Pregnancy loss in small ruminants

Andrés de la Concha-Bermejillo,[a] Juan Romano[b]
[a]Texas A&M Veterinary Medical Diagnostic Laboratory
[b]Large Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences
Texas A&M University, College Station, TX

Abstract

Pregnancy loss in sheep and goats is an important cause of economic loss to livestock producers. Causes of pregnancy loss are noninfectious and infectious. Noninfectious causes include toxic plants and toxins, nutritional deficiencies, medications, and environmental factors. Infectious causes are more commonly diagnosed than noninfectious causes and include bacterial, mycotic, viral, and parasitic infections. Several of the infectious causes of pregnancy loss in small ruminants are transmissible to humans, either directly or indirectly, and pose substantial threats to public health. Identification of the cause of pregnancy loss is of prime importance to implement control programs to reduce economic impact and the risk of human infection. Veterinary diagnostic laboratories help in identifying, preventing, and controlling pregnancy loss. Additionally, laboratory testing is an important part of any surveillance program, in identifying transboundary animal diseases and in testing livestock and their specimens for export purposes.

Keywords: Cattle, abortion diseases, pregnancy loss, embryonic mortality

Introduction

Pregnancy loss in sheep and goats results in reproductive wastage, and thereby making it economically relevant. A wide range of causes, either infectious or noninfectious, resulted in pregnancy loss in small ruminants.[1,2] Some infectious causes of pregnancy loss in small ruminants are zoonotic diseases and may cause serious clinical disease and abortion in humans.[3] Important causes of infectious abortion in small ruminants include *Brucella melitensis*, *Campylobacter* spp., *Chlamydia abortus*, *Coxiella burnetii*, *Leptospira* spp., *Listeria monocytogenes*, *Salmonella* spp., *Yersinia pseudotuberculosis*, *Aspergillus* spp., bluetongue virus (BTV), border disease virus (BDV), Cache Valley virus (CVV), caprine herpes virus-1 (CpHV-1), *Neospora caninum*, and *Toxoplasma gondii*.[1,2] Multiple pathogen infections can occur in some outbreaks of abortion in small ruminants.[4] Some infectious causes of abortion such as *Brucella melitensis*, akabane disease virus, and rift valley fever virus; Wesselsbron disease virus and Schmallenberg virus are exotic and reportable in US. Noninfectious causes of abortion in small ruminants are diagnosed less frequently and include toxic plants, toxins, nutritional deficiencies, and environmental factors.[2,5,6]

Goats are more susceptible to pregnancy loss than sheep because goats require a functional corpus luteum throughout pregnancy. Any cause, infectious or noninfectious that results in luteolysis in goats will terminate pregnancy. In sheep, the placenta is responsible for progesterone production after day 55 of pregnancy and ewes can maintain pregnancy after this period in the absence of a functional corpus luteum.[7]

Although sporadic abortions may occur in any flock or herd, often epizootic outbreaks occur with > 50% pregnancy loss. When several abortions occur in close succession, further investigations must be carried out to determine the cause so that control measures can be implemented. Diagnosis of abortion in small ruminants is difficult, because it is common for fetuses to die in utero and to be expelled after several days. These fetuses exhibit advanced autolysis that hampers the interpretation of postmortem findings. Fetal membranes are often soiled with dirt and hay and contaminated with saprophytic bacteria that can interfere in culturing pathogens responsible for abortion.[2] The objective is to review common causes of abortion in small ruminants, with an emphasis on infectious causes and human health implications, and to outline an approach for investigation of abortion outbreaks, describing available tests for diagnosis. Further information on abortion causes in ruminants is reviewed in the proceedings.[8,9]

Prevention of human infection

Several infectious causes of abortion in small ruminants are zoonotic diseases. Prevention of human infection should be an important goal in the control of these infections. Zoonotic

diseases are transmitted to humans through direct contact with infected animals or fomites, by inhalation of airborne microorganisms, and by ingestion of farm animal products.[3] Most common route of transmission from small ruminants to humans is via consumption of raw milk or fresh cheese from sheep and goats. Only pasteurized milk should be consumed by humans. All milk sold across state lines or internationally must be pasteurized and meet the standards of US Pasteurized Milk Ordinance.[10]

Owners, farm workers, and veterinarians are often exposed to sheep and goat zoonotic diseases during lambing, kidding or by handling aborted fetuses and fetal membranes of infected animals. General precautions that should be taken during lambing/kidding or when handling aborted fetuses include the use of personal protective equipment (PPE). Basic PPE comprises disposable rubber gloves, safety googles, coveralls and rubber boots. Although the use of N95 masks is highly desirable, it is not always possible. To be effective, PPE should have the level of protection needed for the infectious organism involved and should be adequately fitted for the person using it.[11] All dirty clothes should be left at the farm for disinfection and washing or placed in biohazard bags and washed separately. All contaminated trash and rubber boots should be disinfected and trash disposed of properly. Samples sent to the laboratory should be packed and labelled appropriately.[8] Pregnant women, small children, and immunosuppressed individuals should refrain from handling infected animals or assisting in lambing and kidding.[12]

Infectious causes of pregnancy loss in small ruminants

Brucellosis

Brucella melitensis is the main cause of abortion in goats and sheep in many parts of the world where it is also an important cause of human disease. *B. melitensis* infection has been eradicated from the US and currently is a reportable disease.[13] Infection with B. abortus occurs occasionally in small ruminants. Brucella ovis is a less pathogenic species of *Brucella* and more commonly associated with epididymitis and infertility in rams, but occasionally it causes abortion, stillbirth, and increased perinatal mortality.[14] In contrast to other *Brucella* species, B. ovis lacks zoonotic potential.[15]

