Field Investigation of Abortion

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Introduction

Determining the cause of perinatal death is one of the most confounding problems presented to bovine practitioners. Many times a calf or aborted fetus is delivered to the clinic doorstep with little additional information. Because perinatal deaths are many times multifactorial, diagnosis is not a simple problem and a thorough investigation is required to have a reasonable chance of reaching a diagnosis.

Although an aborted fetus quickly gains a producers attention, abortion losses are low compared to other causes of perinatal mortality. Most perinatal losses are due to non-infectious causes that are often overlooked, including nutritional imbalances, genetic problems, birth environment, maternal behavior, or management shortfalls. Infectious causes of perinatal mortality are often difficult to identify because gross lesions are not pathognomonic, and many organisms are difficult to culture or isolate. By working closely with the producer, veterinarians can begin to educate their clients about the process of perinatal death diagnosis.

Investigating Abortions

The first step in the investigation is to establish the number and timing of perinatal losses and the number of at-risk females during the period in question. The veterinarian should aid the producer in keeping a written record of perinatal losses, recording the time of deaths and the cause of death as nearly as can be determined, for each calving season. A complete history backed by accurate records allows the practitioner to identify previous problems, the vaccination program, maternal nutrition during pregnancy, herd exposure to other animals, and management practices that impact fetus or calf survivability.

By examining records and getting a complete history from the client, one can begin to get an accurate account of the stage of gestation when the abortions are occurring, the age of dam that is affected and any seasonal pattern to finding aborted fetuses. Important questions to ask the producer include the vaccination history, movement of cattle into the herd, recent diseases or toxicosis, access to plant abortifacients, history of all injectable products given (especially those that the produce may be reluctant to reveal), whether breeding is by artificial insemination or natural service, and type of feed and pasture. While the history is very important to help direct the diagnostic effort, it is important not to eliminate a disease from the list of possibilities because of history alone. For example, a history of vaccination against a particular disease does not remove that disease from the rule-out list, as any abortogenic disease may cause abortion in spite of previous vaccination.

Necropsy examination of as many aborted fetuses and calves as possible allows the best opportunity to isolate a viral or microbial cause and to establish patterns in pathology. The fetus or calf and the placenta should be sent to the nearest diagnostic laboratory or the practitioner should perform as complete a necropsy as possible. The veterinarian performing the necropsy examination should do the following:

1) Establish the time of death (pre- or postnatal)
   Determine aeration of the lungs
   Determine the presence or absence of milk in the abomasum or small intestine
2) Note evidence of anemia, cyanosis, or jaundice
3) Determine the presence or absence of fluid in the abdomen and then the thorax
4) Examine the liver for size, consistency and for evidence of rupture or necrosis

5) Examine the kidney for gross pathologic changes
6) Examine the small intestine for evidence of enteritis or hemorrhage
7) Examine the large intestine for evidence of atresia or colitis
8) Examine lymph nodes, the thyroid, adrenals and pancreas; taking samples if abnormalities are noted
9) Check the heart for amount of pericardial fat, presence of fluid or fibrin on or around the heart, epicardial hemorrhages, and congenital defects
10) Remove the lungs and trachea and determine the degree of aeration, the presence or absence of petechia or edema and note evidence of bronchopneumonia
11) Assess the limb joints for mobility
12) Note the skeletal muscle color
13) Examine the spinal column for evidence of spina bifida, torticollis, kyphosis, scoliosis or lordosis
14) Remove the brain and examine for congestive hemorrhage, abscess, cerebellar hypoplasia, hydrocephalus, hydranencephaly
15) Sample lungs and abomasal contents for bacterial culture
16) Examine the placenta for evidence of infection. The cotyledons should be examined for size, color, and whether they are uniformly or regionally affected. The intercotyledonary area should be examined for consistency, color and distribution of lesions.

In-hospital diagnostics include, making cotyledon or abomasal content smears. These smears should be stained with Gram and modified acid-fast stains. Phase contrast or darkfield microscopy can be utilized to identify trichomonads, campylobacters, leptospires and fungal hyphae from abomasal contents, cotyledonary smears, placental tissues and uterine fluids. Fetal peritoneal fluid and maternal urine can be examined under phase contrast or darkfield microscopy to identify leptospires. For bacterial culturing, lung, liver and peritoneal fluids are helpful if the necropsy is preformed shortly after death.

