Pregnancy loss in ruminants

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Abstract

Pregnancy loss in ruminants is a major economic loss to producers and highlights the importance of control measures to prevent its cause. Although pregnancy loss may occur at any stage of pregnancy, early loss is usually unnoticed and samples are not submitted for diagnosis. Pregnancy loss is detected by observations for return to estrus, transrectal palpation or ultrasonography, and blood tests for specific antigens. Submissions to a laboratory often consist of 4- to 5-month-old, or older, aborted fetuses and/or maternal serum. Consequently, reports of pregnancy loss in ruminants are biased towards second and third trimester causes of pregnancy loss, because the chance of collecting a fetus or fetal membranes and submitting them to a laboratory improves as pregnancy advances. Causes of pregnancy loss in ruminants are noninfectious and infectious. Among the former are genetic defects, toxins, nutritional deficiencies, iatrogenic, drugs, and environmental causes. Infectious causes include bacteria, fungi, viruses, and protozoa. A major concern of many infectious causes of pregnancy loss in ruminants is their zoonotic potential. A specific cause is identified in only 30 - 70% of late-term abortion samples submitted to a diagnostic laboratory. Adequate specimen collection and proper handling are essential prerequisites for accurate test results. Ideally, multiple concurrent tests are necessary for accurate diagnosis. Main causes of pregnancy loss in ruminants are discussed.

Keywords: Ruminants, abortion, diseases, pregnancy loss

Introduction

Embryonic mortality and abortion are major causes of severe economic losses to the livestock industry.[1,2] Pregnancy loss in ruminants varies with geographic area and period. In only ~ 30% of mid- and late-term pregnancy losses in cattle and 57% in sheep, was a specific cause identified.[3-5] Despite the development of new diagnostic tools and the discovery of new infectious agents that cause abortion in ruminants diagnostic efficiency has not improved.[6]

Infectious agents are reported as major causes of pregnancy loss. Some infectious diseases that cause abortion in ruminants have zoonotic potential and are a public health concern.[7] Noninfectious causes of pregnancy loss, particularly during the embryonic period, may be as important as infectious causes; however, specific causes are rarely determined. Although not strictly a cause of pregnancy loss, neonatal mortality during the first few days after birth may be an indicator of intrauterine disease, and thus, they should be considered among the causes of pregnancy loss.[6,8]

Diagnosis of pregnancy loss in ruminants involves an investigative effort among owner, veterinary practitioner, and veterinary diagnostic laboratory. Veterinary practitioners have an important role in educating farmers about the risks of zoonotic causes of ruminant abortion, and on how to safely submit a fetus, fetal membranes, and maternal serum to the diagnostic laboratory.[5] Furthermore, veterinary diagnostic laboratories have an important role to identify the causes of pregnancy loss and in surveillance of infectious diseases that cause reproductive failure.[9] Adequate specimen collection and specimen handling are essential prerequisites for accurate test results. For any laboratory test procedure, the value of the test may be compromised by using specimens that have not been properly collected, labelled, handled or stored prior to testing.[6,9]

Currently, because many of the infections causes of abortion in small ruminants are zoonotic, examination of small ruminant aborted fetuses and fetal membranes in some diagnostic laboratories are performed in a biosafety cabinet, and the use of N95 or a powered air purifying respirator is mandatory.

In some US laboratories, the standard procedure when pursuing the cause of abortion in small ruminants requires testing for *Coxiella burnetii* by reverse transcription polymerase chain reaction (RT-PCR) before proceeding with other testing. When RT-PCR results are positive for *C. burnetti*, no additional tests are done and all tissues are discarded following biosecurity guidelines.

Main causes of pregnancy loss in ruminants with an emphasis on infectious causes are discussed. Transmission, pathology, and diagnostic methods of infectious causes of pregnancy loss in ruminants are highlighted. More extensive causes of pregnancy loss in cattle and small ruminants are reviewed in other papers included in these proceedings.

Sample submission

Diagnostic success of pregnancy loss in ruminants can be improved by promptly submitting suitable samples by overnight delivery service. When possible, the entire fetus and fetal membranes should be submitted chilled on ice using primary, secondary, and tertiary leak proof containers. The chorioallantoic membrane is most useful for establishing the cause of abortion because it often has microscopic lesions and in some cases the etiologic agent can be demonstrated by microscopic examination or by ancillary tests.[11] If submission of the entire fetus is not possible, a field necropsy should be performed at a site that guarantees convenience, safety, and biosecurity.[12] Fetal brain, heart, lung, thymus, liver, spleen, kidney, adrenal gland, and skeletal muscle should be submitted to laboratory in 10% buffered formalin. A second set of similar tissues should be submitted chilled on ice in individual, labelled Whirl-Pak or Ziploc bags for bacterial culture and molecular analyses.[13] Fetal blood from heart, fetal thoracic or abdominal fluids, and maternal serum should be submitted for identifying antibodies against important causes of infectious abortion.[12,14]

Common causes of pregnancy loss in ruminants

Embryonic mortality and early pregnancy loss are usually unnoticed and often samples are not submitted. Submissions usually consist of aborted fetuses of at least 4- to 5-months of gestational age and/or maternal serum. Consequently, reports of pregnancy loss in ruminants are biased towards second and third trimesters because the chances of collecting a fetus or fetal membranes and submitting them to diagnostic laboratory improves as pregnancy advances.[15] Although infectious agents are most frequently diagnosed, efforts to diagnose noninfectious causes of pregnancy loss are equally important.[16,17]