Brucella melitensis is the most virulent member of the *Brucella* species and accounts for most cases of human brucellosis. Transmission of *B. melitensis* infection from animal to human may occur through direct contact, inhalation of airborne microorganisms, and ingestion of farm animal products. Consumption of raw milk or fresh cheese made with unpasteurized milk from an infected sheep or goat is the main way of transmission from animals to humans.16 Human brucellosis ('undulant fever') is characterized by a nonspecific clinical syndrome with relapsing fever and arthritis and in some cases hepatomegaly, splenomegaly, endocarditis, and abortion.[17]

Abortion due to *Brucella melitensis* in small ruminants commonly occurs during the last 2 months of pregnancy. The organism has high affinity for trophoblast cells and causes placentitis.[18] Diagnosis of brucellosis is the center of any control program and is based on bacterial culture and serological testing. The polymerase chain reaction (PCR) methods provide additional means of detection.[19]

Campylobacteriosis

Campylobacter spp. are important animal pathogens and opportunistic human pathogens. Several species and subspecies of *Campylobacter* cause pregnancy loss and infertility in ruminants. The mammal-associated *Campylobacter* (C.) *fetus* is comprised of 2 subspecies: *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus*, with both being well-known causes of reproductive failures in ruminants.[20]

C. fetus subsp. *venerealis* causes bovine genital campylobacteriosis, characterized by infertility and abortion. *C. fetus* subsp. *fetus* is most frequently isolated from abortions in sheep. Since the 1990s, *C. jejuni* has been associated with ovine abortion in the US.[21]

In sheep, the reproductive disease associated with *C. fetus* subsp. *fetus* and *C. jejuni* is commonly known as epizootic abortion.[22] These bacteria are transmitted orally via fecal contamination or from infected fetal membranes or fluids. Abortion generally occurs in the last trimester and abortion storms occur in naïve flocks.[23] Cases of campylobacteriosis and abortion occur in pregnant women during the second and third trimesters of pregnancy.[24] In pregnant ewes, infection results in placentitis and fetal infection; abortion typically occurs in the last trimester of pregnancy or there is birth of weak lambs. Fetal membranes lesions may be subtle. In some cases, fetal membranes appear edematous, thickened, and leathery. Cotyledons may be covered with brown to red exudate. The fetus is often autolyzed and has red-tinged fluid in body cavities. In some aborted fetuses, peritonitis is present, characterized by a thin layer of fibrin covering liver capsule with strings of fibrin in the peritoneum. In ~ 25% of aborted fetuses, there are white, often circular areas of necrosis in the liver ranging from 1 to 5 cm. Inflammation of fetal membranes, pneumonia, serositis, hepatitis, and encephalitis are common microscopic lesions. Culture of the organism from fetal membranes and fetal stomach content is the preferred diagnostic method.[18] In the US, a vaccine from inactivated cultures of *Campylobacter fetus* and *Campylobacter jejuni* is available for sheep that requires a first dose before breeding and a booster in 60 to 90 days. Annual revaccination is recommended. Antibiotic treatment is used to clear the infection in carrier animals.[13,23]

Chlamydiosis

Chlamydia abortus (*Ch. abortus*), formerly known as *Chlamydophila abortus* or *Chlamydia psittaci* serovar 1 is an obligate intracellular organism that causes abortions in sheep and goats, and

occasionally in deer, cattle and llamas. In sheep, the disease is often referred as enzootic abortion of ewes. It is the most commonly diagnosed infectious cause of lamb loss in many countries world-wide. Ch abortus can be a cause of abortion in humans.[25] Transmission to naïve ewes and goats and to susceptible humans is via contact with tissues and fluids of aborted fetuses, vaginal discharges, and fetal membranes of aborted ewes.[26]

Between > 30 - 60% of pregnant susceptible animals may abort when the organism is first introduced to a naïve flock or herd. Once the infection becomes endemic, the abortion rate decreases and animals that abort are generally newly introduced naïve and young animals. When infection occurs early in pregnancy, sheep and goats may abort late in that pregnancy, but when infection occurs late in pregnancy, infected animals usually abort in the following pregnancy. Lambs and kids infected congenitally may abort during their first pregnancy.[27] Infected ewes and does may give birth to weak lambs or kids that fail to survive beyond 24 hours. Some clinically healthy lambs and kids born from infected ewes/does can be a source of infection for naïve animals and represent a substantial zoonotic risk. The risk of transmission of infection from vaginal discharges of infected ewes/does is substantially reduced or virtually eliminated after several days, once vaginal discharges have ceased.[26]

In advanced stages of *Ch. abortus* fetal infection, there is severe diffuse inflammation of fetal membranes, characterized by cotyledons that are dark red to brown and intercotyledonary areas that are thickened with a leathery appearance. Microscopically, there was loss of trophoblast and suppurative inflammation throughout the hilar zone of placentomes, and vasculitis.[4] Basophilic microorganisms that stain magenta with Gimenez stain are present in trophoblast cells. Focal areas of coagulative necrosis can be observed in the liver and spleen of some aborted fetuses.[7,28] Subclinical infection with Ch. abortus may substantially affect bovine herd health and production.[29] In some countries, a commercial live, attenuated Ch. abortus 1B vaccine strain is used for the prevention of chlamydiosis in sheep. Recently, it became apparent that the unattenuated vaccine strain caused placentitis indistinguishable from placentitis caused by Ch. abortus wild-type.[30]

If Chlamydia is suspected to be present in a flock/herd, treatment with a long-acting oxytetracycline preparation (20 mg/kg) will reduce the severity of infection and losses resulting from abortion. The treatment has to start after 95 - 100 day of pregnancy, the point at which pathologic changes start to occur. Further doses can be subsequently given at 2-week intervals until the time of lambing. Although such treatment reduces losses and limits the shedding of infectious organisms, it does not eliminate the infection nor reverse any pathologic damage already done to the placenta, thus abortions or the delivery of stillborn or weakly lambs can still occur, and the shed organisms are a source of infection for other naıve animals.[31] Moreover, antibiotic treatment has not been critically evaluated and this approach has the potential to create microbial resistance.