Cooperation with a diagnostic laboratory is necessary because gross placental and fetal findings are similar for most causes of abortion - but remember, diagnostic labs excel in identifying infectious agents and many perinatal deaths are noninfectious. If the entire fetus and placenta cannot be sent to the diagnostic laboratory, samples taken from necropsy examination should be collected and sent in a timely manner. The placenta (chorioallantois not amnion), or enough placental tissue to include several cotyledons and the intercotyledonary area are important diagnostic samples. If the placenta is retained, a portion of the placenta that is still attached should be removed and submitted to the laboratory. If the placenta cannot be found, a caruncle should be removed from the cow and both fresh and formalin-fixed portions should be submitted. Fresh and formalin-fixed fetal lungs, heart, liver, kidney, lymph nodes, spleen, brain and intestine should be submitted for bacteriologic culture and histopathologic examination. The lung is the most consistent and diagnostic organ because of the feto-maternal circulatory pattern, the inhalation of infected amniotic fluid and the fact that the lung does not decompose as rapidly as the liver. The most common findings in aborted fetuses are edema and autolysis, both are nonspecific and observed in most cases of abortion, infectious or non-infectious.

The rate of success for a diagnostic laboratory to identify an infectious cause of abortion is highest when the fetus, fetal membranes, maternal serum, serum from 10 in-contact peers, maternal urine and vaginal discharge (if present) are all examined. Serology results need to be interpreted with care and always should be correlated with the herd history, time of abortion, and fetal and laboratory results. Many agents that cause abortion produce their highest antibody titers long before abortion occurs, and the titers may be within the normal range at the time of abortion. Diagnostically significant titers for disease agents should be determined after consulting with the diagnostic laboratory where the samples were sent. Maternal serology on a single serum sample from an aborting cow can determine exposure or lack of exposure to various pathogens, but cannot differentiate between vaccination and natural exposure, or between recent versus previous exposure.[1] Paired serum samples from the cow with the aborted fetus is

only slightly more helpful. Paired serum samples from 10 animals or 10% of cows in contact are more helpful. Serology is most helpful in diagnosing diseases where a titer is closely associated with causation for abortion (i.e. rare, highly-abortagenic pathogens such as brucellosis and some subspecies of leptospirosis). Presence of specific fetal antibodies to an infectious agent provides indisputable evidence of prenatal infection, though infection does not necessarily result in abortion or stillbirth.[1] When antibodies to specific diseases cannot be demonstrated in precolostral serum, Ig levels greater than 20 $^{mg}/_{dL}$ indicate prenatal infection of unknown cause. False negative reactions occur when the fetus dies before it can react immunologically to the pathogen.

Many organisms of relatively low virulence are capable of causing sporadic bovine abortions after invading the gravid uterus. Some examples would be: fungi, *Actinobacterium pyogenes, E. coli,* and *Bacillus* sp. These agents are ubiquitous in the environment, have low virulence and contagiousness and are opportunistic rather than strictly pathogenic. Caution must be taken in interpreting the results of any laboratory examination and results must always be considered along with the history and the rest of the examination. When culturing the fetus, placenta or even the abomasal contents one must remember that bacteria may move from the vagina to the conceptus when the cervix dilates during an impending abortion due to another cause. These organisms may rapidly contaminate the placenta, grow in fetal fluids and even be swallowed by a viable fetus thereby appearing in the abomasal contents.[2]

