History

Between December 2010 and January 2011, abortions occurred in a cohort of 55 Angus-cross replacement heifers in a university herd. A separate cohort of adult cows was present on-site, but no abortions had occurred in that group. The heifers had been artificially inseminated after estrus cycles were synchronized; no natural service was used. Calving was due to begin mid-February 2011. The cohort was vaccinated at 7 to 8 months of gestation with a USDA-licensed product containing modified-live bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV) types 1 and 2, bovine respiratory syncytial virus, and parainfluenza-3 virus. All 55 heifers were confirmed pregnant on the basis of results of ultrasonographic examination at the time of vaccination. The group was vaccinated with multivalent vaccines before breeding on 3 earlier occasions (May 2009, October 2009, and May 2010). A similar vaccination schedule was used uneventfully for other replacement heifers present during the previous 3 years (2007 to 2009). In the cohort of this report, abortions began 32 days after pregnancy examination and vaccination. The herd's health status was otherwise good, and herd management was excellent.

Figure 1—Photographs of the abdominal cavity of a heifer that died as a result of complications of abortion (A) and the liver of a calf fetus aborted by another heifer (B). The heifer was the first to abort in a cohort of 55 Angus-cross heifers; between December 2010 and January 2011, abortions were known to occur in 7 other heifers and were suspected in another 6 heifers. The cohort was vaccinated at 7 to 8 months of gestation with a product containing modified-live bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV) types 1 and 2, bovine respiratory syncytial virus, and parainfluenza-3 virus, and abortions were detected > 32 days after vaccination. In the abdominal cavity of the heifer, there is evidence of fibrinous peritonitis with fibrin overlying the small intestines; the uterus (Ut) is ruptured (a macerated fetus [not shown] was discharged into the abdominal cavity). In the aborted fetus, the liver is faintly mottled because of the presence of multiple pale foci (one of which is indicated [arrow]).

Figure 1—Photographs of the abdominal cavity of a heifer that died as a result of complications of abortion (A) and the liver of a calf fetus aborted by another heifer (B). The heifer was the first to abort in a cohort of 55 Angus-cross heifers; between December 2010 and January 2011, abortions were known to occur in 7 other heifers and were suspected in another 6 heifers. The cohort was vaccinated at 7 to 8 months of gestation with a product containing modified-live bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVDV) types 1 and 2, bovine respiratory syncytial virus, and parainfluenza-3 virus, and abortions were detected > 32 days after vaccination. In the abdominal cavity of the heifer, there is evidence of fibrinous peritonitis with fibrin overlying the small intestines; the uterus (Ut) is ruptured (a macerated fetus [not shown] was discharged into the abdominal cavity). In the aborted fetus, the liver is faintly mottled because of the presence of multiple pale foci (one of which is indicated [arrow]).

Clinical and Gross Findings

The first heifer to develop signs of abortion died 2 days later. At necropsy, it had uterine rupture caused by clostridial metritis; bacterial culture of samples of the postgravid uterus and peritoneal cavity yielded 4+ growth of type A Clostridium perfringens. A fetus protruded through the wall of the uterus into the abdominal cavity and was associated with severe fibrinous peritonitis (Figure 1). The macerated fetus was not examined further. Tissues from the dead heifer were collected for histologic evaluation. Thereafter, 7 other heifers aborted their fetuses; abortions occurred with few or no premonitory signs at 45, 46, 47, 48, 51 (2 heifers), and 55 days after vaccination. There were no other signs of illness in any of the affected heifers, including no retention of fetal membranes. Six fetuses (7.5 to 15.2 kg [16.5 to 33.4 lb]) were submitted for laboratory examination. A consistent feature was moderate autolysis. No lesions were detected in the placentas. There was bloody effusion in thoracic and abdominal cavities and pericardial sacs. The livers of some fetuses were mottled as a result of disseminated tan foci. Of the remaining 47 heifers, 41 calved at term and 6 were found not to be pregnant. Abortion in the 6 nonpregnant heifers probably occurred ≤ 32 days after pregnancy examination and vaccination. Total reproductive loss was 25%. The resultant calf crop was healthy with no unusual illness in the first 3 months after birth.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →
Histopathologic and Laboratory Findings

Specimens of major organs and tissues, including brain and placenta, were collected from the 6 submitted fetuses, fixed in neutral-buffered 10% formalin, routinely processed, embedded in paraffin, sectioned, stained with H&E stain, and examined by use of light microscopy. Sections of formalin-fixed liver, lung, spleen, thymus, and brain tissue that contained lesions were incubated with a polyclonal antibody against BoHV-1 or a monoclonal antibody against BVDV and processed for antigen detection by use of commercial immunohistochemical kits.1-4 Unlike fluorescent antibody tests, which are performed on frozen sections, immunohistochemical analysis of formalin-fixed tissue allows colocalization of lesions with detection of viral antigen.

