

Infectious causes of bovine abortion during mid- to late-gestation

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Abstract

The accurate and prompt diagnosis of infectious abortions in a herd requires cooperation between the herd veterinarian and a veterinary diagnostic laboratory; working together, with good communication and appropriate sampling and testing, the chances of obtaining an etiologic diagnosis are improved. Abortion diagnosis is a challenge as a cause is usually identified in less than half of submitted fetuses. The majority of diagnosed abortions are attributed to infections by a moderate number of bacterial, viral, fungal and protozoal agents. The pathology and other findings used in the laboratory diagnosis of the major infectious agents causing bovine abortion in mid- to late-gestation will be discussed.

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1. Introduction

Bovine veterinarians confronted by a herd abortion problem often seek assistance from a veterinary diagnostic laboratory with the capacity to perform a variety of pathology, microbiology and immunology procedures to identify the cause. Most fetuses submitted to diagnostic laboratories for a diagnostic workup are in the second or third trimester and most diagnosed cases are attributed to infectious causes. Abortion diagnosis is challenging; an etiology is identified in less than half of submissions (Table 1). The surveys listed span four decades in various areas of the United States [1–6]. In these surveys, there are differences in the various infectious agents identified, which may reflect factors such as climate, production type, feeds, management practices, vaccination programs, as well as sampling and laboratory procedures available at the time. With increased knowledge and improved diagnostic proce-

dures, new infections have emerged as important causes of abortion.

2. Abortion diagnosis protocol

Information provided with the submissions about the herd abortion problem may help identify potential causes and exclude others. Some useful information would include estimates of the previous and current abortion rate in the herd, the duration of the abortion problem, and the gestational ages of the abortions. Other useful information would be whether the aborted fetuses are fresh or autolyzed, whether the abortions are occurring in heifers, cows or both, whether there are retained placentae, whether aborting animals appear sick, whether natural or artificial breeding is used, and the vaccination history.

In bovine abortions, pathognomonic gross lesions are uncommon and may be obscured by autolysis. Gross lesions are often undependable guides by which to select specific diagnostic tests so most diagnostic laboratories use a standardized diagnostic protocol

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Table 1
Diagnosis of bovine abortion from veterinary diagnostic laboratory surveys in the United States

	Reference					CAHFS ^a (unpublished data)
	[1]	[2]	[3]	[4,5]	[6]	
Location	North East	Midwest	North East	California	Midwest	California
Survey period	1960–1970	1968–1972	1950–1964	1985–1989	1980–1989	1998–2003
Production	Dairy	Beef	Dairy	Dairy	Mixed	Mixed
Total cases	3812	2544	1421	468	8962	2296
Total etiologic diagnoses (%)	888 (23.3%)	899 (35.3%)	426 (30.2%)	213 (45.5%)	2942 (32.8%)	1019 (44.4%)
Categories of etiologic diagnoses with percentage of diagnosed abortions						
Sporadic bacteria	33.9%	48.9%	46.9%	25.3%		24.7%
Brucellosis	<1%	1.6%	9.6%	0.0%	<1%	0.0%
Listeriosis	2%	1.7%	0.0%	<1%	4.1%	<1%
Salmonellosis	<1%	0.0%	0.0%	3.3%	<1%	1.6%
Leptospirosis	10.7%	0.0%	0.0%	4.2%	2.7%	5.9%
Campylobacter (all species)	10.6%	8.5%	16.4%	1.8%	1.8%	3.7%
EBA	0.0%	2.4%	0.0%	3.7%	0.0%	6.5%
Mycotic	21.9%	10%	5.1%	2.3%	16.2%	3.0%
IBR	12.6%	44.9%	0.0%	8.4%	16.5%	3.0%
BVD	2%	<1%	0.0%	3.3%	13.8%	4.0%
Neospora	0.0%	0.0%	0.0%	41.8%	0.0%	50.9%

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consisting of a panel of diagnostic tests. There may be regional differences in the procedures selected as part of the abortion protocol among veterinary diagnostic laboratories. The following is an example of a bovine abortion protocol which can be modified depending on the condition and type of samples submitted, or other circumstances.

The intact aborted fetus, placenta and serum samples from the dam are the optimal specimens; when available, sample submission from several abortions is recommended. Inclusion of the placenta may be critical in the diagnosis of some mycotic and bacterial abortions where the placenta is the primary tissue affected. A complete necropsy examination is performed on the fetus to identify any gross lesions and to estimate the fetal age and degree of autolysis [7]. If the entire fetus cannot be submitted, preferred samples include a complete selection of formalin-fixed tissues and fresh lung, liver, kidney, placenta, fetal thoracic fluid and abomasal fluid. These samples should be submitted chilled in separate sterile containers and the fluids in sterile tubes (not syringes). There are zoonotic bacterial infections associated with bovine abortion such as Brucellosis, Leptospirosis, Listeriosis, Salmonellosis, etc.; therefore, appropriate precautions should be employed in sampling and shipping specimens. Formalin-fixed tissues for histopathology examination include brain, lung, heart, liver, kidney, adrenal, spleen, thymus, lymph node, skeletal muscle, abomasum, small intestine, eyelid and placenta [8].

