Diagnosis of Infectious Causes of Bovine Abortion

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Abstract

Identifying the cause for a client's herd abortion problem is often a challenge for both practitioners and veterinary diagnostic laboratories. Our success can be improved with an investigation that involves evaluation of the herd situation coupled with thorough, systematic sampling and accurate interpretation of laboratory results. The material to be presented is intended to provide information on sampling for abortion diagnosis and some of the test procedures available at most diagnostic laboratories. Bovine abortion surveys reveal that most of the identified causes are attributed to a moderate number of infectious agents. The specific types of infectious abortion identified in different regions may vary, presumably due to climate, production types, management practices and geographic factors. Also, the type of sampling and diagnostic procedures performed may influence the types of abortions identified. Some causes of abortion have declined in prevalence with improved control measures and, with new knowledge and diagnostic procedures, causes such as *Neospora* infection have emerged as significant causes of abortion. The diagnostic features of some of the most significant infectious causes of bovine abortion identified by veterinary diagnostic laboratories in the United States will be discussed.

Introduction

Abortions cause major economic loss to the cattle industry, and this material is intended to provide information for the practitioner about abortion diagnosis. Surveys of abortion based on veterinary diagnostic laboratory data reveal a variety of causes, most associated with a limited number of infectious agents. The proportion of bovine abortions attributed to a specific abortifacient may vary by region, probably due to differences in climate, production type, management practices, vaccination programs, wildlife populations and rations (Table 1). Sampling and laboratory test procedures may also influence what causes are identified. Significant causes of abortion such as neosporosis have emerged with improvement in diagnostic procedures.

Abortion diagnosis is a challenge for the veterinarian and diagnostician. An etiologic diagnosis is obtained in less than half of aborted bovine fetuses submitted to veterinary diagnostic laboratories, so continued sampling during an ongoing abortion problem is advisable. There are few diagnostic clinical signs or gross changes, so identification of the etiologic agent usually requires a complete diagnostic workup involving a variety of pathology, microbiology and immunology procedures available from a veterinary diagnostic laboratory. Clinical information such as estimated abortion rate, gestational age of abortions, whether the fetuses are fresh or autolyzed, whether both heifers and cows are aborting, and whether natural or artificial breeding is used, may aid in diagnosis.

Maternal serology on a single serum sample from an aborting cow may help determine exposure or lack of exposure to various pathogens, but usually cannot differentiate between vaccination and natural exposure, or between recent versus previous exposure. Leptospirosis and neosporosis are diseases where single serum samples may be useful in a diagnosis. In most other instances, paired acute and convalescent serum samples are needed to identify a significantly rising titer to a particular pathogen. Maternal seroconversion to an abortifacient may precede abortion, so paired serum samples collected at and following abortion may not demonstrate an increasing titer. Maternal serology is most useful when serum from non-vaccinated animals is examined, when several animals are available for testing, and when a detailed history on each animal is provided. Serology on fetal fluids can be useful in detecting fetal antibody levels. Elevated immunoglobulin levels are an indication of an active immune response by the fetus to a foreign antigen, usually an infectious agent. When specific fetal titers are elevated, they indicate fetal exposure to that agent.

Often there are no specific signs or gross lesions that will guide diagnostic test selection, so a broad-based, ("shotgun") standardized diagnostic protocol is utilized. The following is the protocol used by the California Animal Health and Food Safety Laboratory. Other diagnostic laboratories may employ different protocols and obtain similar diagnostic outcomes. The entire fetus with placenta and paired serum samples are the optimal specimens. The placenta is important since in some diseases, such as mycotic abortion and several bacterial diseases, placentitis is the primary lesion. A complete necropsy examination is performed on the fetus and placenta for any gross lesions with estimation of

Table 1. Surveys of bovine abortion from diagnostic laboratory submissions.