Embryonic mortality

Embryonic mortality is a major cause of economic loss in ruminants; it reduces conception rates, thereby impacting production and profitability. Causes of embryonic mortality in cattle are varied and include genetic, inbreeding, low progesterone concentrations, severe postpartum negative energy balance, and infectious diseases.[18] Methods for detection of early pregnancy loss include: observations for return to estrus, transrectal palpation, transrectal ultrasonography, and blood tests for specific antigens.[19]

Noninfectious causes of pregnancy loss

Noninfectious causes of pregnancy loss include genetic, toxic, nutritional, medications, and environmental. Fetal malformation and subsequent abortion, or the birth of abnormal offspring can be due to genetic abnormalities (e.g. arthrogryposis multiplex congenita[20] and bovine arachnomelia syndrome[21]). A nonsense mutation in the APAF1 gene responsible for a lethal effect (Holstein haplotype1), caused an estimated 525,000 spontaneous abortions worldwide over the past 35 years, accounting for ~ $420 million in losses. Holstein haplotype1 was traced to the ancestor Holstein sire Pawnee Farm Arlinda Chief born in 1962, a bull considered the second most influential sire in the Holstein breed history.[22] A description of all genetic abnormalities that cause pregnancy loss in ruminants is beyond the scope of this paper; an extensive review is available.[23]

Toxic plants associated with pregnancy loss in ruminants include juniper (*Juniperus communis*), locoweed (*Oxytropis* and *Astragalus*), Pinus ponderosa, and perennial broomweed (*Gutierrezia microcephala*).[24] Ingestion of *Veratrum californicum*, a plant that grows primarily in the high mountain ranges of the western US, at about the 12 - 14th day of pregnancy can cause congenital cyclopia and other defects of the cranium and central nervous system in lambs, in addition to prolonged pregnancy.[25] Nitrates and mycotoxins are among the toxic causes of abortion in cattle.[26-29]

Malnutrition and negative energy balance cause pregnancy loss.[18] Congenital nutritional muscular dystrophy caused by vitamin E and selenium deficiency is uncommon, but was reported as a cause of pregnancy loss in sheep and cattle.[30] Iodine deficiency and its excess was associated with abortion, stillbirths, and weak newborn calves and goats. Goitrogenic compounds present in several species of *Brassica* spp. and certain pharmacological agents, such as sulfonamides and thiouracil, induced congenital hyperplastic goiter in fetuses when dams were exposed.[31,32] Vitamin A deficiency in pregnant cows was suspected in cases of perinatal calf mortalities.[33] Dams fed a vitamin A deficient ration delivered dead, weak, uncoordinated, and blind calves.[34]

Medications such as prostaglandin $F_{2\alpha}$ can induce luteolysis and pregnancy loss in cattle and goats.[35,36] Ewes treated with netobimin, a benzimidazole compound, on day 17 of pregnancy delivered lambs with fetal skeletal and congenital renal malformations.[37] Heat stress has major effects on fertility and embryonic survival in lactating dairy cows.[38,39] Other causes and more in-depth explanations of noninfectious pregnancy loss and laboratory detection are described.[3,4,10]

Infectious causes of pregnancy loss

Brucellosis

Brucellosis is a zoonotic disease caused by several *Brucella* species and transmitted from animals to humans by ingestion

of contaminated food products, direct contact with infected animals or inhalation of aerosols.[40] Brucellosis in cattle is caused more often by *Brucella abortus* and less often by *Brucella melitensis*. Abortion in the mid-and late-term is the main clinical outcome in cattle, followed by birth of weak calves, perinatal mortality, reduced milk yield, and failure to conceive. Orchitis is the most prominent lesion in infected bulls.[41] Brucellosis in humans is characterized by undulant fever, general malaise, miscarriage, and arthritis.[42]

Brucella melitensis is the most virulent species of the *Brucella* genus and the main cause of abortion in goats and sheep in many parts of the world. It is also the main agent responsible for human brucellosis, predominantly an occupational disease.[43] Clinical, pathological, and epidemiological features of sheep and goat brucellosis due to *B. melitensis* are similar to *B. abortus* infection in cattle.[44] Epididymitis and infertility in rams are most common clinical manifestation of sheep infected with *B. ovis*. Placentitis and abortion occur occasionally in pregnant ewes.[45] In contrast to other *Brucella* species, *B. ovis* lacks zoonotic potential.[46]

Brucella can be transmitted via horizontal or vertical routes. The main way all *Brucella* species are disseminated amongst animals is through contact with fetal membranes, fetal fluids, and vaginal discharges expelled by infected animals. Organisms shed in the milk of infected animals may transmit infection to the newborn. Bulls may spread infection through semen.[43,47]

Pregnant cattle infected with *B. abortus* may develop placentitis and abort during the last trimester of pregnancy, but generally do not abort in subsequent pregnancies. Intercotyledonary areas of fetal membranes are thickened and have a yellow, leathery appearance. Cotyledons appear swollen and covered with yellow to brown exudate. Some aborted fetuses had bronchopneumonia.[47] Infected females that give birth to normal offspring shed the organism in fetal membranes, fetal fluids, and vaginal discharges. In nonpregnant animals, the infection is usually asymptomatic.

