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Investigation of Reproductive Problems in Beef Herds

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Low pregnancy rates and higher than expected abortion rates can result in significant losses to cow-calf producers. Investigation of these complaints is challenging because the underlying cause has often occurred some time before the problem is recognized and there is typically very little diagnostic information available. Many times, cows that are not pregnant and suspect bulls are sold before the full extent of the problem is realized or laboratory samples collected. In other cases, poor herd records may limit the success of the investigation. Despite these frustrations, outbreak investigations can be an excellent opportunity to build a practice and herd investigations can be a change from the routine of every day practice. The basic principles of herd investigations have been reported in many sources¹⁻⁴ and are summarized here.

A herd example⁵

A commercial operator reported a sudden and alarming occurrence of abortions in a herd of 350 cows. The first fetus was recovered 5 days after weaning in the fall. Vaginal discharge and exposed fetal membranes were observed in many other cows. The owners estimated that 20% of the cows aborted during the next three weeks. Three additional fetuses were submitted for laboratory testing during this period. Other partial fetuses were seen on pasture. Because the cattle were far from the home quarter and the coyote population was high, attempts to recover additional fetuses were not successful during the initial outbreak.

Objectives of the investigation

The objectives of the investigation were to measure the extent of the loss in this herd, identify risk factors for the outbreak, and if possible, recommend procedures to reduce the risk of future outbreaks. Risk factors are characteristics of the host, agent, or environment that are associated with an increase in disease probability or reduced performance. For example, common risk factors for impaired fertility in beef cattle include body condition at calving, bull performance, reproductive tract infections, and nutritional deficiencies.⁶ To control an outbreak, the investigator must identify the "key determinants" or risk factors over which management has control that can be altered to affect disease rates or production levels.^{7,8} This can be achieved most consistently by differentiating the characteristics of affected and unaffected animals within the herd with respect to time, place, other potential exposure factors, and environmental influences.²

Procedures for investigating disease outbreaks

The following questions (W5) provide the basic framework for all outbreak investigations:^{1,4,9}

- **What** (and how much)
- **Who**
- **When**
- **Where**
- and finally, **why**.

Obtaining a history

An accurate and complete history requires good listening skills and careful attention when probing for additional information, without using leading or loaded questions. Everyone working with the cattle is consulted. The objective is to obtain a clear chronological picture of events. The history of a reproductive failure investigation should begin prior to the start of breeding and include information from the last calving season. In this case, calendars and old calving records were examined to verify the timeline of events



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and the previous reproductive performance of the herd. It transpired that in this investigation, there was too much information to ensure a complete and accurate history from a single interview. Therefore, a detailed questionnaire was faxed to the herd owner as part of the follow-up to the initial visit. This problem-specific questionnaire was used to verify the information collected during the visit and to obtain more detail on important issues. Example questionnaires for investigating infertility problems have been published⁶ and can be found at www.vdpam.iastate.edu/PAM/outbreak.htm.

The investigators made several visits to the farm to collect information. Herd visits are necessary to collect a detailed history, examine the animals, observe management, and collect appropriate samples and herd records for analysis. Both distant and close examinations of the animals are required. For this case, detailed notes, photos, and video recordings were used to document the visit.

Defining the problem (What?)

A case definition is critical when comparing cases with non-cases to determine the importance of potential risk factors for disease. A simple, easily recognized, and applied definition facilitates the consistency of cases being reported by the herd owner, other farm workers, and veterinarians on the case. The inclusion of unrelated cases can result in errors in recognizing factors that might have contributed to the outbreak.¹⁰