Serological tests including enzyme–linked immunosorbent assay (ELISA) and complement fixation to detect the presence of anti-Ch. abortus antibodies are able to actually prove the presence of an immunological response and thus an infection. Complement fixation titers of 1:16 - 1:32 are considered positive for most laboratories. The titer may increase to > 1:80 in 2 - 3 weeks after abortion. Currently, the best diagnosis of test in aborted fetuses is real time polymerase chain reaction (rtPCR) of fetal membranes and fetal tissues.[25]

In the US, an inactivated vaccine of *Ch. abortus*, abortigenic serovar is available for sheep that required the first dose 60 days prior to breeding and a booster 30 days later. Annual revaccination is recommended. In Europe, some live attenuated vaccines were associated with abortion in sheep (type 1B). Use of live attenuated vaccines presents some drawbacks: require adequate handling, possible transmission to humans, cannot be given to pregnant females and to animals receiving antibiotics and vaccinated animals cannot be commingled with unvaccinated animals.[30]

Coxiellosis

Coxiellosis, also known as Q (Query) fever, is a highly infectious zoonotic disease caused by the intracellular bacterium *Coxiella burnetii* that primarily affects goats, sheep, and less frequently cattle. Other species less commonly affected include dogs, cats, rabbits, a variety of wild and domestic mammals, and birds. In the US, *C. burnetii* is classified as a category B potential aerosolized biological weapon.[12]

Transmission is mainly by ingestion or inhalation of contaminated fetal membranes or uterine discharges, or by inhalation of contaminated dust from barns.[32,33] Ewes and does can shed the bacteria in milk. *C. burnetii* infections in sheep and goats are characterized by late term abortions, stillbirths, and weak neonates that often die during the first few days after birth. In naïve herds, > 50 - 70% of susceptible ewes or goats may abort. In subsequent years, once infection becomes endemic in the herd, the abortion rate may be < 5%. Abortions tend to occur primarily in younger naive animals. Other than losing pregnancy, ewes and goats that abort do not have any clinical signs. The most common lesion is inflammation and thickening of fetal membranes and leathery appearance with large quantities of white–yellow, creamy or reddish-brown exudate at the edges of the cotyledons and in the intercotyledonary areas. Histologically, large amounts of necrotic debris are present on the chorionic surface, with severe necrosis of the chorionic epithelium. Large numbers of microorganisms may be present in the cytoplasm of chorionic epithelial cells, but these microorganisms need to be differentiated from other microorganism that cause

placentitis in small ruminants, including *Brucella* and *Campylobacter* species. The stroma of the chorioallantois is infiltrated with large numbers of mononuclear leukocytes and some neutrophils.[12,33]

Bacterial identity in fetal membranes can be confirmed by immunohistochemistry. Isolation of the microorganism in cell culture, embryonated chicken eggs and laboratory animals is considered dangerous and is rarely used. Currently, the preferred diagnostic method is amplification of *C. burnetii* DNA by rtPCR. Several serologic tests including indirect immunofluorescence, ELISA and complement fixation test are used for the detection of *C. burnetii* antibodies in maternal serum.

C. burnetii IgM and IgG phase II specific antibodies can be detected 2 weeks postinfection and remain increased for up to 13 weeks. Antibodies directed against *C. burnetii* phase I increase generally 4 weeks later compared to phase II antibodies. Serum antibodies in infected animals can be detected for months to years.[12,33]

Leptospirosis

Leptospirosis is a worldwide zoonosis caused by pathogenic spirochetes that belong to the genus *Leptospira*. A large amount of scientific information exists on the disease in cattle, but less information exists about leptospirosis in sheep and even less in goats.[34] Relative to cattle, sheep and goats have been considered resistant to leptospiral infection.[35] Animal infection most frequently occurs through direct contact with leptospira-contaminated urine or indirectly from interaction with contaminated water and/or soil.[36]

Leptospiral infection in sheep and goats occurs in many countries. Affected sheep and goats may act as carriers of the organism. *Leptospira* infection in small ruminants is often subclinical; however, younger animals may have fever, dyspnea, depression, anorexia, jaundice, and may eventually die. In a study in Spain to determine causes of pregnancy loss in small ruminants, 1.7 and 2.6% of abortions were caused by *Leptospira* spp. in sheep and goats, respectively, with the majority of these abortions caused by serovar Pomona.[37]

Laboratory tests are essential to achieve an accurate diagnosis of the infection. Microscopic agglutination test is used to determine the presence of antibodies in serum of infected animals and determine flock/herd status. In aborted fetuses, rtPCR is the recommended test for confirmation of *Leptospira* spp. as cause of abortion.[38]

Listeriosis

Listeria monocytogenes, serovars 4b and 5 (*L. ivanovii*), is the etiological agent of listeriosis in small ruminants. *L. monocytogenes* is a ubiquitous facultative saprotroph present in soil, plants, ground water, and vegetation.[39] Silage is a common source of infection for ruminants. The organism is shed in the feces of healthy carriers and sick animals. *Listeria monocytogenes* is a severe human foodborne infection characterized by gastroenteritis, meningitis, encephalitis, abortions, and perinatal infections.[40]

