion rates appear to be about 2-5% when considering observed abortions only, approximately 5-8% if you consider both unobserved and observed abortions, and about 10% if you consider both unobserved and observed abortions and adjust for changes in population at risk (Kinsel, 1993). As a rule of thumb, annual abortion rates in excess of 10% should be considered significantly increased rates.

Determining what constitutes a significant clustering of abortion has been a difficult issue. One approach to help decide the magnitude of an outbreak has been the use of the *scan statistic* (Wallenstein, 1980; Wallenstein & Neff, 1987). The basis for the scan statistic is that biological events are not uniformly spaced, but are randomly distributed on a timeline. If the number of events expected for a given time interval (i.e. a year) can be estimated from previous experience, then the probability for a given number of events to occur in a smaller time interval (i.e. a 30 day period) can be calculated based on random distribution theory. Figure 1 shows the probabilities associated with 0 to 14 cases in any 30 day time period for herds with 4 to 48 cases expected annually. Based on this figure, the probability that a herd that normally expects 16 abortion cases in a 12 month period could have four cases in a 30 day period is about 0.50 or a 50% chance.

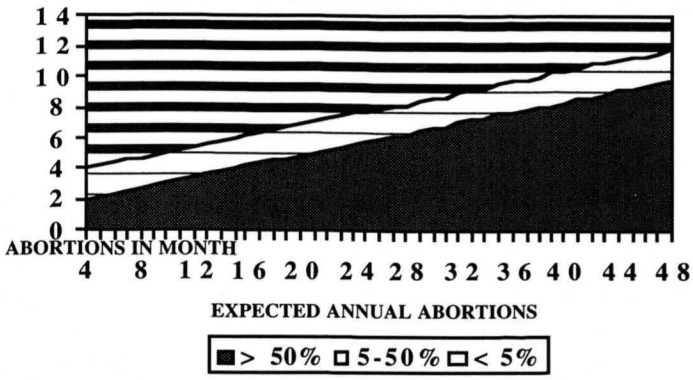


Figure 1. Scan statistic probabilities for clustering.

Collecting the Data

Once the extent of the problem has been determined, a basic set of information is gathered concerning each case for later use in the case-control analysis. The core data set used in the WSU protocol is: animal ID, lactation number, location or string number, freshening date, breeding date, pregnancy diagnosis date, abortion date, mature equivalent milk or relative value, and disease history.

The second set of information that needs to be collected is data concerning management policies of the dairy. This can best be accomplished by administration of 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 forgotten, and it serves as a measure of dairy policy compliance. Topics that should be included in the survey include: general information (farm ID, location, etc.), information concerning new animal arrivals, vaccination history, housing management / facilities, reproductive management, grouping of animals, nutritional management, and waste management / sanitation. An example survey is presented in Appendix 1.

The third step of the investigation should focus on creating a farm event listing. This document is a written record of farmwide events listed by date of occurrence. Events that should be recorded are entry of animals from outside the farm, feed changes, whole herd vaccination, previous episodes of illness, and any herdwide management changes. Information collected for this farm event listing should go back at least three months prior to the start of the abortions.

The last set of data to collect is a record of the bulk tank shipments and milk quality reports. Evaluation of this information may provide evidence of a date of exposure to the inciting event. It is important to remember that shipping weights alone must be carefully interpreted in light of the number of cows contributing to the tank and that the average production per cow is a much 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 six 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 graphical depictions of events allow juxtaposition of abortion case dates with farm level events or changes in production that can provide valuable insight into events that may be associated with the onset of the problem. Plotting of farm events is most easily performed using a horizontal bar graph with each type of farm event listed on the y-axis and time plotted on the x-axis. Events that occur on a single day are best represented by a symbol such as an "X". Abortion cases are best plotted as the first row above the x-axis. Using this arrangement, the investigator can scan for vertical associations of farm events with the start of the abortion problem. A hypothetical farm event temporal plot is shown in Figure 2. This dairy used three different forages prior to the outbreak, vaccinated the whole herd 36 days prior to the outbreak and 16 days after, purchased 10 new springers 17 days before the outbreak,