Ribble and others⁴ have suggested that the first and most critical task in the investigation is to clearly define the problem. One challenge in investigating this particular mid-gestation abortion storm was that the herd had not been pregnancy-tested before the outbreak. Initially, only cows that were observed aborting could be reliably differentiated from those that had failed to conceive. However, this definition failed to account for cows that had aborted, but were not observed. For this investigation, it was decided to palpate all the cows and define a case as a cow confirmed open by palpation before the end of November. The difference between failure to conceive and abortion is important because low pregnancy percentages for beef herds and mid- or late-term abortions are associated with very different risk factors. Many abortions could be verified from the herd records. The owners recorded the eartag and date for most of the cows they observed with either vaginal discharge or visible membranes in the weeks following the recovery of the first fetus. The percentage of non-pregnant cows before the outbreak was probably relatively small. The herd owners observed that only a few animals showed signs of estrus before the outbreak and in the previous two years reported combined non-pregnancy and abortion losses from breeding to calving of 5% and 7%. For this reason, it was assumed that the majority of cows not pregnant in November had aborted, rather than failed to conceive.

Determine the extent of an outbreak or sub-optimal herd production

The extent of, and reaction to, the underlying problem can vary dramatically between different herd owners. Large problems may go unreported, while reproductive losses within accepted or expected rates can be alarming for some herd owners.⁷ Some will seek help after only 1 or 2 abortions in 100 cows.

To determine if reported losses represent a true outbreak, the reproductive performance of the herd is measured by determining the risk of non-pregnancy, abortion, and stillbirth, and by examining the calving distribution pattern. These performance indicators are compared with published or historical expectations or the performance of other neighbouring herds with similar management.

The herd owners in this situation observed and recorded the eartag and date for 61 cows and heifers with obvious clinical signs of abortion; however, there were other cows and heifers that were suspected to have aborted. This could not be confirmed definitively from their records because the losses were too overwhelming during the peak of the outbreak to completely record all suspected abortions. Consequently, all cows and heifers were examined in November – approximately 3 weeks after the first fetus was recovered – to establish a more accurate estimate of the loss and to identify all affected animals. Several cows were identified because the size and tone of the uterus suggested a recent abortion. Mummified and macerated fetuses were also identified during palpation. Overall, 33% (90/270) of the mature cows, and 53% (45/84) of the heifers, were determined to be non-pregnant. Except for the 61 animals that were observed and recorded as aborting, definitive differentiation of non-pregnancy due to conception failure or abortion was not possible in this case. This is substantially higher than expected for a combined “normal” non-pregnant (5-8%) and abortion occurrence (1-2%).

Define the groups of animals affected within the herd (Who?)

Given that there was sufficient evidence to proceed with an investigation, the next step was to answer the question: Which animals or groups were affected (who)? This information was needed to determine what made the cows that aborted different from those that did not.

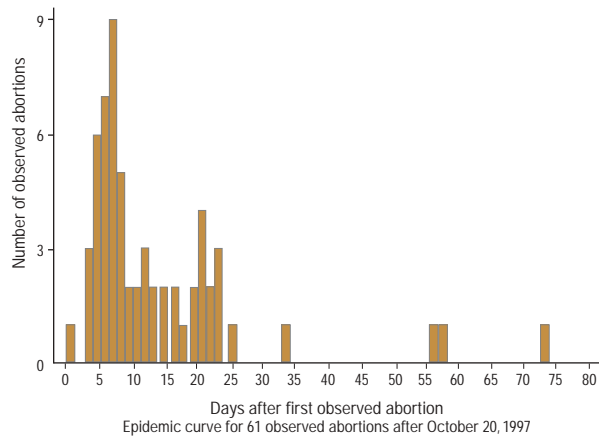
In this cow-calf herd, all the animals were identified and a computerized database system was available to track individual animal histories. During pregnancy testing, the investigators verified the computer herd inventory records and recorded the body condition score for both cows and heifers. Additional information obtained from the records included age and breed; origin (purchased or born on farm); sire and dam; vaccination status; summer pasture location; and history of bull exposure during the recently completed breeding season.

Orient the problem in time (When?)