Diagnosis of brucellosis in infected fetuses is established by isolation of the organism from fetal membranes, fetal stomach content or lung. Several PCR protocols for identification of *Brucella* DNA are used primarily in cultures. *Brucella* antibodies in maternal serum can be detected by serological tests including the Rose Bengal test, serum agglutination tests, complement fixations test, Coombs test, complement fixation tests, and more recently the immunocapture-agglutination technique.[40] B. suis and B. melitensis infections in cattle may interfere with serological diagnosis of B. abortus infection in cattle.[48]

Campylobacteriosis

Campylobacter spp. are important animal pathogens and opportunistic human pathogens. Several species and subspecies of *Campylobacter* cause pregnancy loss and infertility in ruminants.[49] Mammal-associated Campylobacter fetus comprises 2 subspecies: *C. fetus* subsp *venerealis* and *C. fetus* subsp *fetus*, both of which are well-known causes of reproductive failures in ruminants.[50] *C. fetus* subsp *venerealis* causes bovine genital campylobacteriosis characterized by infertility and abortion. *C. fetus* subsp *fetus* was the *Campylobacter* species most frequently isolated from abortions in sheep. Since the 1990s, *C. jejuni* has become an important *Campylobacter* species associated with ovine abortion in the US. Most isolates of *C. jejuni* from sheep abortions are resistant to tetracyclines, the only approved drug for treating infection in sheep in the US.[51]

Transmission of *Campylobacter* spp. is by ingestion of water contaminated with feces of infected animals. Infection of pregnant ewes with *C. fetus fetus* or *C. jejuni* causes late term abortion, stillbirth, and/or the birth of weak lambs. Average abortion rate generally is ~ 25%, but may be as high as 70%. Intercotyledonary fetal membranes of aborted fetuses are edematous and cotyledons are yellow with necrotizing and suppurative inflammation and vasculitis. Some aborted fetuses exhibit fibrinous peritonitis, multifocal areas of hepatic necrosis ranging from 1 mm to 5 cm in diameter, and suppurative bronchopneumonia. Although uncommon, some ewes die due to endometritis and bacteremia.[52] Diagnosis is by bacterial isolation and identification from fetal membranes and tissues of aborted fetuses. PCR and DNA sequencing are valuable tools for confirming phenotypic tests.[53]

Chlamydiosis

Chlamydial abortion is usually due to infection with *Chlamydia abortus* that also causes stillbirth, and birth of weak offspring in sheep and goats. Infection in sheep is known as ovine enzootic abortion and is characterized by vaginitis, endometritis, mastitis, late term abortion, stillbirth, and birth of weak neonates that often die within 48 hours of birth. Abortion usually occurs in the last 2 - 3 weeks of pregnancy, and up to 30% of ewes may be affected in naïve flocks. Infection in goats is similar to infection in sheep. Cattle, pigs, horses, wild ruminants, and humans are infrequently affected.[54] Susceptible animals acquire infection through exposure of oral, palpebral and genital mucosa to fluids, and tissues of infected fetuses and vaginal discharges.

Fetal membranes of aborted fetuses have diffuse purulent inflammation characterized by thickened and dark-red to brown cotyledons. Histologically, there was loss of the cotyledonary and intercotyledonary epithelium and accumulation of necrotic debris, fibrin, neutrophils, and mononuclear leukocytes and thrombotic vasculitis in the stroma. Focal hepatic necrosis, necrosis of the lung, spleen, and infrequently brain was observed in some aborted fetuses.[52]

Chlamydia RT-PCR of fetal membranes and fetal kidney is currently the preferred method to diagnose chlamydiosis in aborted fetuses.[55] Furthermore, the organism can be identified

in fetal membranes and fetal tissues by immunohistochemistry. Several formats of enzyme-linked immunosorbent assay (ELISA) and complement fixation tests are used to detect *C. abortus* in maternal serum.[56]

Coxiellosis

Coxiellosis, often referred to as Q (Query) fever, is a highly infectious zoonotic disease caused by the intracellular bacterium *Coxiella burnetii* that primarily affects goats, sheep, and less often cattle.[7] *Coxiella burnetii* infection of nonpregnant animals is usually asymptomatic, but can cause abortion, stillbirths, endometritis, mastitis, and infertility in pregnant small ruminants and occasionally in cattle. Between 5 - 50% and in some cases up to 90% of pregnant ewes or goats may abort. The organism is shed in urine, feces, milk, vaginal fluids, semen, and placental and fetal fluids.[57]

Self-limited acute febrile disease is the most common manifestation in humans infected with *C. burnetii*. Abortions and stillbirth may occur in pregnant women. In a small percentage of infected humans, Q fever progresses to a chronic form characterized by valvular endocarditis, osteoarthritis, encephalitis, and/or chronic inflammation in other organs.[58]

The main lesion in cases of abortion in ruminants consists of fibrinonecrotic inflammation of fetal membranes. Grossly, intercotyledonary areas are thickened with a leathery appearance and are covered with white-yellow or brownish-red exudate. Cotyledons are swollen and exhibit a yellow or brownish-red discoloration. Microscopically, chorionic epithelium exhibits necrosis and infiltration of leukocytes on the surface. Stroma of intercotyledonary areas have severe infiltration of leukocytes. Large numbers of intracytoplasmic coccobacilli that are visible in hematoxylin and eosin-stained sections can be observed in the cytoplasm of chorionic trophoblast cells. Organisms are gram-negative and have a magenta color when stained with Gimenez stain, but they still must be differentiated from other gram-negative intercellular bacteria, such as Brucella or Campylobacter, by immunohistochemistry.[7,57]