The routine microbiological diagnostic protocol includes an aerobic bacterial culture and *Brucella* culture on the lung, an aerobic culture and *Campylobacter* culture on the liver, and an aerobic culture, *Brucella* culture, and *Campylobacter* culture on the abomasal fluid. The abomasal fluid is also examined with a gram stain and a dark field examination for *Campylobacter* and trichomonads. The abomasal fluid may be cultured for *Tritrichomonas foetus* on appropriate media, based on positive dark field examinations or other indications. A direct smear on the kidney is examined for Leptospire by fluorescent antibody staining. Bacteria cultures of the placenta are dependent on condition and gross lesions. Fungal cultures are performed if there are placental or skin lesions suggestive of fungal involvement [9].

Routine virology procedures vary among laboratories. Routine virus isolation in tissue culture on an organ pool of fetal tissues, usually including lung, liver, spleen, kidney, adrenal and placenta, is performed by some diagnostic laboratories. Other protocols may limit routine virology testing to infectious bovine rhinotracheitis (IBR) virus and bovine viral diarrhea (BVD) virus fluorescent antibody stains on frozen sections of fetal lung (or liver) and kidney. Both IBR and BVD virus immunohistochemistry procedures are relatively specific and sensitive, with the advantage that the pathologist can retrospectively test for the presence of the virus based on the lesions observed in the fetal tissues.

An abortion serology panel on a single serum sample from an aborting cow may help determine if there had been exposure to an agent but usually cannot differentiate between vaccination and natural exposure, or between recent versus previous exposure. Paired acute and convalescent serum samples may identify an increasing titer to a particular pathogen. However, since maternal seroconversion to some agents often precedes abortion, 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 from the herd are tested, and when history on each animal is provided. Routine serology on the dam includes IBR, BVD, *Leptospira*, *Neospora* and *Brucella* serology [8].

Immunologic procedures on the fetus depend on the post-mortem condition and fetal age. A quantitative immunoglobulin assay for bovine IgG can be performed on blood or thoracic fluid. If the fetal IgG is elevated (>20 mg/dL), this is an indication of an active immune response by the fetus to a foreign antigen which is usually an infectious agent [10]. If there is evidence of elevated IgG, serology for IBR, BVD, *Leptospira*, *Neospora*, *Brucella abortus*, Blue-tongue Virus and Parainfluenza 3 virus can be performed. When specific fetal titers are elevated, they suggest fetal exposure to that agent. On occasion, low titers to one or more of these agents may be present in a fetus without other evidence of infection, so a cautious interpretation is warranted. The source of antibody in the fetal fluids, particularly when antibody to multiple agents is present, is not certain, but maternal antibodies may cross to the fetus due to placental lesions.

When available, the placenta should be examined and sampled. In a fresh normal placenta, the cotyledons are red with a clear, translucent intercotyledonary placenta although with autolysis the cotyledons become dull brown and the intercotyledonary placenta less translucent. Intercotyledonary opacity can be associated with edema, inflammation or fibrosis. Exudate on the surface or thickening of the chorioallantois is evidence of inflammation. Cotyledons whose surface is depressed relative to the surrounding intercotyledonary placenta (“cupping”) can be an indication of inflammation in the surrounding stroma. Gross abnormalities in the cotyledons include adherent caruncular tissue, hemorrhage, necrosis and exudation.

An estimation of the gestational age of the fetus can be made from the crown-rump length and the extent and distribution of hair development [7]. Potentially useful

external changes include evidence of meconium staining implying fetal distress and round raised skin plaques that can be associated with mycotic infection. With the fetus opened, an estimation of the degree of autolysis can be made. Evidence that the fetus may have been alive at the time of parturition such as lung inflation, hemorrhage surrounding the umbilical vessels or thrombosis of the umbilical arteries should be noted. A freshly aborted fetus will usually have clear, amber-colored fluid in the body cavities; however, within 1–2 d after death, serosanguinous fluid collects in the body cavities and subcutis. This process is followed by gradual dehydration of the tissues such that by a week after fetal death, the fetus is dehydrated with no abomasal content.