Understanding the time sequence of an outbreak can provide many important clues about the origin of disease in a herd.¹⁰ A graph of events over time (or epidemic curve) describes the pattern of disease and can be particularly useful for investigating abortion problems. The time of each abortion (x-axis) can be plotted in appropriate intervals (days in this example) against the number of abortions recognized at each time interval (y-axis). The epidemic curve for the abortion outbreak in this herd is shown in Figure 1.

The pattern of disease can suggest if the problem resulted from a single point source exposure or from an infectious agent moving from one animal to the next. The slope of the curve indicates whether the whole group was exposed at one time, or if there were multiple exposures over a period of time. In this example, the epidemic curve has a relatively short time

Figure 1: Epidemic curve for abortions in the beef herd under investigation



span of new cases and a dramatic upward slope. This is most consistent with a point source exposure to the causative agent.⁹ This interpretation is also consistent with the fact that the parts of fetuses found later in the outbreak were more decomposed, suggesting the fetuses might have died early and were retained for a period prior to expulsion.

A less steep upward slope, followed by a more defined downward slope, often with one or more additional peaks, would have suggested a propagated agent.⁹ This pattern may indicate an infectious agent moving between animals and groups of animals. The shape and slope of the curve can reflect the incubation pattern from the time of infection to the time the animal becomes contagious and the timing and number of opportunities for transmission.

Examine the spatial pattern of disease (Where?)

To locate potential point sources for exposure, investigators should compare where the affected animals were housed and pastured in relation to the unaffected animals. In this case, the local municipal map was photocopied and used to chart pasture locations. At the beginning of the breeding season, there were 9 breeding groups that were gradually combined into 2 as the pasture season progressed. Information on the occurrence and timing of movement of groups of animals between different pastures is extremely important, but records on this herd's exact location and movements were not complete. There was little potential for contact with cattle from neighbouring herds. There were no apparent differences in the performance of different bulls or in the number of abortions in cows that had been pastured at different locations. Few single point sources for exposure could be verified in this investigation. Many groups of cattle were fed and watered from different sources.

Two common factors were identified: The owners purchased various mineral mixes that were used on all pastures and there was a small creek that ran between two of the pastures that may have been contaminated with offal from local trappers. However, all of the animals from the herd were exposed to both of these factors and so it was not possible to compare the occurrence of abortion between exposed and unexposed groups.

Collect samples

Collecting samples for laboratory examination is a valuable tool to rule-out or confirm a diagnosis. Samples may also determine and define risk and exposure factors, predict the necessity for and appropriate level of intervention, and eventually, assess the success of attempts to control the problem.¹² Laboratory testing is often the most costly part of the investigation. Undirected "fishing expeditions" are particularly expensive and very rarely productive. Tests should be selected based on whether the results are likely to directly affect management or control decisions.

Table 1: Differential diagnosis of reproductive failure in beef herds

Differential diagnosis of abortion		Examples
Infectious causes of abortion	Protozoal causes	<i>Neospora caninum</i> <i>Tritrichomonas foetus</i>
	Bacterial causes	Leptospirosis <i>Campylobacter spp.</i> <i>Ureaplasma diversum</i> <i>Listeria monocytogenes</i> <i>Chlamydia psittaci</i> <i>Coxiella burnetti</i> <i>Haemophilus somnus</i> <i>Brucella abortus</i>
	Viral causes	IBR BVDV
Non-infectious causes:	Mycotic abortion	<i>Aspergillus fumigatus</i> Other molds and yeasts
	Sporadic bacteria abortions	<i>Actinomyces pyogenes</i> <i>Bacillus spp.</i> <i>E. coli</i> <i>Streptococcus spp.</i> <i>Pasteurella spp.</i> <i>Salmonella spp.</i>
	Bacterial endotoxemia	
	Mycotoxins	Ergot alkaloids
	Plant toxins	Pinus species <i>Astagalus spp.</i> (locoweed) Nitrate-containing plants

Necropsy

As few causes of abortion have distinctive gross pathology, laboratory examination is necessary in almost all cases. Whenever possible, submission of the entire fetus and placenta to the laboratory is preferred to examination in the field. In the case under discussion, all the recovered fetuses and placentas from the herd were sent to the lab. The more fetuses submitted, the better the chance for a correct herd level diagnosis based on quantitative pathology.