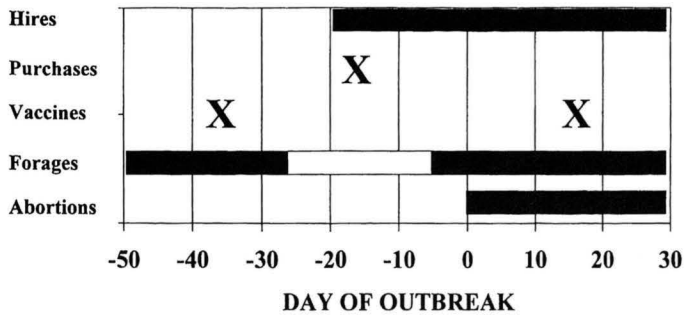


Figure 2. Example farm event temporal plot.

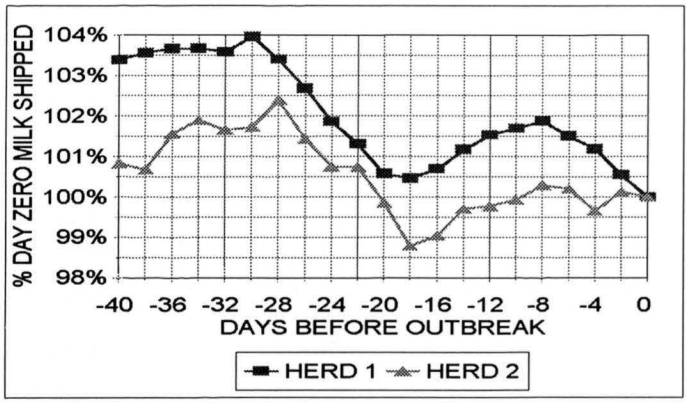
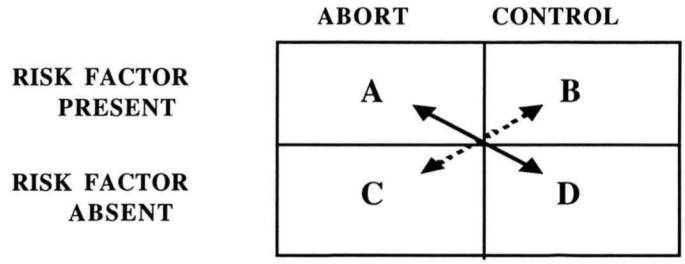


Figure 3. Milk shipments for two Neospora herds.

and hired a new feeder 20 days prior to the outbreak. Possible sources of the problem are the new feed, the new feeder, and the purchased springers.

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 from two Neospora abortion outbreaks. Milk production has been standardized to a percentage of day 0 production to allow comparison across herds. Notice how there is a biphasic pattern with the initial drop approximately 30 days prior to the outbreak and a second decline the week prior to the outbreak. Further questioning of the herd managers on these dairies identified a change to a wet feed associated with the initial drop suggesting the possibility of Neospora outbreaks being initiated by a cofactor.

After construction of temporal plots, a matched case-control analysis is performed. The concept of a case-control analysis is to compare the history of animals which aborted with animals that are pregnant and did not abort. In the WSU 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. Selection of controls is a three step process. Step 1 is to make a table of all abortion cases by month of conception. Step 2 consists of making a similar table of all cows who did not abort by month of conception. In step 3, one control cow from the same month as the aborting cow is randomly selected for each aborting cow. Thus, there should be one control for each case and the same number of controls from each month as cases. After the controls have been selected, the core information recorded earlier for each case should be recorded for each control. Data from cases and controls are then used to create 2 x 2 tables similar to Figure 4 for analysis of the proportion of cases (and controls) found in each risk group.



$$\text{ODDS RATIO} = \frac{A \times D}{C \times B}$$

Figure 4. 2 x 2 table setup for case-control analysis.

Risk Group Based Sampling

The last step in investigating abortion problems in dairy herds is to collect laboratory samples based on risk groups. This risk group based sampling has two benefits. First, it allows comparison of case animals to animals in other risk groups for changes in laboratory values. Second, it will increase the total number of sample submitted which increases the likelihood of identifying the source of the problem. Common risk group categories include age, location, level of production, reproductive status, treatment groups, or feed groups. An example risk group sampling protocol for 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). It is important to remember that at least 10 samples should be collected from each risk group if possible to assure adequate sample size for comparison across risk groups.