Abortifacient organisms invade the dam by a variety of routes including conjunctiva (Brucella), respiratory tract (IBR and BVD), mouth (BVD, Listeria and Leptospira), skin (Leptospira) and vagina (Campylobacter, Trichomonas and Ureaplasma).[2]The placenta is invaded by hematogenous or venereal routes, and reacts with a mild to severe inflammatory response. Placental inflammation puts the fetus at risk since the organism may invade via the umbilical circulation or amniotic fluid or both. Invading organisms cause (depending on virulence and fetal immunocompetence): hepatitis, pneumonia, enteritis, septicemia or death. The clinical manifestations following placental and fetal infection vary depending on the stage of pregnancy when infection occurs. Placental and fetal infection in the first trimester results in: fetal death, resorption, mummification, maceration, or abortion. If infection occurs in the second trimester, fetal death, abortion, or mummification are the sequelae. Placental and fetal infection in the third trimester causes fetal death, abortion, premature birth, fetal maceration, emphysema, stillbirth, a weak nonviable calf, or may cause no clinical disease and a normal calf is born. Maternal sequelae to placental and fetal infection are not uncommon and include retained fetal membranes, endometritis or metritis.

Bovine Abortion Rule-Outs

Once you have the history, herd records, nutrition records, vaccination records and diagnostic laboratory reports, you are ready to narrow down the list of rule-outs. A good method of organizing your thoughts is to list the common causes of pregnancy wastage in bovine along with the history and diagnostic results expected and compare them to the history and diagnostic results of the herd in question. The following tables allow you to begin focusing on the most likely rule-outs, but many exceptions exist and more extensive research may be needed to diagnose some cases.

Table 1. Common infectious ruleouts for bovine abortion[2,3,4,5,6]

<u>*Actinobacterium pyogenes*</u> Sporadic abortion that usually occurs in the second half of gestation. Gross lesions are often not apparent but some feti aborted in the first half of gestation have small (1mm) white to yellow foci in the lungs. Those aborted in the second half of gestation may have fibrinous peritonitis or pleuritis.

Anaplasmosis Late abortions are seen as a sequela to clinical anaplasmosis

Bacillus cereus and *Bacillus licheniformis*

Becoming a common cause of sporadic abortion (up to 3%) in the last trimester.

The fetus is relatively fresh and has a characteristic pericarditis with a thickened and adherent pericardium.

Brucellosis

Abortion usually occurs in the second half of gestation and the abortion rate is high (80% of exposed susceptible cows). The incubation period is 2 wks to 5 mo or longer. This disease is nearly eradicated from the U.S. and the last reported case in Australia was in 1989.

Bluetongue

Birth defects, including: hydraencephaly, arthrogryphosis, dwarfism, and excessive gingival tissue over incisors occur in 15-20% of susceptible non-immune pregnant cows. Infertility occurs due to early embryonic death. Transmission is via the insect vector *Culicoides variipennis*, vertical transmission from viremic dams, and via virus shed in semen also occurs.

Bovine Viral Diarrhea (BVD)

BVD is a sporadic cause of abortion and the rate is usually low but may be moderate to high depending on the number of susceptible cows, the prevailing stage of gestation of the herd and the virulence of the virus. Most BVD-induced abortions occur in the first trimester. Because the fetus becomes immunocompetent about d 180 of gestation, infection before this time may result in immunotolerance and virus persistence (persistent infection).

Persistently infected calves continually excrete tremendous numbers of BVD viruses. They often do not thrive and may die from unrelated causes. Persistently infected heifers that reproduce will produce persistently infected calves.

Frequently BVD virus is present in fetuses aborting from other, lowly-pathogenic causes. This implies that BVD may suppress a cow's immune system allowing her to be infected by diseases that she could normally clear without aborting.

Fungal

Fungi are the most important sporadic cause of bovine abortion in the U.S. *Aspergillus fumigatus* (septate fungi) causes 60-80% of fungal abortions. Non-septate fungi (*Mucor*, *Rhizopus*, *Mortierella*, and *Absidia*) cause the rest of the cases. Transmission is probably via ingestion of fungal spores and is not contagious. Retained fetal membranes are a common maternal sequela.

About one-third of aborted feti have characteristic signs of mycotic dermatitis involving the periorbit, eyelids, shoulders, back and sides. Fungi have a predilection for blood vessels, especially arteries. Thrombosis of placental vessels is common - leading to ischemic necrosis and the characteristic severe necrotizing placentitis.

One-third of fungal abortion feti have titers to BVD,indicating the role BVD may play in increasing maternal or fetal susceptibility to fungal infection.