Histologically, all 6 aborted calves that were submitted for laboratory examination had moderate to severe multifocal coagulation necrosis of the liver. Autolysis notwithstanding, necrotic foci were readily detected as 100- to 1,000-µm-diameter areas in which cellular outlines were lost and karyorrhexis had occurred with various degrees of intracytoplasmic mineralization (Figure 2). Associated inflammation was minimal and, where present, was predominantly peribital. No viral inclusions were detected. Similar, less numerous necrotic foci were detected in sections of the fetuses' kidneys, lungs, spleen, lymph nodes, adrenal glands, thymus, placenta, and brain. Immunohistochemical analysis revealed the presence of BoHV-1 antigen in multiple tissues; it was most abundant in liver, kidney, and lung tissues. Furthermore, BoHV-1 was isolated from pooled tissues (liver, lungs, spleen, kidneys, and placenta) obtained from a fetus that had been expelled 47 days after vaccination of one of the heifers. Viral antigen was widespread in trophoblasts and endometrial epithelium in the heifer that died of complications of abortion. No BVDV viral antigen was detected in fetal or placental tissues. Histopathologic lesions in the gravid heifer comprised severe acute diffuse necrotizing metritis with emphysma and large intraleseional bacterial rods consistent with Clostridium perfringens isolated by anaerobic culture.

Figure 2—Photomicrographs of sections of liver from the calf fetus in Figure 1 (A) and another aborted calf fetus (B). A—Multifocal coagulative necrosis (arrow) is evident; 2 necrotic foci have blue-stained areas of mineralization. The pink, washed-out appearance of the liver is due to autolysis. H&E stain; bar = 200 µm. B—Necrotic foci in the liver tissue are stained brown, indicating the presence of BoHV-1. Immunohistochemical stain specific for BoHV-1 antigen; bar = 100 µm.

Morphologic Diagnosis

Severe acute multifocal necrosis in liver, kidneys, lungs, spleen, lymph nodes, adrenal glands, thymus, placenta, and brain, with detectable intraleseional BoHV-1 antigen.

Comments

Abortion in the heifers of this report was a result of BoHV-1 infection. Apart from the initial case, there was no evidence of the involvement of other infectious agents, including BVDV and pathogenic bacteria, protozoa, or fungi. The diagnosis was supported by detection of BoHV-1 antigen with fluorescent antibody in various tissue specimens and isolation of BoHV-1 from pooled tissues obtained from one of the expelled fetuses.

Abortion due to BoHV-1 infection typically results in expulsion of an autolytic fetus with serosanguineous effusion into its major body cavities.12 Although serosanguineous effusion is not specific for BoHV-1 infection and is often present in aborted bovine fetuses in which no pathogens are found, such effusion should prompt a suspicion that BoHV-1 may be involved. Suspicion should be particularly strong when abortions occur 1 to 3 months after pregnant heifers are vaccinated with products containing modified-live BoHV-1, regardless of whether those products were administered in accordance with manufacturer's instructions or were given in an extralabel manner. Hepatic necrosis was evident grossly in some fetuses but, as is often the case, was subtle. This is characteristic of BoHV-1-induced abortions in cattle; infection with abortifacient alphaherpesvirus, such as equine herpesvirus-1, in other species often results in detectable gross lesions. Few other causes of abortion in beef cattle on range result in disseminated hepatic and renal necrosis. Rule-out diagnoses include systemic bacterial infection, such as salmonellosis and listeriosis, neither of which was detected in the heifers of this report.