The selection of fetal tissues for histopathology examination varies among laboratories (due to a variety of factors). Whereas autolytic changes are common and can be severe, unless the fetus is mummified, histopathology will usually provide some information concerning any evidence of infectious disease and therefore help to evaluate whether the microbiology or immunology results are relevant. Examination of a full selection of tissues is recommended whenever possible. Although autolysis may render the fetal brain to consist of liquefied fragments, collection of this material into formalin is still recommended because the histologic detail is surprisingly preserved. A complete histological examination of the fetal tissues provides information about the distribution of lesions which may also suggest the route of fetal infection and potential causes. Some infections proliferate in the placenta then enter the fetus through the umbilical vessels leading to hepatic and systemic changes. Examples of this pattern of fetal injury can include such agents as *Listeria*, *Salmonella*, IBR and *Neospora*. In other bacterial and fungal infections which initiate a placental infection and then invade via the placental fluids surrounding the fetus the inflammatory lesions are usually associated with lungs, digestive tract or skin. Histologic examination of the eyelid having both conjunctiva mucosa and skin can be a useful and sensitive indicator of infection [10]. On occasion in situations with severe placentitis, there may not be any significant inflammatory lesions in the fetus [11].

3. Diagnostic features of infectious causes of abortion

The following sections discuss the presentation and diagnosis of commonly diagnosed infectious causes of abortion in cattle.

3.1. Sporadic abortions associated with opportunistic bacterial infections

A diverse group of bacterial species are associated with opportunistic infections of the placenta and fetus resulting in abortion. Data from abortion surveys indicates that one-fourth to half of the diagnosed abortions can be associated with infections by bacteria in this group (Table 1). These bacteria are not contagious pathogens but are commonly found in the environment or on mucosal surfaces. A maternal bacteremia is the presumed means by which they reach the gravid uterus and subsequently infect the placenta. Among the bacteria in this group, *Arcanobacterium pyogenes* is the most commonly identified species; *Bacillus* spp., *Escherichia coli*, *Haemophilus somnus*, *Pasteurella* spp., *Pseudomonas* spp., *Serratia marcescens*, *Staphylococcus* spp., *Streptococcus* spp. and other bacteria have also been identified and are included in this group of sporadic opportunistic bacterial abortion [12,13]. Whereas opportunistic bacterial infections are a substantial proportion of diagnosed abortions, their occurrence is sporadic. Abortions storms are not associated with this group of bacteria; multiple abortions in a herd suggest maternal health issues that could enhance hematogenous bacterial infections or there could be additional infectious agents involved in the abortion. In one large survey, up to one-third of abortions associated with *A. pyogenes*, *Bacillus* sp. and mycotic infections had concurrent BVD virus infection [14].

Sporadic bacterial abortions may occur at any stage of gestation, but most are identified in the second half of gestation. There are no specific signs in the dam, although the placenta may be retained. The degree of fetal autolysis is variable. The surface of the placenta may have yellow to brown exudate. Gross fetal lesions may infrequently include fibrin exudation in body cavities. Histologic lesions include a suppurative placentitis and a neutrophilic fetal bronchopneumonia of varying severity. In *A. pyogenes* infection, large clusters of bacterial colonies may be present in the lung with minimal inflammation. As the bacteria involved are common in the environment or mucosa, their presence in fetal tissues might be due to incidental contamination. Therefore, in order to establish an etiologic diagnosis, the bacteria should be isolated in pure or nearly pure culture from abomasal contents or tissues and there should be lesions consistent with a bacterial infection in the fetus or placenta [12,13].

3.2. Brucellosis

B. abortus infection and abortion is now rare in the United States, due to eradication programs. Infection is primarily through ingestion, the bacteria multiply in regional lymph nodes and are then spread hematogenously to other organs, most importantly, the mammary gland, mammary lymph nodes and gravid uterus, usually 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 24–72 h after in utero death and abortion usually occur after the fifth month of pregnancy. Metritis and retained placenta is common. Grossly, there is often a severe placentitis with edema, focal necrosis of cotyledons and thickened intercotyledonary areas with adherent yellowish exudate. The fetus is frequently autolyzed with no gross lesions. Histologically there is a severe placentitis with numerous bacteria visible in chorionic epithelial cells. Fetal lesions consist of bronchopneumonia, which can vary in severity and character from acute neutrophilic bronchopneumonia to more chronic pleocellular bronchiointerstitial pneumonia with periairway infiltrate of mononuclear cells. 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. Various serologic tests have been developed for governmental surveillance and detection of cattle exposed to *B. abortus* [15].