Conclusions

This epidemiologic approach to abortion investigation is intended as a supplemental to, not a replacement for, laboratory diagnosis. Remember that the

probability of an agent diagnosis is highly influenced by the samples you submit. In a 1993 study at WSU, 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 single serology alone (Frech *et al.*, 1993). For that study, a complete kit was defined as paired sera, placenta, and an intact fetus. Although investigating abortion problems is a frustrating situation, having a standard investigation approach will maximize the likelihood of successfully identifying and correcting the underlying cause.

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Appendix 1

ABORTION QUESTIONNAIRE

Abortion is a significant source of economic loss in dairies. The following questionnaire is aimed at identifying management practices that influence the occurrence of outbreaks of abortion. It is important that you try to answer all questions completely even if you do not feel you have a significant abortion problem. All responses should represent your management practices **AT THE TIME OF THE ABORTION PROBLEM**, not what is currently being done. If you do not have an abortion problem, answer questions based on your management practices in **THE LAST 12 MONTHS**. All responses to questions will be kept confidential and your comments are welcome in the space provided on the last page.

GENERAL INFORMATION

Name of Dairy: _____

Address of Dairy: _____

Phone number: _____ Veterinarian for Dairy: _____

What was the maximum number of mature animals on your dairy last year? _____

Was the number of animals on your dairy ever 5% less than the amount above? _ Yes _ No

How many times per day were your cows milked last year? _____

What was your RHA milk production last year? _____ Highest BTSCC last year _____

Do you raise all of your own replacement animals? _ Yes _ No

Have you purchased any replacement animals during the last year? _ Yes _ No

Have you purchased any replacement animals during the last 3 years? _ Yes _ No

VACCINATION PROGRAM

Which of the following vaccines were used in calves 2 - 6 months old?

_ MLV IBR _ Killed IBR _ MLV BVD _ Killed BVD

_ Salmonella _ Brucella _ Leptospira _ Campylobacter

_ Hemophilus _ Other-please specify: _____

Which products did you use in your calves? _____

Which of the following vaccines were used in heifers prior to breeding?

_ MLV IBR _ Killed IBR _ MLV BVD _ Killed BVD

_ Salmonella _ Leptospira _ Campylobacter _ Hemophilus

Which products did you use prior to breeding? _____

Which of the following vaccines were used in lactaing cows prior to breeding?

_ MLV IBR _ Killed IBR _ MLV BVD _ Killed BVD

_ Salmonella _ Leptospira _ Campylobacter _ Hemophilus

Which products did you use prior to breeding?

Which of the following vaccines were used in pregnant animals?

_ MLV IBR _ Killed IBR _ MLV BVD _ Killed BVD

_ Salmonella _ Leptospira _ Campylobacter _ Hemophilus

_ E. coli _ Rota-Corona

Which products did you use in pregnant animals? _____

HOUSING MANAGEMENT

STRING	DIMENSIONS	FREE-STALLS

What type of facility were your animals housed in last year?

_ Free stall _ Tie stall/Stanchion _ Loose housing

Has the total number of stalls available for the herd changed during the last year?_____

Which of the following materials were used for bedding last year?

_ Straw _ Shavings _ Sawdust _ Sand _ Manure solids

Did your housing facilities (for milking cows) have ridge-vents? _ Yes _ No

Did your housing facilities (for milking cows) have walls? _ Yes _ No

Average carbon dioxide levels in barns? _____

Circle the months listed below that dry cows were on drylot?

J F M A M J J A S O N D

Circle the months listed below that lactating cows were on drylot?

J F M A M J J A S O N D

After a heavy rain, how long did standing water remain in the drylot? _____

Was a source of shade available while cows were on the drylot? _ Yes _ No

BREEDING PROGRAM

What percentage of your heifers were bred by artificial insemination last year? _____

What percentage of your cows were bred by artificial insemination last year? _____

What percentage of your heifers were bred by natural service last year? _____

What percentage of your cows were bred by natural service last year? _____

What percentage of services were by a commercial A.I. technician last year? _____

How many breedings are attempted by A.I. before moving cows to a cleanup bull? _____

Were there regularly scheduled vet checks for the herd last year? _ Yes _ No

How frequent were these routine vet checks? _____

Which of the following types of examinations are made during a routine vet check?