Currently, the preferred diagnostic method of Q fever in aborted fetuses is amplification of *C. burnetii*-specific genomic DNA by RT-PCR from fetal membranes and tissues. Several serologic tests including indirect immunofluorescence, ELISA and complement fixation test are used for the detection of *C. burnetii* antibodies in maternal serum. After experimental infection, *C. burnetii* phase II specific antibodies, both IgM and IgG, can be detected 2 weeks postinfection and remain increased for up to 13 weeks. Antibodies directed against *C. burnetii* phase I also increase, but 4 weeks later compared to phase II antibodies. Serum antibodies in infected animals can be detected for months to years.[57] Isolation of the microorganism in cell culture, embryonated chicken eggs and laboratory animals is considered dangerous and is rarely used.[59]

Foothill abortion

Epizootic bovine abortion, also known as 'foothill abortion', is a vector borne disease of cattle that graze in the mountainous regions of California, southern Oregon, and western Nevada. It is caused by *Pajaroellobacter abortibovis* bacteria that is transmitted by argasid tick *Ornithodoros coriaceus* (Pajaroello tick).[60,61]

Abortion usually occurs in the last trimester of pregnancy exclusively in naïve heifers or cows when introduced in endemic areas between ~138 -183 days of pregnancy. Abortion generally occurs three months or longer after exposure of pregnant dams to infected ticks. Affected fetuses may induce their own delivery and may be born weak dying shortly after birth.[62]

Some of the aborted fetuses have severe abdominal distention caused by ascites, severe generalized lymphadenomegaly and splenomegaly, and petechial hemorrhages in the mucous membranes. The thymus has areas of hemorrhage and edema and the liver is swollen and nodular. Multifocal, discrete areas of pale discoloration are observed in many organs, but especially in the heart and kidney. Histologically, the most characteristic lesion is inflammation of the thymus. The lymph nodes and spleen have lymphoid hyperplasia. Severe periportal infiltration of mononuclear leukocytes and multifocal areas of histiocytic infiltration are observed in the pulmonary septa. Vasculitis can be observed in the lung, brain, and meninges.[63]

Recently, a live vaccine under conditional license was approved for cattle 6 months of age and older, and for nonpregnant females 60 days prior to breeding. Foothill abortion vaccine was safe with 100% seroconversion and > 95% protection.

Histophilosis

Histophilosis, caused by the gram-negative bacterium *Histophilus somni*, was associated with numerous clinical syndromes in ruminants including thrombotic meningoencephalitis, bronchopneumonia, polysynovitis, septicemia, mastitis, and sporadic abortion. Aborted fetuses had histologic lesions including cerebral and myocardial vasculitis and thrombosis. Isolation of the organism and detection of *H. somni* DNA by RT-PCR are the routine techniques used in the diagnosis of *H. somni* induced abortion.[64]

Leptospirosis

Leptospirosis is an important zoonotic disease caused by over 260 antigenically distinct serovars belonging to 25 serogroups grouped in 9 pathogenic species, 5 intermediate, and 6 saprophytic species of *Leptospira* a gram-negative bacterium belonging to the Spirochetales order.[65,66] Transmission is by contact with contaminated water or soil or by direct contact with urine from animals or fetal membranes and tissues and fluids of aborted animals.[67]

In the US, chronic infection of cattle with *Leptospira* serovars *hardjo* and *pomona* produce reproductive problems manifested as early repeat breeding, early embryonic death, subfertility, abortions, fetal mummification, stillbirth, retained fetal membranes, premature births, and the birth of weak and/or low-weight calves. Relative to cattle, sheep and goats can be resistant to leptospira infection.[68]

Depending on the serovar, the abortion rate in susceptible cattle may be as high as 50% with *L. pomona* and between 3 to 10% with *L. hardjo*. Aborted fetuses are often autolyzed. Histologically, multifocal renal tubular necrosis or nonsuppurative interstitial nephritis and meningitis can be observed in some aborted fetuses.[52]

Most cases of leptospirosis are currently detected by RT-PCR amplification of bacterial DNA from the fetal kidney. Serologic diagnosis of leptospirosis can be challenging particularly in vaccinated animals and is often used for the determination of the herd immune status, rather than to establish the cause of pregnancy loss. Some cows infected with *L. hardjo* that eventually abort may have high microagglutination test (MAT) antibody titers at abortion, but up to 40% had no detectable antibodies or nonsignificant titers.[69]

Listeriosis

Listeria monocytogenes, serovars 4b and 5 (*L. ivanovii*), are the etiological agent of listeriosis in small ruminants. Listeria have a wide distribution in the environment and may be recovered from dust, vegetation, decaying materials, soil, water, sewage, animal feeds, silage, and natural habitats.[70] The main form of spread among animals is by ingestion of water or food contaminated by infected fetal membranes, feces or vaginal discharges.