L. monocytogenes infections in small ruminants can result in 3 separate clinical syndromes, including encephalitis in adult animals, abortions, and neonatal septicemia. Abortions and neonatal septicemia can occur concurrently with the encephalitic syndrome in the same flock or herd, but more commonly, they occur as separate entities. Abortions in cattle, sheep and goats usually occur in the last trimester of pregnancy and up to 50% of the susceptible pregnant animals may abort in an outbreak.[18,41]

Aborted fetuses are retained in utero for several days before being expelled and may have evidence of autolysis. Retention of the fetal membranes and their inflammation are common. Dystocia, metritis and neonatal septicemia occurs in some cases. Grossly aborted fetuses may have multifocal areas of necrosis ranging from 1 to 3 mm in the liver and less often in the lungs, heart, kidney, spleen, and brain. Fetal membranes exhibit extensive thickening and yellow discoloration of intercotyledonary areas and cotyledons.[18]

Bacterial isolation from fetal membranes, fetal stomach content or fetal tissues is the diagnostic method of choice. Better results are obtained with the cold enrichment procedure.[42] Detection and genetic characterization of *L. monocytogenes* DNA is by rtPCR of fetal brain and other fetal tissues.[43]

Salmonellosis

Salmonellosis is an uncommon disease in sheep, but severe outbreaks may result in heavy animal losses. Sheep can be infected with several species of Salmonella of the family Enterobacteriaceae. Ovine salmonellosis may be an important zoonotic reservoir for human infection.[44] Profuse diarrhea is common and pregnant ewes may abort. *Salmonella abortus-ovis* is a bacterial pathogen that can cause abortions and stillbirths in pregnant ewes, and mortality in neonates.[45] Abortion storms occur in naïve flocks when this organism is first introduced, but once the organism becomes endemic in the flock, ewes generally develop protective immunity, and abortions are sporadic or limited to younger or newly introduced animals. Stress including transportation, comingling, drastic changes in weather, and other factors often precipitate abortion outbreaks in infected flocks. Some ewes that abort may not have other clinical signs of disease apart from abortion; however, some may have fever, anorexia, diarrhea, and may die. Other Salmonella serovars including *S. dublin*, *S. typhimurium*, and *S. montevideo* also can cause abortion in pregnant ewes, with or without systemic illness in the ewe.

Retention of fetal membranes is common. Inflammation of fetal membranes (grossly indistinguishable from inflammation caused by other bacteria) can be apparent. The skin/wool of aborted fetuses is often stained with meconium. Microscopically, evidence of meconium aspiration in the lung is noticed. *Salmonella* spp. can be isolated from the liver, spleen or the gastrointestinal tract of aborted fetuses, fetal membranes, and from vaginal secretions of affected ewes.[18] The PCR allows the detection of *S. abortusovis* DNA up to 3 months after infection in samples that were negative by culture.[46]

Yersiniosis

Yersinia pseudotuberculosis, the cause of yersiniosis is a gram-negative aerobic or facultative anaerobic rod-shaped bacterium of the family Enterobacteriaceae that infects small ruminants, cattle, humans, and several animal species.[47] Clinical disease in 1- to 6-month-old goat kids can be manifested as sudden death or diarrhea. The disease occurs more frequently in winter and spring and appears to be predisposed by stressful situations including drastic changes in weather, transportation, comingling, and starvation. In pregnant goats, infection might result in abortion and the birth of weak kids. The infection has also been associated with mastitis in goats and cows. Transmission occurs mainly through the fecal-oral route, after ingestion of contaminated feed or water.[48]

Fetal infection is characterized by abortion. Fetal membranes are thickened and appear white with opaque white foci on cotyledons. Histologically, suppurative inflammation of fetal membranes and suppurative pneumonia of fetuses. The organism can be isolated from fetal membranes, stomach content, liver and lung. In case of an abortion storm, treatment of goats with tetracycline has been useful. Other broad-spectrum antibiotics may also be useful. Animals receiving antibiotic treatment must undergo the determined milk and meat withdrawal period prior to milk and meat entering the human food supply.[49]

Bluetongue

Bluetongue (BT) is an insect-transmitted, noncontagious disease of ruminants caused by bluetongue virus (BTV), the prototype virus of the genus Orbivirus in the family Reoviridae. BTV exists throughout much of the world between latitudes of ~ 40 - 50 degrees N and 35 degrees S.[50-52] BT clinical disease in North America occurs most commonly in sheep and deer, and sporadically in cattle and goats. It has been described in South American camelids.[53] Abortion has been reported in dogs vaccinated with a commercial multivalent modified live vaccine against canine distemper virus, and adenovirus and parvovirus contaminated with BTV-11.[54] Currently, there are 28 BTV serotypes recognized worldwide. In the US, serotypes 10, 11, 13, and 17 are endemic. BTV-2 is isolated sporadically in the US. Nonendemic serotypes ((1, 3, 5, 6, 9, 12, 14, 19, 22, and 24) normally present in Mexico and Central America may sporadically appear in the US.[53]

Insects of the genus *Culicoides* (biting midges) serve as true biologic vectors for the transmission of BTV infection between susceptible ruminants. In the US, the main vector of BTV is *Culicoides sonorensis* (previously known as *C. varipennis*). After infection, sheep can remain viremic for up to 54 days and cattle for 60 - 100 days. Long term infection with BTV-25 has been reported in healthy goats in Switzerland.[55] Transmission can occur transplacentally or by semen.52 Some of the new BTV serotypes (BTV-25, BTV-26, BTV-27) are thought to also be transmitted horizontally without vector involvement.[56]