The time of loss can be an important clue for infectious etiological agents. The approximate gestational age of the fetus is estimated. Postmortem diagnosis in this case was more difficult because some fetuses were retained *in utero* after death and

others were not found immediately following expulsion. The resulting decomposition obscured lesions and made recovery of the causative organism more difficult in some fetuses.

Four complete fetuses were submitted to the laboratory within the first 3 weeks of the outbreak. No gross lesions were observed. *Neospora caninum* was identified in all 4 fetuses by histopathology and confirmed with immunohistochemistry. The potential contribution of other causes was also considered (Table 1). Tissues were tested for infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), leptospirosis, and a number of other potential pathogens using immunohistochemistry. All samples were negative.

Neospora caninum has not previously been well documented as a cause of abortion outbreaks in beef herds. Further evidence was needed to confirm a herd diagnosis of *N. caninum* abortion, to determine the extent of the infection in the herd, and to explore the role of other potential pathogens.

Other samples

Further investigation of potential point sources through environmental sampling was not possible in this case. There was no remaining mineral mix to test and the creek was nearly dry by the time of the investigation. At the present time, there is no standard method to assess feed sources for contamination with *N. caninum* oocysts. Biological samples examined in this investigation included all the recovered fetuses or placentas, blood, urine, cervical or prepuccial aspirates, swabs of vaginal discharges, and reproductive tracts from slaughtered animals. Feed samples were not available from the period preceding the outbreak.

Serum samples were collected to further examine the potential role of *N. caninum*, IBR, and BVDV in this outbreak. The goal was to look for an association between seropositivity and abortion. Cost will often prohibit laboratory testing of every animal in the herd. The most common approach is to compare laboratory data between cases and controls (non-affected) animals within the herd. To obtain meaningful results, all available cases are often needed, but only a representative sample of the controls. The investigator must select an appropriate comparison or control group. In this case, the remaining pregnant cows were used.

The selection of individuals to sample is very important. Formal probability or random samplings using random number generators or random number tables are the preferred options for selecting representative animals. A frequently acceptable compromise is to use systematic sampling. Systematic sampling involves the selection of animals at equal intervals. The first animal is selected randomly from within the first interval. If 10% of 100 animals are required, a number between 1 and 10 is randomly selected. If this number were 6, then the 6th, 16th, 26th, 36th, ... 96th animal through the chute would be sampled.

Probably the most common question and often the most difficult to address is "How many samples are necessary?" In the present example, blood samples were collected from all cases and controls, but complete herd

sampling is often cost prohibitive. There is no single correct number of samples suitable for every situation. The required number of animals is based on:

- the acceptable degree of uncertainty in the final estimate
- the expected prevalence of the factor of interest, or, for continuous measurements, the variation in the factor within the population
- the size of the population examined.

The number of samples required also depends on the question being asked. In most cases, the investigator must take what is available. If the number of cases is limited, there are some statistical advantages to having more controls than cases up to a ratio of about 4 controls to 1 case. WIN EPISCOPE 2.0 can be used to calculate the required sample size.

Serum banking

In many investigations, there is only one opportunity to collect laboratory samples from the herd during the critical time frame. The clinician cannot possibly direct the laboratory to analyze the samples for every potential question that could arise during the investigation because of cost. If the expenses for sample collection are less than analysis, one potential answer is serum (or sample) banking. Samples are collected and stored with sufficient precautions to ensure that they can be analyzed later with minimal loss of sample integrity and quality. In this example, serum was frozen from each of the cows and was analyzed later for leptospirosis antibodies.

Summarize and analyze the herd data (Why?)