In small ruminants, infection with *Listeria monocytogenes* may cause abortion, encephalitis, meningitis, septicemia, and gastroenteritis. More often, these syndromes occur separately. Although serovar 5 (*L. ivanovii*) is less common, it is highly pathogenic in sheep and caused abortion in cattle.[52] Abortion in small ruminants and cattle generally occurs during the last trimester of pregnancy. Aborted fetuses are often autolyzed. Inflammation of fetal membranes and pinpoint areas of necrosis can be observed in the liver, heart, lung, kidney, and brain of aborted fetuses. Bacterial isolation from fetal membranes, fetal stomach content or fetal tissues is the diagnostic method of choice. Better results were obtained with a cold enrichment procedure.[71] Severe disease characterized by abortion and encephalitis is more often observed in pregnant woman and immunocompromised individuals.[72]

Salmonellosis

Salmonella spp. are frequently associated with enteritis and diarrhea in ruminants, but are also a cause of abortion in cattle, sheep and goats, and are zoonotic agents. *Salmonella abortus-ovis* and *Salmonella montevideo* are bacterial pathogens that can cause abortions and stillbirths in pregnant ewes and goats, and mortality in neonates.[73,74] In naive flocks, as many as 60% of all susceptible ewes and does may abort as a result of Salmonella infection. If this disease agent becomes endemic in a flock, abortions are usually sporadic; only young animals and new sheep introduced into the flock tend to be affected. Ewes and does may become carriers after aborting.

Ewes and does that abort may be asymptomatic prior to aborting, or have fever, depression, and diarrhea. Metritis and retained fetal membranes, bacteremia and death can occur after abortion. Fetal membranes are thickened, gray to red or yellow due to necrotizing suppurative inflammation and vasculitis with presence of coccobacilli in the cytoplasm of trophoblast cells. Autolyzed fetuses had signs of fetal stress characterized by diffuse yellow-green staining of the skin with meconium.[52]

Salmonella enterica subsp. *enterica* serovar Dublin (S. Dublin) may, in the course of a systemic infection, colonize the placenta and fetus and cause placentitis, abortion, and stillbirth in cattle.[75] Organisms can be isolated from fetal membranes and fetal abomasal contents.

Ureaplasma diversum infection

Ureaplasma diversum is a common inhabitant of vagina and prepuce of cattle. This organism was isolated from field cases of calf pneumonia, keratoconjunctivitis, mastitis, seminal vesiculitis, granular vulvitis, endometritis, salpingitis, and abortions.[76] Abortions, stillbirth, and birth of weak calves occurred sporadically; however, outbreaks involved multiple animals.[77]

Yersiniosis

Yersinosis is a zoonotic disease caused by *Yersinia pseudotuberculosis* that infects sheep, goats, cattle, humans, and other animal species. Transmission occurs by ingestion of contaminated food or water.[78] Abortions in small ruminants generally occurred in the last 2 weeks of pregnancy. Inflammation of fetal membranes and multifocal hepatic necrosis are the most frequent macroscopic lesions in aborted fetuses. Fetal membranes are thickened, edematous and yellow. Multifocal areas of hepatic necrosis are observed in some aborted fetuses. Some aborted fetuses had microscopic evidence of necrotizing inflammation of fetal membranes with vasculitis and bronchopneumonia.[79-81] This organism can be isolated from stomach contents, fetal membranes, lung and other fetal tissues, and from the uterus of aborting sheep and goats.[81]

Mycotic abortion

Mycotic abortions in cattle are usually sporadic. *Aspergillus fumigatus* is the most common cause of mycotic abortion in cattle with other fungi of the genera *Absidia* spp., *Mucor* spp.,

Rhizopus spp., *Mortierella wolfii*, *Candida* spp., and *Torulopsis* being less common.[82]

Severe inflammation of fetal membranes is a frequent lesion characterized by necrosis, thickening and cupping of the cotyledons and leathery thickening and yellow discoloration of the intercotyledonary area.[83] Fetal dermatitis, characterized by raised circular epidermal plaques occurs in ~ 25% of the cases.[84]

Diagnosis is established by culture and isolation. Microscopically, fungal hyphae can be observed in the lesions in hematoxylin and eosin-stained sections or sections stained with Gomori's methenamine silver and Periodic acid-Schiff stains. Fungi can also be identified in fresh tissues, or in paraffin-embedded sections by panfungal PCR.[85]

Bluetongue virus

Bluetongue virus (BTV) is an orbivirus of the family *Reoviridae* family composed of 28 serotypes. BTV is transmitted to ruminants by several species of biting midges (*Culicoides* spp.) and caused thrombo-hemorrhagic fevers mainly in sheep. In the US, the main vector of BTV endemic serotypes is *Culicoides sonorensis* (*C sonorensis*; previously known as *C varipennis*). *C. insignis* was identified in the southeastern US. Apparently, some new BTV serotypes (BTV-25, BTV-26, BTV-27) were transmitted horizontally without the involvements of the vector.[86]

Clinical disease is more common in sheep and is characterized by fever, depression, salivation, facial swelling, panting, nasal discharge, hyperemia of the muzzle, lips, ears, oral ulceration, and coronitis. Morbidity can be as high as 100% and mortality can range from 0 - 30%.[87]

