

PRACTITIONER'S APPROACH TO INVESTIGATION OF ABORTIONS IN BEEF CATTLE

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Abstract

Investigation of abortions is one of the greatest challenges of cattle veterinarians. Worldwide, diagnostic laboratories only make a specific diagnosis in about one third of submissions (range of 23 to 46% in 5 studies). How can we increase our success rate in diagnosis of abortions? We can, through a combination of application of modern epidemiologic and pathologic principles with a goal of identification of the risk factors that are active in the herd experiencing the abortions. This presentation focuses on obtaining a thorough history to define the problem in epidemiologic parameters, collecting as complete a set of samples as possible and implementation of the case-control method of sampling and risk factor analysis.

Causes of Abortion

A background or abnormal rate of abortions occur in the absence of disease outbreaks. Background abortions are considered to be 2 to 3% in beef herds, and up to 10% in dairy herds. In Texas, the background abortion rate in beef herds in the Standardized performance Analysis database is approximately 2.5%. Abortions cause significant economic losses to beef and dairy cattle. They can be very frustrating to client and veterinarian because they are very difficult to diagnose. Laboratories generally only make a diagnosis in about 1/3 of submissions. *Infectious agents are thought to be the cause of around 50 to 60% of abortions in cattle.* Non-infectious causes include genetic problems, hormonal imbalances, nutritional deficiencies or excesses, and toxic plants or chemicals. Diagnostic laboratories usually diagnose infectious agents when they do make a diagnosis. They are capable of diagnosing some of the non-infectious causes of abortion if the proper samples are sent in. The following list contains most of the presently known causes of abortion of cattle. No doubt there will be more causes discovered in the future, like the *Neospora caninum* breakthrough, that will clarify the muddy waters of bovine abortion.

Infectious

Epidemic

Brucella abortus / *Leptospira* sp. / *Campylobacter fetus* / IBR virus / BVD virus / BRSV / *Trichomonas fetus* / *N. caninum*

Sporadic

Hemophilus somnus / *Listeria monocytogenes* / *Corynebacterium pyogenes* / Bluetongue virus / Cache Valley virus / *Ureaplasma diversum* / mycotic

Genetic

Inbreeding / problem sires / Robertsonian defect

Hormonal

Estrogens (silage/poultry litter) / Progesterone (high pasture protein affects synthesis or clearance)

Nutritional

Deficiencies

Protein / vitamin A / iodine / selenium

Excesses

Protein / urea / copper / iodine

Toxins

Bacterial

Embryonic mortality evidenced by prolonged interestrus intervals and increased services per conception occurs in cases of coliform mastitis of dairy cows.

Endotoxin in gram negative bacterial vaccines, esp. first 2 months and last 2 months of gestation. Dairy cattle highest risk, then purebred beef and crossbred beef least risk.

Increased risk if multiple gram negative vaccines given and if ADE also given.

Plants

Pine needles / broomweed / locoweed / narrow leaf sumpweed

Plant estrogens

Pastures with >30% clover

Mycotoxins

Aflatoxin / ergotamine / fusarium toxin (zearalenone)

Chemicals

Nitrate / OP fertilizer

Investigation of Abortions

An abortion investigation should proceed through a series of steps that include a detailed history, examination of animals, examination of the environment, analysis of data, pathologic diagnosis and epidemiologic diagnosis. *The underlying goal of the investigation is to identify the risk factors active in the herd that are associated with the abortions. The abortions can then be controlled the next year by eliminating or minimizing those risk factors.*

History (Define problem, collect information on herd management)

Problems in past? Lab work done? Diagnoses? Resultant management changes based on diagnoses?

Index case, dates of all calf losses

Numbers of calves lost / number females at risk

Weak calves? / clinical signs?

Fetal defects? (cerebellar/ocular = BVD; *arthrogryposis / scoliosis=bluetongue or Cache Valley*)

Females sick? / retained placentas?

Vaccination program (heifers and cows: what / when)

Nutrition program – mineral program

Herd recently worked? (Gram negative bacterins?)

Access to pine needles?

Recent weather: Heat stress?

Abortions in neighboring herds?

Examination and Sampling of Animals

Dam

(sick / febrile? / retained placenta / hemoglobinuria?/ Cu, I, Se status?)

placenta (fresh on ice and fixed in formalin)

- Send in large amount, not just 1 cotyledon
- *Uterine caruncle* - can be torn off if there is no placenta

Serum

- Paired samples for titers: abortion and 3 weeks later are of *limited use*; occasionally yields diagnosis; best if also sample cows that had normal calves (2 to 3 times the number of affected cows) to compare titers – **(CASE:CONTROL METHOD)**.
Prospective serology: whole herd sampled at preg checks and serums are frozen; at abortions sample abort and non-abortion cows
- Mineral and/or vitamin concentrations if indicated: affected and non - affected
- Nitrate/BUN if indicated: affected and non-affected

Fetus – Necropsy

(Formalin fixed) (Fresh-on ice)

Tissues

Lung	Lung
Liver	Liver
Kidney	Kidney
Spleen	Spleen
Heart	Heart
Brain (half)	Brain (half)
Skeletal muscle	Stomach contents
Thymus	Ocular fluid
Eyelid	
Any abnormal tissues	

Serum - Thoracic fluid or heart blood (FETAL SEROLOGY)

Tests: Abortion screen titers
Total IgG

Examination of Environment

Nutritional assessment and a search for plant or chemical toxins.

Analysis of Data

Data analysis consists of evaluation of the epidemiology and pathology findings.

