Failure of pregnancy in ruminants

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Abstract

Failure of pregnancy in ruminants is a substantial source of economic loss around the world. A small number of abortions should be expected even in a healthy herd or flock. Identification of the cause can be challenging and often requires extensive laboratory diagnostic support. Success requires an accurate history, and a complete tissue sample collection, including placenta and serum from the dam. Testing on more than a single case in an outbreak may be necessary. Multiple bacterial, viral, and protozoal agents have a role and there are also noninfectious causes such as vitamin and mineral deficiencies, and intoxications.

Keywords: Abortion, failure of pregnancy, cattle, sheep, goats

Introduction and general approach to investigating failure of pregnancy

Approaching ruminant abortion requires having as complete a sample set as possible. Whereas there are a few pathognomonic lesions, abortions are notoriously unrevealing grossly and even microscopically. Autolysis is usually advanced and lesions are often subtle. We frequently have to resort to laboratory testing for a diagnosis and even so, a disappointing number of cases will result in no diagnosis. Certainly, in the face of an outbreak, multiple aborted fetuses from multiple dams may provide a better chance at finding a diagnosis.

A complete history, including nutrition, parity, and health history of the dam, is critical. A minimum sample set would include:

1. Fetal membrane (chorioallantois, not amnion), cotyledonary and intercotyledonary, fixed and fresh
2. Lung, fixed and fresh
3. Liver, fixed and fresh
4. Kidney, fixed and fresh
5. Brain, fixed and fresh
6. Thyroid, fixed and fresh
7. Tongue, fixed
8. Skin, fixed (eyelid)
9. Spleen, fresh
10. Thymus, fresh
11. Stomach contents, fresh
12. Dam serum, acute (at abortion) and convalescent (3 - 4 weeks later)

Alternatively, fetal thoracic fluid can be used for antibody detection, though hemolysis in the sample can substantially impair many assays.

Note that even though samples appear autolyzed, they will still have value, especially for molecular testing. Brain is often especially liquefied due to autolysis and incomplete myelination, but will congeal when poured into formalin and lesions can be identified histologically. Small ruminants and cattle have similar reproductive anatomy and physiology; however, do not necessarily have similar lesions nor respond equally to similar pathogens.[1-18]

Whereas, every abortion should be noted by the herd or flock manager, and the value of a full diagnostic necropsy assessed, a certain rate of abortions is expected. In a dairy herd, for instance, a rate of 3 - 5% fetal loss is expected.[19] Deviations from a herd's expected rate is often the impetus for an investigation.

Infectious causes of abortion

Lesions associated with infectious pathogens generally lead to either inflammatory lesions or teratogenic effects (viruses). History, gross findings, and histopathology will often provide sufficient evidence for the experienced diagnostician to make a presumptive diagnosis, but laboratory confirmation is usually necessary, including culture, immunohistochemistry, and/or polymerase chain reaction (PCR).

Viruses

Among the viruses, herpesviruses are the most ubiquitous and include infectious bovine rhinotracheitis (bovine herpesvirus-1) and caprine herpesvirus-1. Gross and histologic lesions are multifocal necrosis of the liver, lung, adrenals, and kidneys, but with no placental lesions.[17,24] Confirmation is generally by PCR or rising serum titers in the dam. Bovine

herpesvirus-4 is associated with a higher risk of abortions, but a clear connection is elusive.[5]

More commonly, however, viral agents disrupt development and teratogenic effects are the presenting complaint. Pestiviruses, including bovine viral diarrhea virus, border disease, and classical swine fever, lead to embryonic death and resorption early in pregnancy, but abortion in late pregnancy, abortion, stillbirth, or neonatal death are the result, with fetal developmental abnormalities, mummification, and weakness.[20] Arthrogryposis, a condition where the limbs are contorted and stiff, is usually associated with brain lesions such as hydrancephaly, porencephaly, and hydrocephalus. Border disease can affect the hair as well, causing hair-like deformities in wool breeds of sheep. Cache valley virus and some strains of bluetongue virus can cause similar lesions in cattle and small ruminants. Foreign animal disease viruses such as Schmallenberg, Wesselsbron, and Akabane viruses should also be considered if appropriate. Differentials for limb and musculoskeletal developmental anomalies such as these would include toxic plants such as *Veratrum californicum* (sheep), *Lathrhyrus* spp., lupines, and locoweed.[2,15] Vitamin A deficiency has also been identified as a cause of developmental lesions in ruminants.

Bacteria

Bacteria are likely the more common cause of abortion. Importantly, several are zoonotic and appropriate precautions are warranted when conducting postmortem investigations. Ideally, all ruminant abortions should be conducted in a biosafety hood, but this is rarely practical, so gloves, boots and apron, goggles, and a properly fitted respirator are reasonable precautions.