_ Pregnancy _ Postpartum _ Prebreeding _ Problem Breeders

GROUPING OF ANIMALS

Did you separate dry cows from the lactating cows? _ Yes _ No

Did you separate sick cows from the lactating cows? _ Yes _ No

Did you separate close-up cows from the lactating cows? _ Yes _ No

Did the dry cows and sick cows ever share a pen? _ Yes _ No

Did the close-up cows and sick cows ever share a pen? _ Yes _ No

FEEDING/NUTRITION PROGRAM

What percentage of your ration was made up of the following forages last year?

_ Alfalfa hay _ Alfalfa haylage _ Grass hay

_ Grass haylage _ Corn silage _ Green chop

Circle the forages listed above that you raised on your dairy.

What percentage of your ration was made up of the following concentrates last year? _____

_ Corn _ Barley _ Wheat _ Oats _ Other:_____

Circle the concentrates listed above that you raised on your dairy.

What percentage of your ration was made up of the following commodities last year?

_ Urea _ Whole cottonseed _ Delinted cottonseed _ Cottonseed meal

_ Soybean products _ Sunflower products _ Beet pulp _ Cannery waste

_ Brewers grains _ Bakery waste _ Wheat fines _ Other:_____

Was a mineral/vitamin supplement added to any rations? _ Yes _ No

What form of supplemental selenium was added to the dry cow ration?

_ Special dry cow ration _ Lactating cow ration _ TM salt _ Special mineral

Were dry cows given selenium injections? _ Yes _ No

Were any other cows given selenium injections? _ Yes _ No

Did you feed a total mixed ration (TMR)? _ Yes _ No

How many times per day did you feed the TMR? _____

Were cows fed grain from a computer grain feeder? _ Yes _ No

How many times per day did you feed concentrates? _____

How many times per day did you feed roughages? _____

Did you feed any grain in the parlor? _ Yes _ No

How often were the mangers/feed bunks cleaned out? _____

PASTURE MANAGEMENT

Circle the months listed below that dry cows were on pasture?

J F M A M J J A S O N D

Circle the months listed below that lactating cows were on pasture?

J F M A M J J A S O N D

Could cows on pasture drink from streams, ponds, ditches, or other sources of standing water?

_ Yes _ No

Was the pasture fertilized in the last year? _ Yes _ No

Which of the following methods were used to water the pasture?

_ Sprinkler irrigation _ Rainfall only _ Flood/ditch irrigation

For irrigated pasture, which of the following sources of water were used for irrigation?

_ Irrigation ditch _ Spring water _ Lagoon water

_ Well water _ Pumped from river directly

What was the shortest period between irrigation and grazing/harvesting of fields? _____

Did you spray slurry on your pasture last year? _ Yes _ No

Did you spray slurry on any fields harvested for forage last year? _ Yes _ No

Did you spread any manure on your pasture last year? _ Yes _ No

Did you spread any manure on fields harvested for forage last year? _ Yes _ No

What was the shortest period between application of wastes and grazing/harvesting? _____

SANITATION PRACTICES

Did you use a flush system to clean your pens last year? _ Yes _ No

What source of water did you use for your flush system? _ Fresh _ Recycled

Did you manually scrape manure out of any pens last year? _ Yes _ No

How many times per day did you scrape/clean your pens and alleyways? _____

Which of the following were used for manure disposal last year?

_ Lagoon _ Pit _ Hauled away _ Spread on field

NEW ADDITIONS TO HERD

For each animal purchased in the last year, list the following:

Cow ID	Lactation	Date purchased	Source of animal

ABORTION TIMELINE

For each cow that was diagnosed pregnant, but failed to produce a calf last year, list the following information (columns with ? are yes/no questions):

Cow ID	Date diagnosed pregnant	Breeding date	Abortion observed?	Date of abortion	Samples submitted?