Reference #	5	8	4	1, 2	6	7	3
Location	NE USA	Midwest USA	NE USA	California USA	Australia USA	Midwest USA	California USA
Production type	Dairy	Beef	Dairy	Mixed	Dairy	Mixed	Mixed
Total abortion cases	3812	2544	1421	468	265	8962	2296
Year	1960-1970	1968-1972	1950-1964	1985-1989	1981	1980-1989	1998-2003
% etiologic diagnosis	23.3%	35.3%	30.2%	45.5%	37%	32.8%	44.4%
Ranking							
#1	Mycotic	IBR	*Campylo*	*Neospora*	Mycotic	IBR	Neospora
#2	IBR	Mycotic	*Streptoco*	*Arcanobact*	*Salmonella*	Mycotic	EBA
#3	*Streptoco*	*Campylo*	*Staphyloco*	IBR	*Campylo*	BVD	*Leptospira*
#4	*Leptospira*	*Arcanobact*	*Arcanobact*	*Streptoco.*	*Arcanobact*	*Arcanobact.*	*Arcanobact*
#5	*Campylo*	EBA	Brucellosis	*Leptospira*	*Leptospira*	*Bacillus*	BVD
#6	*Arcanobact*	*Leptospira*	*Leptospira*	EBA	Protozoa	*Listeria*	*Campylo*

fetal age and degree of autolysis. The routine histopathology samples examined are brain, lung, heart, liver, kidney, adrenal, spleen, thymus, lymph node, skeletal muscle, abomasum, small intestine, colon and placenta. Routine bacteriology includes cultures of the lung, liver and abomasum. Abomasal contents are examined by dark field microscopy for *Campylobacter* and tritrichomonas species. A *Leptospira* fluorescent antibody (FA) is performed on kidney impression smears. Fungal cultures are performed if placental, skin or other lesions are suggestive or fungal hyphae or yeast cells are seen on dark field examination. Virus isolation is not routinely done but lung, liver, spleen, kidney, adrenal and placenta are the usual tissues collected. Routine immunology tests include AGID test for bovine IgG1 on fetal fluids (usually use thoracic fluid, <20mg/dl considered normal). If fetal IgG levels are elevated the infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), *Brucella abortus, Leptospira, Neospora* serology is performed. Dam abortion serology includes IBR, BVD, *Leptospira, Neospora* and *Brucella abortus* serology. Fluorescent antibody examinations can be performed on frozen sections of lung, liver, kidney, adrenal, spleen and/or placenta for IBR, BVD, and *Leptospira* as needed.

In situations where an intact fetus cannot be submitted to the diagnostic laboratory, most of the abortion diagnostic protocol can be completed if the veterinarian submits a complete set of fresh and formalin-fixed samples. Fresh (cooled, not frozen) samples from the fetus should include; lung, liver, kidney, sterilely collected abomasal content, fetal blood (usually pleural fluid sample), dam serum sample and placenta. As a minimum, the formalin fixed samples should include brain, lung, heart, liver, kidney, spleen, thymus, skeletal muscle, abomasum, small intestine and placenta. Other formalin fixed samples such as eyelid, trachea, lymph node, thyroid and adrenal may also be useful in some cases.

The laboratory identification of a potential abortifacient based on tests performed on submitted samples requires the interpretation of the veterinary practitio-

ner to establish whether this constitutes a valid diagnosis for the herd's abortion problem. Errors in assessing blame for an abortion problem may occur when sampling is incomplete (only serology performed) or the sample is inadequate (single fetus submitted from an abortion storm). The following information concerns some of the significant causes of bovine abortion identified by veterinary diagnostic laboratories in the United States.

Sporadic Bacterial Abortion

Various bacteria may cause isolated sporadic abortions in cattle. Of these, *Arcanobacterium (Actinomyces) pyogenes* is most common. Abortion can occur at any stage of gestation, but most are in the latter half of gestation. The placenta may or may not be retained. These bacteria are not contagious pathogens, but are in the environment or on mucous membranes. A maternal bacteremia is the presumed means by which they reach the gravid uterus and subsequently infect the fetus. Since the bacteria are common in the environment, their presence may be indicative of an incidental postmortem contamination of the fetus or placenta. Therefore, to establish a diagnosis, the bacteria should be isolated in nearly pure culture from abomasal contents or tissues, lesions consistent with a bacterial infection must be present, and other causes for abortion should be ruled out. Placentitis, often accompanied by a bronchopneumonia in the fetus, are the most frequent pathology findings. Due to their sporadic nature, no specific control measures are recommended.