Brucellosis in cattle is caused by *Brucella abortus*; in sheep *Brucella ovis* and in sheep and goats, *Brucella melitensis* are the most important agents in this genus.[10] However, there are likely other closely related species that are sporadic causes. These are reportable diseases. Severe placentitis is the most common lesion, characterized by granular necrotic debris on the cotyledons and an opaque thickened and leathery intercotyledonary chorion. Thick adherent pasty brown to tan material fills the intercotyledonary regions. The fetus is usually fresh. Microscopically, neutrophils and macrophages fill the edematous connective tissue and the walls of chorionic vessels. Trophoblast cytoplasm is frequently stuffed with small short gram-negative rods. Culture, PCR, and immunohistochemistry can specifically identify the organism. Serological tests are also used to identify infections, particularly on a herd or flock basis; however, caution should be exercised when interpreting these tests in regions where vaccination is used as false positives can provoke unwarranted regulatory concerns. Mastitis can be a sequela to infection and can lead to chronic shedding and human exposure in the milk.

Coxiella burnetii is quite common in small ruminants, but is also a possible cause of abortion in cattle, though much rarer.[14,26] This organism is zoonotic and considered a 'select agent' by the CDC due to its infectivity and potential for use as a bioterrorist weapon. In small ruminants, late-term abortion, stillbirths, and neonatal death are the common presentations. Usually there are no fetal lesions, but thick suppurative cotyledonary and especially intercotyledonary plaques are characteristic. This is a condition where examination of fetal membranes is critical to the diagnosis. Histologically, trophoblasts are also packed with small rods that can be noted with HE stains, but Gimenez stains can highlight them. Routine serology is not widely used to investigate this disease in the US.

Chlamydia abortus (previously known as *Chlamydophila abortus* or *Chlamydia psittaci* immunophenotype 1 is also a common cause of infectious abortion in small ruminants and rarely in cattle.[4] Similar late-term abortions, stillbirths, and neonatal deaths are typical, and the gross and histologic lesions are very similar, including the presence of small rods within trophoblasts. Unlike coxiellosis cases, however, fetal lesions such as hepatic, splenic, and lymph node necrosis are sometimes observed. Immunohistochemistry and PCR are the best methods to confirm the diagnosis – serology can be misleading due to the ubiquity of the organism in the environment. In cattle, the lesions are typically much more subtle and may consist only of vasculitis and edema in the chroioallantoic stroma.

Campylobacteriosis in small ruminants is an entirely different disease than in cattle.[23] In sheep and goats it is caused by *Campylobacter fetus* subsp. *fetus*, *Campylobacter jejuni* subsp. *jejuni*, and *Campylobacter lari*, and is spread by the fecal oral route leading to bacteremia in the dam and subsequent placentitis and fetal sepsis. The lesions are primarily cotyledonary, with necrosis and suppurative exudate; the intercotyledonary space is often edematous, but there are not dense mats of necrotic and suppurative debris as in chlamydiosis, coxiellosis, or brucellosis. Similar to these diseases, trophoblasts may contain dense mats of organisms within trophoblasts. Fetal targetoid regions of liver necrosis are typically noted, along with peritoneal and pleural fluid accumulation and fibrin. These organisms can be cultured with special media (modified Cary-Blair, for instance) and there are specific immunohistochemical stains to make a diagnosis. Wet mounts of abomasal contents or cotyledonary imprints may show these organisms darting motility with darkfield or phase contrast microscopy. In contrast, *Campylobacter fetus* subsp *venerealis* is a venereally transmitted disease which is primarily associated with infertility, early embryonic death, and only rarely, abortion in cattle, though cattle can rarely be infected by other species of Campylobacter.[16] Fibrinous intercotyledonary placentitits and fibrin in the thoracic, pericardial, and peritoneal cavities, and splenomegaly are usually observed. Culture is again possible, but PCR and IHC are often more commonly used in laboratory diagnosis.

Listeria monocytogenes and *Listeria ivanovii* are considered pathogens in silage-fed animals and usually in the winter, though cases are reported in pasture raised ruminants.[6] Infections can result in neurological disease in adults or abortion, but rarely both. Regardless, there is septicemia in the dam and they are usually ill. Retained fetal membranes postabortion and metritis can also be a problem. Edema in the intercotyledonary placenta and necrosis in the cotyledons are present, and fetal lesions include necrotizing hepatitis, nephritis, pneumonia, myocarditis, with pleuritic, peritonitis, and pericarditis. Cultures (especially if the lab can use cold enhancement) are usually diagnostic, and gram staining will demonstrate gram-positive organisms on cytology or histology.