Results of immunohistochemical analysis are helpful to corroborate fluorescent antibody detection of BoHV-1 antigen in aborted fetal tissues.1 It is generally possible to disclose colocalization of viral antigen with necrotic foci, even when fetuses are rotten. Isolation and characterization of the virus from tissue samples provide a definitive diagnosis for
BoHV-1.4 Fluorescent antibody detection of viral antigen in frozen sections is an inexpensive and rapid means of diagnosis, but results can be hampered by nonspecific cellular fluorescence due to autolysis. Other methods of laboratory diagnosis, such as PCR assays, are generally not done in abortion workups because of the associated costs, unless the abortion episode is large or unusual in some respect.

In the cohort of heifers of this report, there was a temporal association between abortion and recent vaccination with a polyvalent vaccine containing modified-live BoHV-1. Similar linked events have been identified recently by veterinarians and diagnosticians in Colorado, Texas, South Dakota, and Wyoming. Such postvaccination abortions affected erinarians and diagnosticians in Colorado, Texas, South Dakota, and Wyoming. Such postvaccination abortions affected such syndromes may increase as modified-live BoHV-1 vaccines continue to be used in pregnant cattle, such as oophoritis and perinatal loss, also associated with field or vaccine strains of BoHV-1 infection. In 1 reported episode affecting a small herd, no cattle pregnant for 5.5 months or less aborted following BoHV-1 vaccination, whereas 9 of 16 cows in their sixth, seventh, or eighth month of gestation did abort following BoHV-1 vaccination.5 This may be pertinent to the cases described in the present report because the 2010 heifer cohort was vaccinated at 7 to 8 months of gestation, instead of at 6 to 7 months as in previous years. The high incidence of BoHV-1 abortions in the United States decreased in the 1980s and 1990s once veterinarians realized the risks of using modified-live BoHV-1 vaccines in pregnant cattle.6 In the authors’ laboratory, diagnosis of >1 episode of BoHV-1 abortion annually was uncommon during the 1990s and in the early part of this decade. Recently, there was an apparent increase in the number of postvaccination abortions following use of products licensed for use in appropriately vaccinated pregnant cattle. Other syndromes associated with field or vaccine strains of BoHV-1 infection in pregnant cattle, such as oophoritis and perinatal loss, also developed and are poorly characterized.6-11 The importance of such syndromes may increase as modified-live BoHV-1 vaccines continue to be used in pregnant cattle.

An etiologic diagnosis is established in <40% of bovine abortions in the United States.12 Diagnostic success rates increase with the submission of multiple fetuses from outbreaks, particularly when serum from the dams and placental tissues are also submitted. There is regional variability in the importance of abortion-causing agents in North American cattle. Neosporosis, considered one of the most important causes of abortions in dairy herds, has never been confirmed in cattle in Wyoming, of which most are located in extensive beef cow-calf operations. By contrast, infection with BVDV or opportunistic bacteria and ingestion of pine needles are commonly diagnosed causes of abortion in cattle in Wyoming. Most published data on bovine abortions are biased toward the last third or half of pregnancy because the chance of collecting a fetus or fetal membranes for submission to a laboratory improves as gestation progresses. Chromosomal abnormalities and unidentified infectious agents are probably responsible for some abortions that occur during the first half of pregnancy in cattle.

Veterinarians are often discouraged by the low diagnostic success rate for bovine abortions, even when losses are large and multiple carcasses are submitted. There is a tendency to assume that submission of an autolytic fetus is of little value and that waiting for a fresh fetus or placenta to be submitted to the laboratory favors obtaining a definitive diagnosis. This becomes a problem when an infection is of a type that almost invariably results in an autolytic fetus or when the organism targets tissues, such as brain and spinal cord, that are rarely submitted for analysis as part of abortion workups. During food animal abortion episodes, submission of an entire fetus with placenta is most useful and ensures that a full set of tissue samples is available for virologic, bacteriologic, toxicological, and pathological examinations.

A letter published in JAVMA in 2010 included comments regarding an apparent increase in the number of bovine abortions following administration of BoHV-1 vaccines in the United States, which was in part due to confusion about the appropriate use of modified-live virus products. Although most postvaccination abortions appear to reflect inadvertent extralabel use of BoHV-1 vaccines (ie, giving vaccine to pregnant cattle that were not vaccinated in the previous 12 months with an appropriate modified-live BoHV-1 vaccine) by owners and veterinarians, some involve appropriately vaccinated heifers. Until there is a method to distinguish vaccine strains from field strains of BoHV-1, diagnosticians will be unable to confirm whether an episode like that described in the present report reflects a wider problem due to vaccine-induced reproductive failure.

References