Brucellosis

The incidence of *Brucella abortus* infection and abortion has been reduced in many countries due to the government eradication programs. Abortions usually occur after the fifth month of pregnancy and metritis and retained placenta is common. Transmission of the infection is primarily through ingestion. The disease is chronic in nature. Bacteria multiply in regional lymph nodes nearest the site of entry and then spread via the blood stream to other organs, most importantly the mammary gland, mammary lymph nodes and gravid uterus. Uterine infection occurs during the second trimester. Bacteria invade the placental trophoblasts and cause chronic placentitis and fetal infection, resulting in fetal death due to placental disruption and endotoxemia. Fetuses abort 24 to 72 hours after *in utero* death. Bacterial isolation is necessary to confirm the diagnosis. *B. abortus* can be isolated from various sources including fetal abomasal fluid, lung, placenta, uterine fluid and milk. A placentitis with edema, focal necrosis of cotyledons and thickened intercotyledonary areas with adherent yellowish exudate may be present, and the fetus is frequently autolyzed with no gross lesions. Histologically there is a severe placentitis, with numerous bacteria visible in chorionic epithelial cells and bronchopneumonia in the fetus. Various serologic tests have been developed for governmental surveillance and detection of cattle exposed to *B. abortus*. Interpretation of these serologic tests and identification of "reactor" cattle is under the direct supervision of governmental agencies responsible for the disease eradication program. In endemically infected herds, a vaccination program will offer some protection against infection and spread of the disease. The RB51 vaccine reduces the occurrence of vaccine titers, which might be confused with titers resulting from field exposure. The aborted fetus, placenta and uterine discharges are heavily infected with *B. abortus,* so disposal of infected tissues is required to prevent both animal and human exposure.

Listeriosis

Listeria species are widespread in the environment, and abortion due to *Listeria monocytogenes* and *L. ivanovii* infections are encountered throughout the United States. Most abortions are sporadic but abortion storms can occur. The placenta is usually retained at abortion and some cows may have a fever with anorexia due to metritis. While listeriosis in adult cattle can occasionally cause encephalitis, this is rarely seen in association with abortion. Ingestion of poorly fermented silage may be associated with infection. Fetuses are usually in the third trimester and autolyzed. Gross lesions are often absent, but in some cases pinpoint white foci are found in the liver. Additional findings suggesting a bacterial infection include small pale foci in placental cotyledons, and fibrin in body cavities. Diagnosis is based on isolation of *Listeria* species, which may be present in multiple tissues, along with histopathology changes that include placentitis and hepatitis. Immunohistochemistry is also available to identify the bacteria in fixed tissues.

Leptospirosis

The most significant serovars of *Leptospira* associated with abortion in US cattle are *L. pomona,* maintained in swine and wildlife species, and the hardjo serovar that is maintained in cattle. The hardjo serovar is separated into *Leptospira interrogans* serovar hardjo (type: hardjo-prajitno) and *Leptospira borgpetersenii* serovar hardjo (type: hardjo bovis). Abortion is frequently the only clinical sign observed in a herd. *Leptospira* hardjo serovars are associated with infertility, abortion of fetuses four months to term, and weak calves

at birth. Abortion due to *L. pomona* usually occurs in the last trimester. The herd abortion rate seldom exceeds 10% with hardjo serovars, but can approach 50% with severe herd infections of *L. pomona.* Signs of acute leptospirosis including fever, hemolytic anemia, hemoglobinuria, icterus and high mortality can be seen in younger cattle. In lactating cattle, agalactia and mastitis can occur with flaccid udders and thick yellow to occasionally blood-tinged secretions. Leptospires can be shed in urine for several weeks and survive in wet environments for up to 30 days. Prolonged shedding is observed with the hardjo serovars. The organisms can penetrate intact mucous membranes or abraded skin. Establishing a diagnosis of *Leptospira* abortion is difficult. Identification of leptospires by darkfield microscopy of fetal fluids or silver stains of fetal tissues is rarely successful. Fluorescent antibody examination of fetal kidney using multivalent antisera is a rapid means for diagnosis, though specific serovars cannot be determined. Maternal serology using the microscopic agglutination microtiter test may assist in diagnosis of leptospirosis, though caution must be used to distinguish between vaccination, previous exposure and recent infection. Herd vaccination two or more times a year using a multivalent bacterin is recommended. Other measures, such as isolating infected animals and eliminating exposure to contaminated water or aborted fetal tissues are recommended.