Leptospirosis is caused by a variety of serovars, often with regional and host-specific variations in susceptibility and vector. In cattle, *Leptospira borgpetersenii* is specifically adapted to cattle; in sheep, abortions attributed to leptospirosis are much rarer and most are due to *L. hardjo*, *L. pommona*, *L. catellonis*, and *L. icterohaemorrhagiae*.[13] Maternal ingestion and bacteremia are the usual routes for the bacterium to get to the placenta and fetus, though placental lesions are usually subtle. Grossly, fetuses are usually markedly autolyzed, making diagnosis challenging. Fetal lesions are often restricted to kidney or liver, and may be minimal. PCR on dam urine, fetal membranes, or fetal kidney is the confirmatory test of choice, though some labs can still perform the challenging fluorescent antibody assays on kidney impression smears.[27] Serology is challenging, in that each serovar must be individually assayed, and vaccination and past infections may result in deceivingly high titers. Note that aborting dams may shed large numbers of organisms in the urine and uterine discharge and these organisms are zoonotic.

Salmonellosis is a fairly common cause of abortion in cattle, especially in dairy, but is very rare in small ruminants. *Salmonella enterica* serovar Dublin is most commonly associated, but other serotypes have been reported. Infection is usually via the digestive tract, with maternal bacteremia resulting in placental and fetal infection. Retained fetal membranes and severe fetal autolysis are common with these infections. What lesions might rarely be present are likely obscured by the autolysis. Microscopically, mild inflammation of fetal membranes and necrotizing hepatitis are observed, with other organs less frequently affected.

Bovine foothills abortion (epizootic bovine abortion) is a disease of local, but significant, importance, causing abortion and neonatal deaths in cattle grazing pastures in the foothills of California, Nevada, and Oregon. *Pajaroellobacter abortibovis* has been implicated in the most recent research, and are carried to the cows by the pajaroello tick (*Ornithodoros coriaceus*).[1] The dam is not affected, but fetal mortality can be as high as 90%. Fetuses have markedly enlarged and firm livers, spleens, and lymph nodes, and petechiae around the eyes, mucous membranes, and in the thymus. Ascites is also commonly reported.

Mycoplasma sp. and *Ureaplasma* sp. (especially, *Ureaplasma diversum*) are commensal organisms in the female reproductive tract and are attributed to some abortions.[7] However, the difficulty in identifying these organisms and the likelihood of contamination during sampling make it difficult to confirm their role in many of these cases. Identifying the organism in fetal lung or some other protected site is important to establish it as a cause in a particular case. Lesions ascribed to *Mycoplasma* spp. and *Ureaplasma* spp. are primarily associated with vasculitis, including edema, necrosis, hemorrhage, and fibrin exudation. Conjunctivitis has been reported as well. Interestingly, the amnion is often the most affected part of the placenta, which is unique among the abortifacient agents described here.

Arguably, the most frequent bacteria isolated from abortions are opportunists. Maternal bacteremia can result in spread to the uterus and infection of the placenta and fetus. Suppurative placentitis and fetal septicemia are often observed. Rumen acidosis in the dam is a frequently associated condition, with subsequent spread of the bacteria via ruminal ulcers into the bloodstream and then to the placenta. To confirm a bacterial cause of an abortion, the bacterium of interest should be isolated in nearly pure culture and the bacteria from compatible lesions via gram stain or immunohistochemistry.

Fungi

In cattle and to a lesser extent, sheep and goats, mycotic infections are generally hematogenous in origin and do not ascend through cervix as is common in horses.[29] Portals of entry into the dam are likely in the digestive tract, respiratory tract, or skin wounds. *Aspergillus* sp. are most common, but others include *Mucor* spp. *Rhizopu*s spp. and *Candida* spp. These are typically sporadic, but can be epidemic when there is a common insult to all the pregnant females in a herd (such as an outbreak of rumen acidosis). Placentitis is usually very severe with thick plaques of necrotic debris, suppurative and fibrinous exudate, vasculitis, and fibrosis. Often there are no fetal lesions, but if present, bronchopneumonia or dermatitis are the most common. Identifying the mycotic organisms in the lesions is usually not difficult microscopically, but Gömöri methenamine silver stain or Periodic acid–Schiff can be used to characterize the fungus. Speciation is possible by culture of fetal membranes or abomasal contents.

Protozoa

Protozoan are economically important causes of ruminant abortion. In cattle, *Neospora caninum* is the most important with a world-wide distribution and up to 60% seroprevalence rate in US herds.[8] It is especially important in that it can be maintained in the cow chronically, repeatedly transmitted to the fetus during repeated pregnancies, and carried congenitally by asymptomatic calves into the next generation. Infection can also occur horizontally via ingestion of oocytes from dogs or coyotes. Abortions generally occur during the second trimester (an important comparative point to other causes) and the fetus is markedly autolyzed with abundant serosanguinous fluid in the peritoneal, pleural, and pericardial cavities. Subtle white streaks or foci are sometimes present in the heart or tongue. Histologically, foci of central necrosis surrounded by mononuclear cells are characteristic, though IHC or PCR is usually required to identify organisms in the lesions. Thoracic fluid can also be used to document an antibody response in the fetus. Care should be taken, however, since the incidence of neosporosis is so high in the cattle population, simply identifying organisms (or a serologic response) in an aborted fetus does not ascribe causation.