This analysis begins with organizing and summarizing the data for the entire herd. The next step is to quantitatively describe the breed and age structure of the population, document the physical examination and the laboratory results, and then characterize the problem using descriptive statistics. The risk of abortion is compared across different age, breed, or management groups within the herd to determine what is different about the animals that aborted. For example, if the test reflects an important risk factor for fetal loss, the *N. caninum* seropositive animals should have a significantly higher frequency of non-pregnancy or abortion than their negative herd mates.

These comparisons form the initial foundation for the answer to the final question: Why did the outbreak occur? Sometimes these comparisons will identify one or more key determinants for the disease outbreak. Other times, this process targets specific questions that can be addressed by obtaining additional information from the herd owner or laboratory testing.

Attack rate tables

The attack rate is the proportion of the group that is affected during a given period. The attack rate is compared between those that are exposed and those that are not exposed to each individual potential risk factor. To easily visualize this comparison across many different risk factors, an attack rate table (Table 2) is used to help identify the exposures most likely to be associated with disease.

Table 2: Partial attack rate table for the abortion problem in this cow-calf herd.

Suspected risk factors	Exposed animals				Unexposed animals			
	Number affected (not pregnant)	Number not-affected (pregnant)	Total number	Attack rate (%)	Number affected (not pregnant)	Number not-affected (pregnant)	Total number	Attack rate (%)
Age. Heifers (exposed columns) versus mature cows	45	39	84	53%	90	180	270	33%
Neospora status								
– All cows	122	160	282	43%	7	58	65	11%
– Heifers	38	30	68	56%	2	9	11	18%
– Mature cows	84	130	214	39%	5	49	54	9%

The application of the appropriate statistical test allows the clinician to avoid over interpreting a difference between groups that could have been due to chance.^{9,13} In the example above (Table 2), a chi-squared test shows that *Neospora* seropositive heifers were significantly more likely to be open (56%) than seronegative heifers (18%) ($P < 0.025$).

The importance of different risk factors can be objectively compared by measuring the magnitude of the association between each exposure and the outcome of interest or disease. The two common indices used to measure the magnitude of effect of a risk factor are the relative risk and odds ratio (Table 3). The relative risk is the ratio of the risk (or cumulative incidence or attack rate) of disease in the animals exposed to the factor of interest to the risk (or cumulative incidence or attack rate) of disease in those that were not exposed.

Table 3: Relative risk and odds ratio for abortion in cows exposed or not exposed to *N. caninum* as determined by serology

Item	Aborted	No Abortion	Totals
Exposed	38	30	68 (all exposed)
Non-exposed	2	9	11 (all non-exposed)
	40 (all diseased)	39 (all non-diseased)	

$$\text{Relative risk} = [38 / 68] / [2 / 11] = 3.1$$

$$\text{Odds ratio} = [(38 / 2) / (30 / 9)] \text{ or } [(38 / 30) / (2 / 9)] = 38 \times 9 / 2 \times 30 = 5.7$$

In this example, the relative risk is 3.1, suggesting an association between exposure and abortion. If the relative risk were < 1 , then exposure would be associated with a decreased risk of non-pregnancy. If the relative risk is 1, there is no association. The farther the relative risk is from 1, the stronger the mathematical association is between the exposure and pregnancy status. The 95% confidence interval for heifers in this example was 1.2 to 8. As this range does not include 1, the association is statistically significant. Confidence intervals for either the relative risk or odds ratio can be calculated by using shareware programs such as WIN EPISCOPE 2.0.

The odds ratio can also be used to measure the association between exposure and disease for any study type. It is the method of choice for expressing the magnitude of an effect in case-control comparisons. The interpreta-

tion of the odds ratio is similar to that of relative risk, but it may slightly overestimate this value in some cases.

Provide a written report to the herd owner

Preliminary reports summarizing initial findings in this case were issued within a few weeks of the initial herd visit. A final report was issued later when all laboratory analyses were completed.