In the US, BTV-induced brain malformations and abortion in sheep and cattle occur infrequently and are the result of infection with live-attenuated BTV strains present in vaccines licensed only for sheep.[88] During the 2006 European BTV outbreak, the ability of the field strain BTV-8 cross the placenta and infect the fetus was a major concern because of transplacental transmission as high as 33% and an increase in the numbers of abortions, stillbirths and fetal deformities in cattle, including hydranencephaly.[89-91] In these cases, BTV can be detected by RT-PCR in fetal splenic tissue. Isolation of infectious virus or the presence of BTV antibodies in fetuses was reported in a few cases. Immunotolerance was observed in a few animals.[92-93]

Serological assays for the detection of BTV antibodies in maternal serum include complement fixation, virus neutralization, agar gel immunodiffusion test, and ELISA. Virus isolation is by inoculation of susceptible sheep or embryonated chicken eggs with heparinized blood or homogenized lymph nodes, spleen, or lung. Subsequent adaptation to cell culture and serotyping of the virus may be necessary. Blood and tissue samples should be kept at 4°C. The most common diagnostic test is RT-PCR which has a reported high sensitivity and specificity; however, virus was not present in some positive RT-PCR cases.[87]

Infectious Bovine Rhinotracheitis

Primary infection with bovine herpes virus -1 (BHV-1) may result in several clinical manifestations including infectious bovine rhinotracheitis (IBR), abortion, infectious pustular vulvovaginitis, and systemic infection in neonates.[94] After initial infection with BHV-1, the virus establishes a lifelong latent infection in the nervous sensory ganglia and pharyngeal tonsils. Reactivation of BHV-1 from latency can occur after stressful situations including transportation, calving and treatment with corticosteroids.[95,96] The primary immune response developed after BHV-1 natural exposure or vaccination is able to successfully control viral recrudescence in a latent carrier.

Abortion and fatal systemic disease in neonates are the most severe consequences of respiratory infections of seronegative cows with virulent strains of BHV-1.[94] After infection in pregnant cows, BHV-1 may stay latent in the placenta and invade the fetus after several weeks. Once the fetus is infected, it dies quickly and remains in utero for several days before expulsion. Fetal autolysis is usually present because of rapid death of the fetus. The subcutis is edematous and red-tinged. Large amounts of red-tinged fluid were present in the thoracic and abdominal cavity and pericardium.[52] Small, discrete white nodules are observed in the liver. Histologically, discrete areas of necrosis were identified in the liver, kidneys, spleen, lungs, and adrenal glands. Intranuclear inclusions characteristic of herpesvirus infections are difficult to find in hematoxylin and eosin-stained tissue sections including sections from the adrenal glands. Fetal membrane lesions consist of necrosis and vasculitis. Abortion generally occurs between 5 to 8 months of pregnancy.

Use of live modified BHV-1 vaccines in pregnant animals that did not have antibodies from a previous vaccination were at increased risk of pregnancy loss.[97] In the US, a number of bovine abortions occurred following the use of BHV-1 vaccines that in part caused confusion about safety of modified-live virus products. Use of inactivated vaccines is safer for pregnant animals and animals with an unknown pregnancy status. Immunization of females with inactivated BHV-1 vaccine prior to breeding built protection against pregnancy loss.[97] Prevalence of abortion has diminished in regions that have fewer BHV-1 naïve populations.

The most important practice for the control of BHV-1 transmission is detection and elimination of BHV-1 semen samples. Virus isolation and BHV-1 DNA amplification by RT-PCR from fetal lung or liver or from EDTA whole-blood or semen of adult animals are the preferred methods of diagnosis. Detection of BHV-1 maternal antibodies is by virus neutralization or ELISA.[98]

Bovine viral diarrhea

Bovine viral diarrhea is an important infectious production disease in most cattle-producing countries worldwide

caused by bovine viral diarrhea virus (BVDV), a member of the genus *Pestivirus* in the Flaviviridae family. Currently, phylogenetic analysis has identified 21 *Pestivirus* subtypes (BVDV1a-u) and 4 *Pestivirus* subtypes 2 (BVDV2a-2d). Four *Pestivirus* H subtypes (HoBi a - d) have been identified in Europe. Bovine viral diarrhea viruses also are of cytopathic (cp) and noncytopathic (ncp) biotypes.[99-101]

In adult immunocompetent cattle, BVDV infection often causes subclinical disease with manifestations ranging from a mild transient infection to more severe respiratory disease lasting 2 - 3 weeks characterized by fever, nasal discharge, pneumonia and even death. Animals that recover from this form of BVDV infection clear the virus and develop lifelong immunity. The infection also has been associated with diarrhea and hemorrhagic syndrome.[102] Countries that have implemented control and/or eradication programs have, on average, 1.5 times lower pooled BVDV prevalence at animal and herd levels compared to countries without intervention measures.[103]

The outcome of BVDV fetal infection is complex and varies depending on the stage of pregnancy, the ability of fetus to mount an immune response and the BVDV biotype. Apparently, embryos are resistant to infection until they hatch from the zona pellucida ~ day 10 of pregnancy.[104] During embryonic development and up to fetal differentiation, BVDV infection causes embryonic death. In general, when infection with ncpBVDV occurs after fetal differentiation and up to 6 months of pregnancy, embryonic death, mummification, fetal malformation, abortion, immunotolerance, the birth of persistently infected calves, stillbirth or the birth of weak or undersized or apparently normal calves, and fetal malformation may occur. Later stages of pregnancy have limited susceptibility to infection and calves are born with precolostral antibodies to BVDV.[105, 106]