From 5 to 50 -75% of susceptible animals may have clinical signs when BTV is first introduced into a naïve population; however, once the infection becomes endemic, the morbidity and mortality may be as low as 1 - 2%. Clinical disease in sheep is characterized by fever, depression, salivation, facial swelling, panting, nasal discharge, hyperemia of the muzzle, lips, ears, oral ulcerations, and coronitis.[53]

BTV vertical transmission from dam to fetus in enzootic areas is considered negligible. In these areas, BTV-induced pregnancy loss and congenital malformations have been associated with BTV strains from modified live virus vaccine strains. Infection of cattle, sheep, and goats with the European BTV-8 wild-type strain demonstrated a high incidence of transplacental transmission and pregnancy loss in natural circumstances.[57,58] Transplacental transmission of wild-type BTV-8 is approximately 10 - 41.7% in cows and up to 69% in sheep.[59] Infection of pregnant cows with wild-type BTV-8 can cause abortion, stillbirth, and fetal malformations (hydranencephaly).[60] Goats are less susceptible to BTV-8 infection than sheep and cattle.[61]

Modified live virus (MLV) vaccines are used in California to protect sheep against infection with BTV serotypes 10, 11, and 17 and an MLV vaccine to BTV serotype 10 is licensed for use in sheep throughout the US. Adverse effects of BTV MLV vaccines are fetal malformations and recombination of BTV vaccine strains with field strains giving rise to new strains of virus.[53]

Serologic diagnosis of BTV relies upon the detection of BTV antibodies by competitive ELISA, virus neutralization, agar gel immunodiffusion, and complement fixation tests. BTV can be isolated from heparinized blood, semen or tissues (spleen, lymph nodes, and lung) of infected animals by inoculation of susceptible sheep or embryonated chicken eggs. Subsequent adaptation to cell culture and serotyping of the virus may be necessary. Assays based on rtPCR are used to detect BTV RNA in clinical samples (e.g. blood or spleen).[62]

In the US, a nationally licensed commercial modified live vaccine is commercially available for sheep and goats to prevent Type 10 Bluetongue virus infection. Lambs are vaccinated > 3 months of age and ewes are vaccinated 3 weeks prior to breeding or after lambing. Additional vaccines in CA to Types 10, 11, and

17 for sheep and Types 10,11, 13, and 17 for wildlife are available through a state license. Other states use inactivated BTV provisionally to control outbreaks.

Border disease

Border disease is caused by BDV that belongs to the genus Pestivirus in the family Flaviviridae, together with bovine viral diarrhea virus types1 (BVDV-1) and 2 (BVDV-2) and classical swine fever virus (CSFV).[63] It is known that many pestiviruses exhibit a broad species tropism.[64] Small ruminants can abort with bovine viral diarrhea virus (BVDV).[65] Sheep persistently infected with BDV readily transmit the virus to calves seronegative to BVD virus.[66]

Several subgenotypes of BDV have been described to date. BDV infections occur mainly in sheep and goats but also in cattle, pigs, and wild even-toed ungulates.[63] Infection of pregnant ewes and goats with BDV during the first trimester of pregnancy often results in fetal death and resorption. Infection during the second and last trimester of pregnancy causes fetal malformation, fetal mummification, abortion, stillbirth, and birth of weak or normal lambs.[18] Some of the lambs infected congenitally had low birth weight, abnormalities in wool pigmentation and severe neurological signs including ataxia or rhythmic contractions of the hind limbs, ears, tail, and head (hairy shakers).[2] Examination of the brain reveals cerebellar hypoplasia, porencephaly, hydranencephaly, cerebellar dysplasia, nonsuppurative meningoencephalomyelitis with hypomyelinogenesis, and arthrogryposis.[67,68]

Diagnosis of pregnancy loss by BDV in sheep and goats is complex. Virus isolation, detection of BDV antibodies in fetal serum or fluids from cavities and amplification of BDV nucleic acid by rtPCR should be performed concurrently for accurate diagnosis.[18]

Cache Valley virus

Cache Valley is an arthropod-borne viral infection, afflicting a variety of domestic and wild ruminants including sheep, goats, deer, cattle, caribou and also pigs, horses, raccoons, woodchuck, turtles, rabbits, foxes, and humans, caused by CVV, a bunyavirus in the family Bunyaviridae, Bunyamwera group.[69,70] Other North American bunyaviruses including LaCrosse virus, San Angelo virus, and Main Drain virus and other exotic bunyaviruses such as Akabane, Schmallenberg, and Rift Valley fever viruses cause similar congenital malformations in sheep and goats.[2,71]

CVV is endemic throughout the US, Canada, Mexico and some countries of Central America.[69] Transmission of CVV to ruminants occurs through bites of infected mosquitos, including several species of *Aedes*, *Psorophora*, *Anopheles*, *Coquillettidia*, *Culex*, and from *Culiseta inornata*. Majority of infections in adult ruminants are subclinical, but CVV infection in pregnant ewes and goats and less often in other ruminants may result in embryonic mortality, fetal mummification, stillbirth, and various degrees of musculoskeletal or central nervous system abnormalities including arthrogryposis, hydranencephaly, hydrocephalous, microcephalous, porencephalia, cerebellar hypoplasia, scoliosis, torticollis, and lordosis.[70]