Some general instructions regarding the report:

- The report must be written for the intended audience.
- A report is prepared for the herd owner, but in some cases, it may be important that a banker or lawyer be able to understand it.
- The report should be well-organized, concise, and explain the findings and recommendations of the study without excessive scientific or industry-specific terminology.^{10,14}
- Tables and graphs can be used to summarize results when possible.
- The recommendations should be presented with sufficient detail to avoid confusion.
- Both short- and long-term recommendations may be necessary.
- The potential costs and benefits should be explained to the herd owner.

In this case, recommendations were limited by our current understanding of *N. caninum* transmission and control. *N. caninum* is recognized as an important cause of abortion in some parts of the world, but was not commonly recognized as significant cause of abortion in beef cattle until recently. Control recommendations are limited, in part because the life cycle of *N. caninum* is poorly described. Preliminary data suggest that dogs are one definitive host. Because an environmental point source exposure was suspected in this case, general strategies for minimizing the potential for fecal contamination of feed sources were recommended. The impracticality of eliminating fecal contamination by wildlife in grazing areas, surface water sources, and forage could be a substantial limiting factor in preventing new infections. New carcass disposal policies were implemented to minimize scavenging by domestic dogs, coyotes, or wolves. It was recommended that all open cows and heifers be separated from the main herd and culled when practical. Options for testing and culling positive replacement heifers were also considered, but no strong data were available to support the effectiveness of culling positive animals in the face of

an unidentified environmental exposure and, as a result, culling of pregnant positive animals or yearling heifers was not pursued. Positive animals, however, were identified and were excluded as recipients for embryo transfer programs.

Investigation follow-up

Relatively few complex problems are completely resolved with a single report. Many investigations are done retrospectively with insufficient information to reach definitive conclusions. In these cases, protracted herd health and productivity monitoring and occasionally planned follow-up studies are required to address outstanding questions.

When there is a rapid solution to the problem and control measures can be introduced, follow-up field studies may be required to determine if the control measures were effective. In this example, a planned randomized clinical trial was used to determine the success of some specific recommendations. This herd was followed over time in an effort to measure the long-term economic consequences and to evaluate control strategies.¹⁵ At the end of the first breeding season following the outbreak, 13.5% of the heifers and 22.2% of the cows were open (not pregnant). Cows with live calves and replacement heifers that were still serologically positive in the spring were more likely to be open in the fall (odds ratio: 2.0, 95% confidence interval, 1.1 to 3.7). No associations with increased risk of abortion, stillbirth, or non-pregnancy were identified in the following two years. Without additional evidence, culling the positive and pregnant beef herd replacements based solely on serological status is not recommended.

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Abstract of Interest

Reproductive performance of a cow-calf herd following a *Neospora caninum*-associated abortion epidemic

WALDNER CL, HENDERSON J, WU JTY, BREKER K, CHOW EYW. This study examines the long-term impact of a *Neospora caninum*-associated abortion outbreak in a large cow-calf herd in northern Alberta. Blood samples were collected 4 times from all bred females and heifer calves born during the spring before the outbreak: (1) at the time of the outbreak, (2) the following spring, (3) the subsequent fall, and, finally (4) the second spring after the outbreak. The samples were analyzed using a commercially available enzyme-linked immunosorbent assay for *N. caninum*. Calves born immediately following the outbreak were also monitored. Individual calving or abortion records were available from all cows for 2 calving seasons. All cows and heifers were pregnancy tested after the 2 subsequent breeding seasons. At the time of the abortion outbreak in 1997, 81% of all bred females and 87% of the heifer calves were serologically positive. In spring 1998, 49% of cows and 47% of the heifer calves remained positive. In fall 1998, 48% of the remaining cows and heifers were serologically positive. After the first breeding season following the outbreak (1998), 13.5% of the heifers and 22.2% of the cows were open (not pregnant). Animals that were serologically positive in the spring were more likely to be open in the fall (odds ratio, 2.0; 95% confidence interval, 1.1 to 3.7). No subsequent associations with increased risk of abortion, stillbirth, or nonpregnancy were identified. *Can Vet J* 2001;42:355-360.

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