Histophilus somnus

Reproductive manifestations of *H. somnus* infections include: severe vulvitis, vaginitis, infertility, postpartum endometritis, placentitis and abortion. Abortion is sporadic in occurrence and occurs late in gestation.

Infectious Bovine Rhinotracheitis (IBR)

A major cause of bovine abortion in many parts of the U.S. Abortions occur from 4 mo to term. Often, no other signs of infection are noted in infected cows. Retained fetal membranes are common.

Histopathologic identification of small necrotic foci in the liver without significant inflammation is a consistent finding. An infected cow has her maximum serum neutralizing titer at or before abortion; therefore, paired serum samples are not usually helpful.

Leptospirosis

Pomona and hardjo are the primary serovars of concern in U.S. Although abortions can occur as early as day 90, abortion in the third trimester, stillbirths or weak calves are more commonly seen. The producer may see maternal fever, hemoglobinuria, anemia and icterus prior to abortion. The fetus usually dies before expulsion, therefore it is autolytic.

Listeriosis

Listeria organisms may occur in large numbers in poorly preserved silage. Abortions usually occur in the winter (Jan - April) during the last trimester. Cows may have pyrexia or anorexia before or during abortion.

The fetal liver is shrunken and soft, with small, pin-point yellow-gray foci of necrosis, mainly on the right half.

Neosporosis[7]

Protozoal disease causing abortion, stillbirth, weak, or paralyzed calves. Also seen is a syndrome where calves become paralyzed within 4 wks of birth. The dams are clinically normal. Anti-*N. caninum* antibody in presuckling serum or fetal fluids is indicative of congenital neosporosis - lack of antibody is non-diagnostic. Microscopic lesions are found in brain, spinal cord, heart, and rarely other organs.

Salmonella abortus

Transmission is by ingestion.

The aborted fetus dies *in utero*, and is edematous and autolytic.

Trichomoniasis

Venereal disease characterized by infertility, embryonic mortality and occasionally early abortion. The fetus is not attacked until d 50 of gestation and abortion occurs 2 to 5 mo. following the infected service.

The protozoan is found in great numbers in the fetal fluids associated with trichomonad-induced abortion. Scrapings of smegma can be cultured in Diamond's medium or examined directly at 100X magnification.

Ureaplasma diversum

A common inhabitant of the vagina of the cow and prepuce and urethra of the bull. Has been associated with granular vulvitis, salpingitis, endometritis and abortion.

The relationship between granular vulvitis and infertility is unclear, although some studies suggest an adverse affect on fertility.

Infection results in embryonic death with a return to estrus or third term abortions or the birth of weak calves.

The placenta has characteristic areas of white, opaque, amniotic fibrosis due to multifocal-to-confluent necrosis on the inner surface of the amnion with vasculitis, fibrosis and occasionally mineralization. An interstitial pneumonia with monocytic airway cuffing frequently occurs. A

lymphocytic infiltrate in the lamina propria of fetal conjunctiva with goblet cell metaplasia may also suggest a *Ureaplasma* abortion.[8]

Vibriosis - *Campylobacter fetus ss venerialis*

Primarily an infertility inducing disease characterized by embryonic mortality and irregular cycles. Abortion rate is low (<10%) and occurs between 5-7 mo. Transmission is venereal and infected cows spontaneously recover, usually by 4 mo. Bulls do not spontaneously recover. Artificial insemination using properly treated semen prevents the transmission of this infection. Diagnosis is by herd history and response to vaccination or by isolating Campylobacter from cervical mucus collected from open females exposed for the first time to suspected bulls 3 wks. to 3 mo. previously or from smegma collected from suspected bulls. The smegma and cervical mucus should be shipped to a diagnostic laboratory in Clark's medium.

Vibriosis - *Campylobacter fetus ss fetus or ss jejuni*

Causes sporadic abortion in herds with no history of infertility. Abortions can occur at any stage of pregnancy, usually in the second or third trimester. The transmission is by ingestion, therefore, utilizing AI does not prevent infection.

The aborted fetus is usually fresh, and liver rupture is not uncommon.

Yeast – Candida

Sporadic midterm (5-6 mo) abortions occur with a necrotizing placentitis.