CVV-experimentally induced fetal malformation in sheep occurs when the virus infects the fetus between days 27 and 54 of pregnancy. After the second month of pregnancy, the ovine fetus becomes immunocompetent and develops a cellular and humoral immune response that controls and clears CVV without apparent consequences to the fetus.[72] Dystocia may be a sequela in ewes that deliver full-term malformed offspring. Ewes that are CVV seropositive at breeding are resistant to reinfection with CVV, but not against other related bunyaviruses.[72,73]

Because fetuses infected in second and third trimesters develop an immune response that clears CVV, demonstration of CVV-neutralizing antibodies in the serum or thoracic or abdominal fluid of malformed fetuses is the best method to establish diagnosis. Virus isolation from full-term aborted fetuses with CVV-induced malformations is unsuccessful. Presence of CVV antibodies in maternal serum is an indication of exposure but not a proof that fetal malformation was caused by CVV. Lack of antibodies in maternal serum rules out CVV as the cause of fetal malformation. Presence of serum CVV antibodies before breeding in ewes and goats is protective of transplacental fetal infection with CVV, but not against other related bunyaviruses.[69,70]

Some diagnostic laboratories offer a gel-based PCR for the demonstration of CVV-RNA in tissues of aborted fetuses. Cross contamination in gel-based PCR platforms is common and its use is discouraged. An rtPCR assay has been developed for the detection of California (CAL) serogroup viruses and CVV, for use in human surveillance, but is not routinely used for CVV in small ruminants.[74] Until, rtPCR methodology is validated in small ruminants, the detection of CVV-RNA in late term malformed ruminant fetuses, the results of gel-based PCR are difficult to interpret.

Breeding ewes outside of the mosquito season may help reduce CVV fetal infections. However, short-term changes in weather patterns during a particular season may result in renewed vector activity and increased risk of fetal infection.[69]

Caprine herpesvirus-1

Caprine herpesvirus 1 (CpHV-1) belongs to the subfamily of alphaherpesviruses that contains seven genetically-related viruses. CpHV-1 is closely related to bovine herpesvirus 1 (BoHV-1), responsible for infectious bovine rhinotracheitis (IBR).[75]

CpHV-1 infection of goats can cause vulvovaginitis and neonatal systemic infection. CpHV-1infection of pregnant goats may result in abortion without other clinical signs of infection.[76]

Microscopic lesions in aborted fetuses consists of multifocal areas of necrosis with minimal cellular infiltration in liver, necrotic bronchiolo-alveolitis and necrosis in other organs including thymus, spleen, kidneys, intestine, and adrenal gland. Characteristic herpesvirus eosinophilic intranuclear inclusions are difficult to find, but can be observed more frequently in cells surrounding the areas of necrosis especially in adrenal glands. Necrotizing vasculitis may be present in fetal membranes' villi.[18,77,78] Diagnosis can be established by observation of characteristic microscopic lesions in conjunction with virus isolation and amplification of CpHV-1DNA by rtPCR.[77]

Neosporosis

Neosporosis is caused by *Neospora caninum*, an Apiclomplexa parasite closely related to *T. gondii*. *Neospora* was initially misidentified as *Toxoplasma*, but was subsequently differentiated based on host preferences, etiology, morphological, and genetic differences.[79] Dogs and other canids (coyotes, wolves, other) are definitive host.[80] Cattle, sheep, goats, white-tailed deer, other ruminants, and horses are intermediate hosts.[81]

Neosporosis is a major cause of abortion in cattle, sheep and goats. Infection in small ruminants occurs either by ingesting sporulated oocysts shed by dogs and other canids in feces that contaminate food and water, or vertically from infected ewes and goats to the fetus after recrudescence of a chronic infection during pregnancy. Transplacental infection may result in abortion, fetal mummification, birth of a congenitally-infected offspring with clinical neuromuscular signs, or a live and healthy progeny. Fetal membranes may have areas of necrosis and mineralization, but the intercotyledonary areas are unremarkable. Multifocal areas of necrosis may be apparent in the brain and liver of few of the *N. caninum* aborted fetuses.[18,82] The clinical outcome of *N. caninum* in pregnant sheep and goats is dependent to some extent on the stage of pregnancy when animals are infected.[83]

Diagnosis in aborted fetuses is established by demonstration of characteristic microscopic lesions in the tissues of aborted fetuses, together with demonstration of the organism by immunohistochemistry or by amplification of *N. caninum* DNA by rtPCR in brain, spinal cord, or other tissue of aborted fetuses.84 Detection of antibodies to *N. caninum* is by ELISA or immunofluorescent antibody tests.[85]

Toxoplasmosis

Toxoplasmosis is caused by *Toxoplasma gondii*, a coccidia – intracellular protozoan parasite of the phylum Apicomplexa. The parasite is present virtually in all warm-blooded animals including sheep, goats, and in nearly one-third of humans. Goats are very susceptible to infection. Cats (Felidae) are the only known definitive hosts and thus, the main reservoirs of infection and main source of infection for small ruminants. Unsporulated oocysts are shed in the cat's feces. Small ruminants are intermediate hosts and contract the infection by eating contaminated grass and/or animal feed with feces of infected felines. Small ruminants do not shed oocysts in feces. Ewes and does that abort cannot infect 'clean ewes' or does in the flock or herd, unless naïve pregnant ewes and does ingest fetal membranes and fetal fluids of infected animals. Cats become infected after consuming intermediate hosts harboring tissue cysts. Cats may also become infected directly by ingestion of sporulated oocysts.[86] In humans, *T. gondii* is most frequently transmitted horizontally following ingestion of environmentally resistant oocysts excreted by cats, or via ingestion of undercooked meat containing persistent asexual stages (bradyzoites) residing in intermediate hosts' tissues.[87]