Fetal infection with ncpBVDV before the development of immunocompetence (between ~ days 45 and 145 of pregnancy) can result in fetal immunotolerance and persistently infected (PI) calves that are born alive.[107] These in utero-infected calves have no antibodies against BVDV, continuously shed large amounts of virus and are the main source of the virus for other herd mates. PI animals generally die by 2 years of age, often of mucosal disease that occurs when ncpBVDV mutates into cpBVDV causing super infection. BVD superinfection also result when PI animals are superinfected with field strains cpBVDV or are vaccinated with cytopathic modified live virus vaccine. After the fetus develops immunocompetence (~ 150 days of pregnancy), it is possible to have the birth of clinically normal calves with BVDV precolostral serum antibodies.[108]

In aborted fetuses, a battery of diagnostic tests including virus isolation, RT-PCR and demonstration of BVDV antibodies in serum or fluid from the thorax or abdomen should be done in parallel. Immunohistochemistry, RT-PCR and antigen capture ELISA in ear notches are used to detect PIs. Virus neutralization tests are used for the detection of BVDV antibodies in serum.[109] Detection of PIs, implementation of biosecurity measures and vaccination are important practices for the control of BVD in herds. BVDV vaccines available in the US include killed virus and modified live virus.[110]

Cache Valley virus

Cache Valley virus (CVV) is an arbovirus of the family *Bunyaviridae* that is endemic in North America and infects a wide range of domestic and wild animals, and humans. The virus is transmitted by the bite of competent vectors of several species of *Aedes*, *Anopheles*, *Coquillettidia* and *Culiseta* genuses including *Ae. japonicus*, *Ae. scapularis*, *Ae. sollicitans*, *Ae. taeniorhynchus*, *Ae. vexans*, *An. punctipennis*, *An. quadrimaculatus*, *Co. perturbans* and *Cu. Inornata*.[111]

CVV infection is usually asymptomatic in adult sheep, goats and cattle. CVV infection during pregnancy may result in embryonic mortality, fetal mummification, fetal malformation, abortion, stillbirth and pregnancy loss in sheep and goats and less often cattle.[112,113] A limited number of human case reports have described severe illness including meningitis.[111]

Congenital malformation in sheep and goats may include one or more of the following: arthrogryposis, kyphosis, torticollis, pelvic limb hemimelia, maxillary prognathism, hydranencephaly, and cerebellar hypoplasia.[114] In utero experimental infection of pregnant ewes at day 35 of pregnancy resulted in necrosis of the central nervous system and skeletal muscles at 7 - 14 days post-infection, and hydrocephalus, micromyelia and muscular loss at 21 - 28 postinfection.[115]

CVV infections in aborted fetuses and stillbirths can be diagnosed using serum neutralization tests because fetuses are able to mount an antibody response and clear the virus. Experimental infection of pregnant ewes with CVV ~ 35 days of pregnancy had low virus antigen and RNA signal in tissues by day 56 of pregnancy and cleared the virus by day [75.116] Virus isolation in full-term malformed fetuses is unsuccessful.

Experimental infection of pregnant sheep with two California serogroup bunyaviruses (LaCrosse virus and San Angelo virus) and a Bunyamwera serogroup member (Main Drain virus) induced a range of lesions including arthrogryposis, hydrocephalus, fetal death, axial skeletal deviations, anasarca, and oligohydramnios.[117] Fetal teratogenesis in sheep, goats, and cattle have been described in natural and experimental infections with other related exotic Bunyaviruses including Akabane virus, Schmallenberg virus, Rift Valley fever virus and Wesselsbron disease virus.[118,119]

Caprine herpesvirus-1

Caprine herpesvirus -1 (CpHV-1) belongs to the subfamily of

alphaherpesviruses that contains 7 genetically-related viruses. CpHV-1 is closely related to BoHV-1, the cause of infectious bovine rhinotracheitis. Late term abortions and gastroenteric and respiratory disease in 1- 2-week-old kids have been associated with CapHV-1 infection.[120] Up to 50% of pregnant does in a herd may experience late term abortions or the birth of stillborn kids. Numerous white, pinpoint foci ranging from 1 to 2 mm may be observed in the liver and lungs of aborted fetuses. Microscopically, discrete areas of coagulative necrosis can be observed in liver, lung, thymus and less frequently other organs. Intranuclear inclusions bodies in the periphery of the areas of necrosis. In adult goats, CpHV-1 infections are general subclinical, but vulvovaginitis and balanoposthitis occurs infrequently.[121]Amplification of CpHV-1 by RT-PCR and virus isolation are used for diagnosis. The virus neutralization test is used for the demonstration of CpHV-1antibodies in maternal serum.[122]

Neosporosis

Neospora caninum, the cause of neosporosis, is an apicomplexan parasite and one of the most important causes of pregnancy loss in cattle. *N. caninum* also causes abortion in sheep and goats. The life cycle of *N. caninum* is similar to that of *Toxoplasma gondii* in small ruminants, but cattle are the intermediate host and dogs and coyotes the definitive host of *N. caninum*.[123] There are 2 modes of transmission: vertical from dam to offspring; and horizontal from its definitive host, the dog, to cow, sheep or goat, with vertical transmission being more common. Dogs generally acquire infection by ingesting fetal membranes or tissues from infected aborted fetuses.[124]

Abortion in cattle due to *N. caninum* is more common between 5 and 7 months of pregnancy. In naïve herds, 30% or more of pregnant heifers or cows abort over a period of several months. Once, the infection becomes endemic in a herd, the abortion rate is about 5% per year and persists for several years.