Microscopic intracytoplasmic yeasts are seen within trophoblasts.

As stated previously, it is important to note that most perinatal mortality is not due to an infectious cause, but rather noninfectious causes such as: genetic, immunologic, teratogenic, nutritional, toxic plants, hormonal, and physical insults.

Table 3. Noninfectious causes of bovine perinatal mortality

Genetic

Losses attributed to genetic causes generally occur early in gestation (less than 90 days) and are beyond the routine capability of most diagnostic laboratories.[2] Genetic causes of abortion or perinatal death include lethal genes, chromosomal aberrations (numerical or structural), major histocompatibility genes or genetic-hormonal interactions

Teratogens

Exposure to chemical or plant teratogens in early pregnancy may result in embryonic death, abortions, stillbirths, dysmaturity and defective calves.

Nutritional

Primary deficiencies are more commonly associated with infertility and neonatal calf mortality than abortion.

Acute, severe starvation

Protein deficiency - results in prematurity, dystocia, neonatal mortality and the "weak calf syndrome"

Vitamin A deficiency - results in late term abortion, weak, uncoordinated, or blind calves.

Iodine deficiency - thyroid hypofunction resulting in hairless, weak calves with a high mortality.

Phosphorus deficiency - dystocia and weak calves

Cobalt deficiency - weak, non-viable calves

Selenium / Vitamin E deficiency - prematurity, stillbirths and weak calves. Excess selenium will also cause abortion

Toxic Plants

Toxic plants can cause sporadic to epidemic abortion losses concentrated in a short period of time, particularly if the herd has a concentrated breeding season. Drought or other feed restriction can lead to the consumption of toxic plants that are usually avoided or not preferred.

Hormonal asynchrony

Hormonal asynchrony in the early postpartum period may be caused by infectious insults such as metritis or endotoxemia. Uterine infections can cause altered corpus luteum (CL) function via altered prostaglandin production by the endometrium. Endotoxemia may produce CL lysis directly by evoking a generalized synthesis of PGF_2.[9] Noninfectious factors such as excess dietary protein and energy restriction have also been shown to be detrimental to fertility through their effects on hypothalamic and ovarian function.[10,11] These causes of hormonal asynchrony are considered by many to be a major cause of reproductive wastage and prolongation of the calving interval.[2]

Exogenous Corticosteroids

Glucocorticoids given for therapeutic reasons cause a high percentage of treated females to abort.

Physical

Dystocia, causing delayed parturition or trauma to the fetus is an important cause of perinatal loss. Dystocia as the cause of perinatal death is often diagnosed by the lack of evidence of infectious causes combined with evidence such as a history of a difficult birth, congestion and edema of the head, contusions, fractures, partial expansion of the lung, or excessive amniotic fluid in the trachea and bronchi. Management that assures the proper maturity and size of heifers at first calving and assures calf size that matches maternal pelvic size by utilization of birth weight and calving ease EPDs (expected progeny differences) can greatly decrease losses due to dystocia.

Other physical causes of perinatal losses include:

Ruptured amniotic vesicle - early abortion <60 d
Severe trauma to fetus - early abortion <60 d
Umbilical cord displacement or torsion (rare in cattle)
Severe maternal stress (anemia, transportation, surgery) occasionally results in abortion.
Allergies and anaphylactic reactions
Malformed calf not due to infectious causes.
Examples are osteopetrosis, hydrocephalus and arthrogryposis

Endotoxins

Bacterial endotoxins released from maternal infections cause sporadic abortion by causing the release of prostaglandins. The fetus expelled is usually normal and diagnostic investigation of the fetus and fetal membranes are futile. A known or suspected gram-negative maternal infection is the only clue leading to a presumptive diagnosis.

High Environmental Temperature

High environmental temperature during or shortly after conception can significantly increase embryonic mortality in cattle.[12]

Because diagnosis of perinatal death is often difficult, a thorough process of history taking, necropsy, laboratory assistance and follow up is often required to be successful. Beginning with a list of rule-outs and comparing your results with expected characteristics of infectious and non-infectious causes of perinatal mortality can guide you to a definitive diagnosis.

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