Epidemiologic Data Analysis

Management or weather events can be recorded above a time line showing dates of abortions (*temporal pattern*) 6 months prior and as long after abortions began as data permits to visualize associations between the abortions and events such as new feed sources or herd additions. For dairies, a graph with the abortion time line as the x-axis and average milk production per cow as the y-axis may reveal the timing of a reproductive insult. Attack rates should be calculated for

cattle of different age groups, pasture groups, pen groups, breeds and origins (*animal and spatial patterns*).

Interpretation of Serologic Results

Fetal serology - Fetal serum abortion agent titer screens and total IgG may be helpful in arriving at a diagnosis. Depending on age of fetus, infectious agent and duration of in-utero infection, fetuses may produce antibodies to reproductive pathogens. In one study, greater than 20 mg IgG/dl was found in 55% of aborted fetuses and only 2% of abattoir fetuses (1). They also observed that 92% of 86 fetuses with greater than 20 mg IgG/dl had histologic lesions. Mean serum IgG concentration of aborted fetuses was 128 mg/dl and only 1.3 mg/dl for abattoir fetuses.

Paired Titers in Dam

Evaluation of serum titers in individuals that have aborted rarely is helpful. Significant: 1) A change from negative to positive, 2) a four-fold increase or 3) a high titer. Having a high titer or even a four-fold increase in titer is not indicative of abortion due to a specific agent. The chances of useful results from titer data of dams increases when they are used in a prospective or in a case – control manner. A titer must be classified as negative, weak or positive for each animal entered into a 2 X 2 table. The following guidelines can be used to classify titers against *Leptospira spp.*, infectious bovine rhinotracheitis (IBR) virus and bovine viral diarrhea (BVD) virus (2).

Agent Titer Classification

Lepto. <100 Negative or exposure > 90 days
100-400 Weak positive or vac. expos <90 days
>400 Positive; field exposure <90 days

IBR <5 Negative or exposure > 90 days
5-40 Weak positive or vac. expos. <90 days
>40 Positive; field exposure <90 days

BVD <5 Negative or immunotolerant
5-160 Weak positive or vac. expos. <365 days
>160 Positive; field exposure <90 days

2 X 2 Table

A 2 X 2 table can be used in a *case-control* manner to evaluate the significance of a specific risk factor, eg. vitamin A deficiency, location on the ranch or positive serology. Sampled cows are classified into 4 groups: aborted and non-aborted, and within each: vitamin A deficient and vitamin A adequate. The number of cows in each category are then entered in the 2 X 2 table.

	Aborted Cows	Cows calved normal
Risk factor present (Vitamin A deficient)	A	B
Risk factor present (Vitamin A adequate)	C	D

A 2 X 2 table can be used to calculate 2 statistics: the odds ratio (OR) and the chi-square test of association. The OR is an estimate of the relative risk associated with the risk factor being evaluated. An OR of 1 implies that there is no association between the risk factor being evaluated and abortion. An OR greater than 1 implies that the risk factor being evaluated is associated with abortions. The equation for the OR is:

$$OR = \frac{A \times D}{B \times C}$$

The odds ratio is meaningless, however, unless it is proven to be statistically valid by the chi-square test of association. That is because often it is calculated from too few numbers of animals. The chi-square value can be calculated by hand or with computer software programs. The equation for the chi-square statistic (X^2) is:

$$X^2 = \frac{N(AD-BC)^2}{(A+C)(B+D)(A+B)(C+D)}$$

N equals the total number of animals in the 2 X 2 table. The larger the chi-square statistic, the less likely the observed results could have occurred by chance alone. Interpretation of the chi-square involves use a Chi-square table. The probability that the observed association could have occurred by chance alone is represented by the *P* value above the chi-square statistic that is closest in value to but less than the calculated chi-square statistic. The following table lists *P* values for various chi-square values derived from a 2 X 2 table.

	<i>P</i> = 0.05	<i>P</i> = 0.10	<i>P</i> = 0.20
Chi-square statistic	3.84	2.71	1.64

Generally, P values of 0.05 or less are considered to indicate a true relationship that did not occur by chance. This looks hard, but it is easy. *You can do it with a hand calculator in 5 minutes. Try it!!*

Pathologic Diagnosis

A positive agent diagnosis should be based on serologic or cultural findings that indicate a specific agent *plus* gross and/or microscopic lesions characteristic of the agent. Care must be taken in diagnoses based solely on serology unless results of case-control samples are evaluated in a 2 X 2 table.

Epidemiologic Diagnosis

The pathologic diagnosis is more of a preliminary diagnosis than a “case closed - final diagnosis”. If the pathologic diagnosis is an infectious agent, the question that must be answered is “what allowed the agent to produce disease in this operation?” That leads to the epidemiologic diagnosis which may be several *key determinants* (risk factors alterable by management). Multiple risk factors may be active in the herd. For most infectious diseases, more than the agent is necessary for disease to result. Biosecurity may be lax, vaccination programs may be poorly carried out, the ranch may be overstocked or there may be deficiencies of minerals necessary for an effective immune response. Control of the abortions can be accomplished by changes in herd management that eliminate or minimize the causative risk factors.

New Approaches to Diagnosis of Abortions

Defining the temporal, animal and spatial patterns of abortions will increase our success in diagnosis. We now know that serology of cases only is generally of no diagnostic value and that we must include control animals in our sampling strategies. *Case-control analyses are capable of identifying the degree of association of a variety of risk factors with abortions.*

Serology of fetal blood or thoracic fluid (3) is capable of separating cases of abortion into infectious and non-infectious categories. Modern genetics is contributing greatly to the diagnosis of abortions. Polymerase chain reaction (PCR) tests for specific infectious agents are much more sensitive than culture and growth because they are able to positively identify agents in samples containing dead organisms. New tests that identify toxic damage to nuclear DNA will eventually be used to sort abortions into toxic or non-toxic categories. Our success in diagnosis of abortions will increase proportionally to the degree that we apply new diagnostic methodology and testing.

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