T. gondii infection in pregnant sheep and goats may cause early embryonic death, fetal mummification, abortions, stillbirth, and the birth of weak kids or lambs.[88] *T. gondii*-induced abortion can occur in ewes and goats of all ages, although maiden ewes and goats are most affected. Most ewes and does are asymptomatic before and after abortion. When a flock or herd of naïve pregnant ewes or does are first infected with *T. gondii*, the infection results in an epidemic outbreak with high abortion rate. The pregnancy loss is less drastic in subsequent lambing/kidding seasons. Sheep and goats that abort as a result of *T. gondii* infection develop immunity and are more resistant to abort a second time, but subsequent pregnancies may result in the birth of weak offspring and shedding of the organism in fetal membranes, fetal fluids, and milk.[82,88]

Histologic examination of fetal tissues and fetal membranes, detection of *T. gondii* in tissues by immunohistochemistry, amplification of T. gondii DNA by rtPCR, and serological examination of the fetus and the dam for the presence of antibodies are all useful in diagnosis of toxoplasmosis. *T. gondii* is ubiquitous in the environment. Presence of antibodies in maternal serum is an indication of exposure and not a confirmation of the cause of abortion.[88,89]

Prevention of toxoplasmosis involves keeping cats away from feed, feed troughs, and pens with pregnant ewes or goats. Maintaining clean bedding and clean pen areas is important. Grain and feed should be stored in covered containers. Barn cats may play an important role in rodent control, but the population, particularly of kittens must be kept under control. Kittens younger than 6 months are more likely to be carriers of *T. gondii* than adult cats. Delivery/nursing of kittens by queens must not be permitted in pens with pregnant ewes/goats and feed barns. All adult cats should be neutered or spayed. Cats should not be allowed to consume fetal membranes or fetuses and should not be fed raw meat.[90]

There is no approved treatment for toxoplasmosis in small ruminants; however, feeding the coccidiostat decoquinate during pregnancy may reduce lamb losses.[91] A live vaccine for the protection of sheep against abortion due to *T. gondii* is used in some countries, but the vaccine is not licensed in the US.[92] The vaccine available commercially contains live attenuated

toxoplasma (strain S48) for the prevention of abortion in sheep. The vaccine provided life-long protection; however, its downsides are potential risk to people that handle the vaccine, expensive, short shelf-life and could revert to a pathogenic strain.[92]

Noninfectious causes of pregnancy loss

Veratrum californicum

Veratrum californicum is a plant that grows primarily in the high mountain ranges of the western US. *Veratrum californicum* fed to ewes ~ at days 12 - 14 of pregnancy can cause congenital cyclopia and other defects of the cranium and brain in lambs, in addition to prolonged pregnancy. Ingestion of the plant between days 19 - 21 of pregnancy causes embryonic death. Maternal exposure between days 24 - 30 results in fetal cleft palate. Metacarpal, metatarsal, and tracheal cartilage defects result from maternal exposure during days 27 - 36 of pregnancy.[93]

Astragalus pubentissimus

Sheep and cattle grazed on *Astragalus pubentissimus* (locoweed) plant early in pregnancy may abort or give birth to congenitally deformed offspring including arthrogryposis, aplasia of the lower jaw, hypermobility of the hock and stifle joints, and contracted tendons, especially the ankles.[94]

Dexamethasone

Ewes experimentally treated with 0.25 mg/kg body weight on days (1, 3, and 5) during first trimester, days (51, 53, and 55) during second trimester, and days (101, 103, and 105) during the third trimester had decreased plasma progesterone concentrations and aborted. Abortion appeared to be a consequence of the adverse effects of decreased placental progesterone concentrations.[95]

Prostaglandin $F_{2\alpha}$

Goats depend on progesterone from the CL throughout pregnancy. Exogenous treatment or endogenous release of $PGF_{2\alpha}$ at any time during pregnancy will result in pregnancy loss in goats. Two doses of 5 mg $PGF_{2\alpha}$ given intramuscularly 24 hours apart at ~ 3 months of pregnancy in does results in abortion and 70% of does retained fetal membranes.[96] Pregnancies in ewes carrying a single embryo can be terminated by 1 dose of 100 µg of cloprostenol given ~ day 21 of pregnancy, but 2 injections given about 7 days apart are necessary when multiple embryos are present. However, the ewe placenta takes over progesterone production at ~ day 55 of pregnancy and ewes will not abort when exogenous $PGF_{2\alpha}$ is given or endogenous release after 60 - 70 days of pregnancy.[97]

Benzimidazoles

Benzimidazole compounds have teratogenic effects in domestic and experimental animals. Giving ewes netobimin, a benzimidazole compound, on day 17 of pregnancy resulted in fetal skeletal and congenital renal malformations.[98] Albendazole, a broad-spectrum anthelmintic, should not be given to female cattle during first 45 days of pregnancy or to ewes or does during the first 30 days of pregnancy.

Vitamin E and selenium deficiency

Congenital nutritional muscular dystrophy caused by vitamin E and selenium deficiency is uncommon but has been reported in sheep and cattle.[99] Affected lambs may have bilateral symmetrical chalky-white streaks in skeletal muscles and in the heart. Histologically, necrosis, degeneration, and calcification of myofibers are observed.