Campylobacter fetus

Campylobacter fetus subspecies *venerealis* (formerly *vibrio*) is a widespread, venereally transmitted cause of infertility due to early embryonic death and occasional abortions. Most abortions are noted at four to six months gestation. Following initial infection in cows, bacteria are cleared from the uterus but may persist for longer periods in vaginal mucus. Reinfection can occur, though some immunity exists following infection in the cow. This immunity explains why there is a higher incidence of infertility among heifers within chronically infected herds. As with trichomoniasis, infected bulls are subclinical carriers, and when a herd infection is established, it is more prevalent in older bulls. The disease can also be transmitted by contaminated semen at artificial insemination and with contaminated semen collection equipment. Successful identification of the bacteria from the reproductive tract or aborted fetuses is necessary for diagnosis. Culturing of preputial smegma is an effective way to isolate the agent, but bacteria can also be identified or cultured from aborted fetuses and vaginal secretions, especially from recently bred heifers. *Campylobacter fetus* ss. *venerealis* is fastidious, requiring careful sample collection and proper transport. Aborted fetuses can be fresh, autolyzed, or mummified. Fibrin may present in the thorax, heart sac or abdomen. *Campylobacter* may be identified in the abomasal fluid using direct darkfield microscopy and cultured from several sources, including lung, placenta and abomasal fluid. Abortion may occur with *C. fetus* subspecies *fetus* and *C. jejuni* as a sporadic event, so identification of the specific *Campylobacter* isolated from an aborted fetus is needed to establish whether it is a venereal infection. Control measures include artificial insemination where practical or annual vaccination of cows prior to breeding. Vaccination of bulls may also be effective, though protection is more difficult to achieve in bulls.

Tritrichomonas foetus

Tritrichomonas foetus is a highly prevalent, venereally transmitted protozoal pathogen that is a major cause of infertility similar to *C. fetus* ss. *venerealis*. Infection causes early embryonic death, occasional abortions and pyometra. Most fetal loss is in the first five months of gestation. Infection in the cow usually is transient and bulls, especially older bulls, are inapparent carriers. Clinical history of infertility in bull-bred cows leaves suspicion for trichomoniasis or campylobacteriosis. The parasites are most efficiently obtained and identified by aspirating smegma from the preputial fornix of infected bulls using a dry insemination pipette. Diagnosis may also be made from aspirated cervical or vaginal mucous of infected cows. Uterine fluid from infected cows with pyometra can be an excellent sample. The protozoa are very fastidious, so special care should be taken to properly collect, maintain and transport collected fluid according to the needs of your diagnostic laboratory. To minimize false negative results, three weekly cultures of suspect bulls that have been sexually rested for at least 10 days are recommended. Aborted fetuses are often autolyzed, with no gross lesions. The placenta may be edematous. Protozoa are present in placental or abomasal fluid. In some instances, trichomonads can be seen microscopically. Control procedures focus on eliminating transmission of the disease through artificial insemination, segregation of animals into infected and non-infected groups and/or possible removal of infected animals. A killed, whole-cell vaccine is available but data on protection from pregnancy loss is not clear.

Mycotic abortion

The proportion of abortions caused by fungal infection varies depending on climate and housing. The majority of mycotic abortions in cattle are caused by *Aspergillus fumigatus*. Mycotic abortions usually occur as sporadic, third-trimester abortions. Clinical signs in

the dam are infrequent, aside from retained placentas. Lesions consist of placentitis involving both the cotyledons and intercotyledonary placenta, often resulting in a diffusely thickened, leather-like placenta. Diagnosis is based on the lesions and demonstration of fungus by culture (placenta, abomasal fluid, lung); direct identification of the fungal hyphae with KOH wet mount exam of scrapings from skin lesions; or histology. No specific control measures are available, but if an outbreak should occur, the amount of fungal contamination in the feeds and environment should be evaluated. Practices that predispose to rumenitis might contribute to the problem.