Transplacental infection may also result in, fetal mummification, birth of weak compromised calves, or the birth of clinically normal infected neonates. These normal infected neonates remain persistently infected and eventually transmit infection transplacentally to their progeny and preserve neosporosis in the herd. Cows that abort due to Neospora infection do not show other clinical signs. Apparently, risk of abortion in subsequent pregnancies is lower.[123]

Aborted fetuses are often autolyzed or may be partially or fully mummified. Focal areas of inflammation can be observed microscopically in the brain, brain stem, skeletal muscle, and heart. Rarely, the organism can be recognized in the brain by standard hematoxylin and eosin staining, but can be demonstrated by immunohistochemistry. Fetal membranes have necrosis of the trophoblast in cotyledons, with normal intercotyledonary areas. Congenitally infected full-term calves may be born weak, show ataxia or are born clinically asymptomatic but have high serum titers of precolostral serum antibodies.[52]

Numerous diagnostic tests including RT-PCR, histopathology and fetal serology should be done concurrently in abortion cases for the accurate diagnosis of neosporosis. Serum antibodies in maternal serum detected by ELISA are used to determine the infectious status of individual animals, but the presence of maternal antibodies alone is not a proof of the cause of pregnancy loss.[125] The most important measure for the control of neosporosis is to identify and eliminate congenitally infected heifers and to reduce postnatal transmission from definitive hosts.[126]

Sarcocystosis

Sarcocystois is a genus of cyst-forming coccidian and the cause of sarcocystosis. *Sarcocystois* spp. are obligatory 2-host life cycle Apicomplexa parasites that includes herbivores as intermediate hosts and carnivores as definitive hosts. Infection is common but the majority of infected cattle, sheep, and goats are asymptomatic. Acute fatal disease develops occasionally and is characterized by fever, edema, and jaundice. Animals exhibit inappetence, weight loss, decreased milk production, endometritis, and neurological signs. Pregnant animals may abort during the acute phase.

Abortion may be indirect by premature induction of parturition due to the release of prostaglandin $F_{2\alpha}$ during acute disease or by direct infection of the fetus. Aborted fetuses exhibit extensive microscopic lesions consisting of multifocal areas of necrosis and mononuclear cell infiltration in the brain and meninges. Similar lesions can be observed in the heart, kidney, liver, lung, and fetal membranes. Organism can be identified by immunofluorescence in frozen sections of tissues.[52,127]

Toxoplasmosis

Toxoplasmosis, caused by the apicomplexan parasite *Toxoplasma gondii*, is a major cause of abortion in sheep and goats.[128] Simultaneous infection with *Chlamydia abortus* and *Coxiella burnetii* occurs in some cases. Most sheep and goats become infected with *T. gondii* by ingesting food or water contaminated with sporulated oocysts shed by cats. Less than 4% of persistently infected sheep transmit the parasite in utero to the fetus.[129,130] Infection during the first part of pregnancy is likely to result in early embryonic death and resorption. Later in pregnancy, infected ewes and does may exhibit fetal mummification, abortion, stillbirth, and birth of weak offspring. Infection of pregnant ewes and does with T. gondii in naive herds may result in abortion storms. Sheep and goat develop humoral and cellular immune responses after infection that provide effective protection against pregnancy loss in subsequent pregnancies.[129]

On gross examination, the cotyledons of aborted fetuses have numerous small white nodules ranging from 1 to 3 mm.

In few affected fetuses, there is evidence of focal myocarditis, pneumonitis or encephalitis. The organisms can be demonstrated in formalin fixed, paraffin embedded tissues by immunohistochemistry. The preferred technique for the diagnosis of *T. gondii* in aborted fetuses is RT-PCR.[52] Presence of maternal serum antibodies is an indication of exposure, but does not prove *T. gondii* as the cause of abortion.

Trichomoniasis

Trichomoniasis is an economically important infectious venereal disease of cattle caused by *Tritrichomonas foetus*, a protozoan flagellated parasite. Infected bulls are asymptomatic but carry the protozoa in their preputial sheath and penis and are the source of infection for cows and heifers.[132] In some bulls, the protozoa are harbored in the distal urethra. Infection occurs at coitus and continues for some time after infection. Most infected animals clear the infection spontaneously within a few weeks to a few months. After clearing the infection, cows can become pregnant and carry a fetus to term. The infection may cause embryonic mortality, pyometra, and infrequently mid-pregnancy abortion.[133,134] Lesions include, mild inflammation of fetal membranes characterized by edema, and small amounts of white to yellow exudate and mild necrosis of cotyledons. Generally, fetal lesions are not present, but large numbers of organism can be detected in the fetal fluids and stomach.[52,135] Some aborted fetuses had suppurative bronchopneumonia.[16]

In aborted fetuses, diagnosis is by detection of *T. foetus* DNA by RT-PCR in fetal membranes, placental fluids, fetal stomach content or in uterine washings or vaginal discharges. Required samples for detection of trichomoniasis in bulls is a preputial/penis scraping.[136]

Conflict of interest

Authors have no affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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