Congenital goiter

Calves, lambs, and kids with goiter are born partially or completely hairless and are either born dead or die soon after birth.[100-102] Congenital hypothyroidism can be the result of iodine deficiency or excess, due to goitrogenic compounds that interfere with thyroid hormone synthesis, or due to hereditary defects in thyroid hormone synthesis. Common goitrogenic compounds include thiouracil, sulfonamide, and plants of the Brassica group. Affected and aborted full-term fetuses/newborns exhibit severe, bilateral thyroid enlargements, alopecia, myxedema, swelling of the tongue and laryngeal edema, the latter causing asphyxia.[18]

Body condition score

Reducing body condition (BCS) score in the last 45 - 60 days prior to lambing or kidding increase the risk of pregnancy loss and/or pregnancy toxemia. The most efficient and accurate method to assess energy adequacy in ewes and goats is to perform and record the BCS by using an objective 1- 5 scoring system, with 1 being extremely thin and 5 being extremely obese.[103] The BCS is determined by palpating the amount of fat and muscle covering on the spinous processes and transverse processes in the lumbar region. Most healthy productive ewes and goats will have a score of 2.5 - 3.5. Ewes or does with a score of 1 - 2 should be examined and fed to attain a higher score, whereas those with a score > 3.5 should be fed less. Dietary changes should be carried out slowly and abrupt reduction in total energy intake should always be avoided. It is convenient to assess the BCS at prebreeding, at breeding, and 3 months postbreeding. At prebreeding, animals < 2.5 are fed more, whereas those > 3.5 are fed less to arrive a score of 2.5 - 3 at breeding. Changes in feed management are avoided during the first month of pregnancy. At 3 months of pregnancy, evaluation is required to arrive at an ideal score of 3.5 prior lambing/kidding. The primary predisposing cause of pregnancy toxemia is inadequate nutrition during late pregnancy. Animals may not be fed according to the number of fetuses or because of insufficient energy density of the ration and decreased rumen-reticular capacity due to

fetal growth. Fetal growth is 70% in the last 6 weeks prior to delivery; therefore, the requirements need to be satisfied accordingly. Moreover, the increase in the number of fetuses will affect the energy needs of the dam. Females with a poor BCS (≤ 2) or that are over conditioned (≥ 4) and carrying more than 1 fetus are at highest risk of developing pregnancy loss and toxemia, although the condition can occur even in ideally conditioned ewes on an adequate ration.

Gastrointestinal parasites

Many species of nematodes and cestodes cause parasitic gastritis and enteritis in sheep and goats. The most important of these are *Haemonchus contortus*, *Teladorsagia* (*Ostertagia*) *circumcincta*, *Trichostrongylus axei*, intestinal species of *Trichostrongylus*, *Nematodirus* spp, *Bunostomum trigonocephalum*, and *Oesophagostomum columbianum*. *Cooperia curticei*, *Strongyloides papillosus*, *Trichuris ovis*, and *Chabertia ovina* also may be pathogenic in sheep. Even subclinical infections depresses appetite, impairs gastric digestion, and reduces use of metabolizable energy and protein with the consequent loss in BCS. Clinical signs associated with gastrointestinal parasitism are shared by many diseases and conditions; however, a presumptive diagnosis based on signs, grazing history, and season is often justified. Infection usually can be confirmed by demonstrating nematode eggs or tapeworm segments in fecal examination.

In small ruminants during late pregnancy and early lactation, the presence of gastrointestinal parasites appears to be more deleterious due to the higher energy requirements with reduced capacity of the digestive system. Presence of parasites with inadequate feeding program in pregnant females create ideal conditions to begin an outbreak of pregnancy loss.

'Diagnostic attitude' for a pregnancy loss case

An adequate history is paramount for the diagnostic investigation of pregnancy loss, specifically the following information: the total population at risk, number of females that have pregnancy loss, category of females, age, stage of pregnancy, fresh or decomposed fetus, movements of animals (male and female) within the property or outside the property and time, purchase of new animals, change in source of water, food (quality and quantity), vaccination (type and time), deworming (product and time), and medications.

To optimize reaching a diagnosis, correct sampling from more than 1 abortion is required. Fresh material is crucial and the following material is required: fetus, fetal membranes, and maternal blood. Contaminants, such as feces, urine, or dirt material should be removed gently from the specimens under biosecurity protocols without washing the sample with water or with antiseptics. To avoid mistakes, contact diagnostic laboratory about submitting samples, samples required and preservation, and shipping method.

Managing the pregnancy loss in the flock/herd

Females with pregnancy loss are kept away from healthy pregnant females until a diagnosis is determined. Reduce or avoid any movement of animals. Measure of biosecurity and biocontainment need to be in place immediately.

Pregnancy loss is considered infectious until it is proven to be noninfectious. Therefore, a good clinical history, assessment, and submission of adequate samples to the laboratory are paramount. Every person involved with animals needs to wear gloves, boots, protecting clothing, and masks. It is important to have the order of work from healthy to sick, from young to old, and from clean to dirty for cleaning or feed management purposes. No child, pregnant women, elderly, or immunocompromised person should assist. Problem females should not go for auction or to another flock/herd as breeding animal until the cause is determined.

Conclusion

It is obvious that pregnancy loss in small ruminants is an important cause of economic losses for producers. Additionally, some infectious causes of pregnancy loss in small ruminants are transmitted to humans and pose an important human health risk. Rapid and accurate identification of the cause of pregnancy loss is of prime importance. The diagnosis of small ruminant pregnancy loss involves an investigative effort among the owner, the veterinary practitioner, and the veterinary diagnostic laboratory. For an accurate diagnosis, a battery of diagnostic tests is conducted in parallel including histopathology, rtPCR, bacterial culture, virus isolation, fetal antibodies in serum or fluid from thorax or abdomen, and maternal serology.

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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