Infectious Bovine Rhinotracheitis (Bovine Herpesvirus Type I)

Clinical disease associated with infection includes abortion, vulvovaginitis, balanoposthitis, respiratory disease, conjunctivitis, encephalomyelitis, and fatal systemic infections in neonatal cattle. Exposure of previously unexposed pregnant cattle can result in abortion storms, with 25-60% of cows aborting. Experimentally, abortion occurs at any stage of gestation, but in field conditions abortions are usually seen in the second half of gestation. Infection occurs through contact with infected cattle shedding virus from respiratory, ocular, and reproductive secretions. Fetuses are autolyzed and there are usually no gross lesions. A presumptive diagnosis is made from histopathology and confirmed by viral isolation, by detection of viral antigen in fetal tissues by FA, or immunohistochemistry. Vaccination is the major method of control.

Bovine Virus Diarrhea

BVD virus can cause a variety of clinical disease problems, including abortion and congenital infection. Two biotypes, noncytopathic and cytopathic, of BVDV are known. Noncytopathic BVD infections in fetuses prior to four months gestation can result in the birth of persistently infected live calves, which are a source of infection for other cattle. Results of fetal BVD infection vary, depending on type of BVD virus and gestational age of the fetus infected. First-trimester infections can cause infertility, embryonic death, fetal resorption, mummification, or abortion. Infections between two and four months, gestation may also result in persistently infected live calves. Fetal infections beyond approximately four months gestation often result in transient fetal infections, with the development of a fetal immune response, specific fetal antibody production, and elimination of the virus. However, abortions can also occur during later gestational infections. Mid-gestational infections can result in congenital anomalies. Diagnosis of BVD abortion is complicated, in that the infection may be present and not be the cause of abortion. Fetal infection can be determined by detection of virus in fetal tissues or fetal antibody production indicating prior fetal infection. In order to diagnose BVD as the cause of abortion, the fetal infection needs to be combined with evidence of compatible fetal pathology and/or herd history. Herd screening procedures, utilizing serology and skin immunohistochemistry, can assist identification of persistently infected animals. Control is focused on detecting and eliminating contact with animals shedding the virus, particularly persistently infected animals or acutely infected cattle.

Neosporosis

Neosporosis is a coccidian parasite that has a worldwide distribution and is a major cause of abortion. An important feature of the disease is that the parasite can be maintained in cattle as a chronic, apparently lifelong, infection that can be passed on to the fetus during pregnancy. Fetal infection has a variable outcome and may result in abortion, although most infected cows give birth to a healthy, congenitally infected calf. Diagnosis of the infection is assisted through examination of aborted fetuses and serologic testing of cattle for evidence of infection. *Neospora* infected cattle that abort have no signs of clinical illness other than abortion. The majority of the abortions occur during the second trimester of pregnancy and the fetus is autolyzed. Pathology examination of aborted fetuses and serology can be used to confirm *Neospora* infection as the cause of abortion. Pathologic diagnosis is based on presence of the typical lesions, identification of the parasite and other serologic and microbiologic tests to eliminate other causes. Several serologic tests are available to assist in diagnosis of neosporosis. The assays utilize *Neospora caninum* tachyzoites or specific derived antigens. Specificity and sensitivity of the various serologic tests are comparable, depending on the minimum antibody titer that has been established as the cut-off for a positive result. Laboratories utilizing any of the serologic tests for *Neospora* should establish appropriate cut-off titers using sera from known infected and noninfected cattle. Enzyme-linked immunosorbent assays (ELISA) for detection of *Neospora* antibodies are widely used because the procedure is rapid, inexpensive and consistent. *Neospora* serology can be used as part of the routine abortion screen, to estimate herd infection rate, to assess the proportion of abortions that can be attributed to neosporosis and to evaluate routes of transmission.