In contrast to neosporosis, toxoplasmosis is much more common in small ruminants and is associated with feed contaminated with cat feces containing *Toxoplasma gondii*.[9] Depending on the stage of pregnancy, the outcome may be embryonic death and resorption early in pregnancy or mummification, stillbirth, or neonatal death later in gestation. Gross lesions are typically restricted to the placenta, with multifocal region of cotyledonary necrosis and mineralization. Histologically, lesions are in the brain and the placenta: mononuclear inflammation in the cotyledons surrounding regions of min-

eralization and necrosis and regions of necrosis and gliosis in the brain. Keep in mind that even autolyzed and liquefied brain tissue can be diagnostic histologically after formalin fixation. Identification of fetal antibodies in fetal thoracic fluid can also be helpful.

Tritrichomonas foetus is another important protozoan cause of abortion and infertility in cattle with similarities in transmission and pathogenesis to *Campylobacter fetus* subsp. *venerealis*. Infertility and early embryonic loss are typical, whereas abortion and pyometra are lesser common manifestations.[22] The disease is largely maintained via inapparent bull carriers, so it is observed most frequently in range situations where natural service is used. Placental and fetal lung lesions are noted when abortion does occur. Histologically, intralesional trichomonads are within the edematous placental stroma and bronchopneumonia with abundant intra-alveolar trichomonads in the lungs. Multinucleated giant cells are often also present. Immunohistochemistry can aid in finding organisms in tissue samples, but culture using specific media (e.g. InPouch™) or PCR of fetal membranes, fetal abomasal contents, uterine or vaginal fluid, or preputial scrapings from an infected bull is the most definitive. Ideally, purification of the sample by culture followed by PCR is the best approach.

Noninfectious causes of abortion

Veterinarians tend to focus on infectious causes of disease in animals, yet often noninfectious causes can be more prevalent and may indeed act as a predisposing or exacerbating factor for infectious pathogens.[11] Nutrition has an important role in development and growth of the fetus and deficiencies can have a serious impact on fertility. Specific deficiencies that can lead to failure of pregnancy include copper, selenium, vitamin E, vitamin A, and iodine.[15] Copper deficiency can lead to implantation failure, early embryonic death or later in pregnancy can lead to demyelinization, depigmentation, and abnormal haircoat or wool. Iodine deficiency is associated with goiter, and usually results in stillbirths and weak neonates.[3,15] Iodine concentrations are best measured in the thyroid gland itself, so a laboratory should have developed reference values in this organ. True iodine deficiencies are possible, but often are associated with goitrogenic plants such as Brassica spp., kale, or beets (and beet by-products).

Vitamin E and selenium deficiency have long been known for causing white muscle disease, though this is observed much more rarely today. More likely, are subclinical deficiencies that result in decreased fertility, decreased immunocompetence of the dam and fetus, and other more subtle physiologic issues (Yamini B, personal communication).[28]

Vitamin A deficiency has been associated with multiple developmental anomalies of the bones, eyes, brain, immunocompromise, and infertility.[18] In sheep, this can be associated with the use of beet pulp as an energy supplement for ewes. Vitamin A levels are normally lower in fetuses, so appropriate reference values should be used.

Toxic plants can also be a factor in abortion and developmental anomalies in ruminants. Many defects are associated with plant toxicities at a specific point in pregnancy. For instance, *Veratrum californicum* must be ingested by the ewe on day 14 or 15 of pregnancy to produce the characteristic cyclopia that is described with this toxicity.[15] Ponderosa pine causes both systemic illness and abortion with retained fetal membranes in cattle.[25] Locoweed (*Astragalus* and *Oxytropis* sp.) can lead to abortions and skeletal and neurologic abnormalities. Lists of possible toxic plants are long, regionally specific, and often speculative, so it is important to examine the pasture and stomach contents carefully when investigating possible plant intoxications.[12,21]

Conclusion

Failure of pregnancy in ruminants can be a substantial cause of financial loss to producers. Diagnosis can be frustrating as gross lesions are often inapparent and laboratory testing can take time and additional resources. Additionally, a diagnosis is not always possible. However, a thorough knowledge of the possible causes, a good history, and a complete sample set, including fetal membranes and dam serum, from multiple cases will usually provide an answer that can guide management changes to alleviate the abortion problem.

Conflict of interest

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