There are several ways that cattle may acquire *Neospora* infection, either by horizontal infection or by vertical transmission of the infection transplacentally during pregnancy. Recently, McAllister *et al* and later

Lindsay *et al* were successful in demonstrating that the dog is a definitive host for *Neospora* infection, and there is epidemiological evidence that suggests dogs have a role in transmission of neosporosis. Vertical transmission is an important feature of bovine neosporosis. The parasite can be maintained in cattle as a chronic infection that can be passed on to the fetus during pregnancy. In some cows, this fetal infection may result in an abortion. However, most infected cows give birth to a healthy, but congenitally infected, calf. A heifer calf born congenitally infected is capable of transmitting the infection on to the next generation when she becomes pregnant, thus maintaining infection in the herd.

Control of this infection must take in to account that a major method of *Neospora* transmission in herds is through infection of fetuses in chronically infected cattle. Infected cows can be identified from serologic titers or from a history of previous *Neospora* abortion or congenital infection. With this knowledge, control could be focused on reducing the number of infected cows in the herd and limiting the introduction of infected replacement cattle into the herd. One recommendation for control in herds utilizing embryo transfer procedures is to insure that all recipient cows are seronegative. Various antimicrobial agents have been tested against *Neospora caninum in vitro* and have been used to treat clinical infections in dogs. Recently, Toltrazuril-sulfone (ponazuril) has been shown to have efficacy in experimental *Neospora* infections in calves, but it has not yet been proven to be effective in naturally infected cattle. Currently, there is no proven chemotherapeutical method whereby an infected cow can be cleared of the infection. A killed vaccine (NeoGuard, Intervet Inc) is available for *Neospora* but its efficacy is not established in terms of reducing fetal infections, reducing abortions, or in preventing postnatal infections.

There are no proven methods to prevent postnatal infection from ingestion of oocysts but, based on experimental and epidemiological evidence that the dog can be a definitive host, it would be prudent to take measures to reduce potential for this type of transmission. Removal of all potentially infected tissues, such as aborted fetuses and placentas, from the environment that might serve as a source of infection for susceptible hosts would be advisable. In addition, fecal contamination of feed and water sources by other animals should be minimized.

Epizootic Bovine Abortion

Epizootic bovine abortion (EBA), also known as Foothill Abortion, is a regional abortion problem and is the most common cause of abortion in California beef cattle. It is an infectious fetal disease transmitted by the Argasid tick, *Ornithodorus coriaceus*, to pregnant cattle grazing the foothill pastures in California, Nevada and Oregon. The disease is seen in heifers or cows exposed to endemic areas for the first time. Infection in the first half of pregnancy causes a chronic fetal infection and the abortions, either sporadic or as an outbreak, occur in the last trimester. The fetus is usually not autolyzed and may be born alive and premature. Diagnosis of EBA in an aborted fetus is based on characteristic gross and histologic lesions. There is generalized enlargement of lymph nodes and spleen. Often, there is abdominal distension due to ascites and liver enlargement. The thymus may be reduced in size, with hemorrhage and edema. Histologic examination of fetal tissues, particularly the lymphoid organs, is required to confirm a diagnosis of EBA. Fetal immunoglobulin levels are usually elevated.

The cause of EBA is still under investigation after over 50 years of research. During the past 15 years, Jeff Stott and coworkers at UCD and University of Nevada - Reno have established a protocol to reproduce the disease in pregnant cattle by injection of thymus from EBA fetuses. Although numerous attempts to isolate a bacterial agent from infected tissues have been negative, using molecular techniques a bacterium within the delta-proteobacterium group has been implicated as the cause based on specific bacterial rDNA sequences that are consistently associated with the disease. This has led to histochemical and immunohistochemical stains by which a bacterium has been identified in the fetal tissues.

Current recommendations for prevention of the disease involve limiting exposure of susceptible females to the tick during the first six months of pregnancy. In northern regions of the tick range, more severe winter weather limits tick activity to a defined period during the warmer months. This has enabled some ranches to have some success reducing EBA problems by changing to a fall calving season that limits tick exposure of cows in early gestation. In areas where the active tick season is more prolonged (i.e. southern ranges of the tick), it may be difficult to find a sufficient period of time during which animals can reach an advanced stage of gestation without also being exposed to ticks. In these ranches, introduction of heifers to endemic areas before their first breeding season may induce immunity.

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