3.3. Listeriosis

Listeria species are widespread in the environment and abortion due to *Listeria monocytogenes* and *L. ivanovii* infections occur throughout the United States. The proportion of diagnosed abortions attributed to *Listeria* species infection in diagnostic laboratory data is low, although in a large Midwest survey, *Listeria* species infection constituted 4.1% of diagnosed abortions [12]. Most abortions have a sporadic occurrence, but in some conditions, abortion storms may occur. In some instances, there may be illness in aborting cows with fever and anorexia due to metritis. Listeriosis in adult cattle can occasionally cause encephalitis but this is rarely seen in association with abortion. The infection has been associated with ingestion of poorly fermented silage.

Fetuses are usually aborted in the third trimester and often markedly autolyzed. The placenta is usually retained at abortion. Gross lesions are often absent or

obscured by autolysis but in some, pinpoint white to yellow foci are present in the liver. Additional gross findings may include small pale foci in placental cotyledons, and fibrin in body cavities. The histopathology changes include suppurative placentitis and multifocal necrotizing or suppurative hepatitis. In addition, meningitis and intravascular bacterial colonization in many organs with or without associated inflammatory lesions is sometimes encountered. In most aborted fetuses, *Listeria* spp. are present in multiple tissues and do not require cold enhancement for successful isolation. In fresh tissues, liver impression smears or abomasal fluid can be gram stained to identify Gram-positive coccobacilli. In fixed tissues, gram staining is often useful in multiple tissues particularly liver sections. Immunohistochemistry stains for *L. monocytogenes* utilizing commercially available antibody can assist the diagnosis when a positive culture result is unavailable [13].

3.4. Salmonellosis

In the United States, abortions attributed to *Salmonella* species infections are not common and usually present as sporadic occurrences so perhaps could be grouped with other opportunistic bacterial infections causing sporadic abortions. However, in the United Kingdom and some other regions of the world, salmonella abortion is an important cause of both enzootic and epizootic abortion [16]. Most bovine abortions due to *Salmonella* species infections are associated with *Salmonella dublin*, but other serotypes can be involved. The infection is presumed to originate from the intestinal tract. Bacteremic episodes can lead to the localization and proliferation of the infection in the placentome causing destruction of fetal villi which may lead to abortion without bacterial invasion of the fetus.

The abortions are usually in the second half of gestation and the placenta is often retained. The chorioallantois is thickened with fibrinous fluid with a diffusely grey to red chorionic surface. Portions of caruncular tissue may be adherent to the cotyledons. The fetus is usually quite autolyzed and may be emphysematous. Usually no remarkable gross lesions can be identified, although indistinct pale foci may rarely be present in the liver. In the placenta, there is neutrophilic placentitis and mineralization with bacterial proliferation in cotyledonary villi. In the liver, there can be a multifocal suppurative hepatitis. Lung lesions may be minimal consisting of neutrophilic bronchial exudate. *Salmonella* and *Listeria* abortions have

similarities in that the bacterial infection proliferates in the placenta followed by massive infection of the fetal liver, septicemia and death, often without development of bronchopneumonia typical of some other bacterial infections of the placenta.

3.5. Leptospirosis

Leptospirosis is likely an under-diagnosed cause of abortion in cattle and occurs worldwide. The most important serovars of *Leptospira interrogans* associated with bovine abortion are *Leptospira hardjo* and *L. pomona*, though rarely *L. interrogans* serovars *icterohaemorrhagiae* and *grippotyphosa* have been associated with bovine abortion. *L. hardjo* serovars are adapted to cattle who serve as the maintenance host, whereas other serovars of *Leptospira* involved in bovine abortions are maintained in other domestic or wildlife species. Leptospire can be shed in urine for several weeks and more prolonged urine shedding can be observed with *L. hardjo* infections. Leptospire can survive in wet environments for up to 30 d and can penetrate intact mucous membranes or abraded skin. Abortion is a manifestation of chronic leptospirosis in adult cattle, and is frequently the only clinical sign observed in a herd. 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 [17,18].

Abortion can occur 1–3 mo after initial infection with *L. hardjo* serovars and 1–6 wk after infection with *L. pomona*. *L. hardjo* infection is associated with infertility, abortions from 4 mo to term, and weak calves. Abortion due to *L. pomona* usually occurs in the last trimester. The herd abortion rate seldom exceeds 10% with *L. hardjo* infections but can be higher with herd infections of *L. pomona*. The aborted fetus is usually autolyzed. Icterus may be seen in late gestation fetuses infected with *L. pomona*. Histologic lesions may not be observed but in some cases, renal tubular necrosis and interstitial nephritis is present [13]. Bile retention within liver canaliculi may be present. Non-suppurative meningitis has also been reported.

Because leptospire are labile and difficult to culture, bacterial isolation is impractical for routine diagnostics. Identification of leptospire by darkfield microscopy of fetal fluids or silver stains of fetal tissues is rarely successful. Fluorescent antibody examination of fetal kidney smears using multivalent antisera is a convenient, rapid procedure although not highly sensitive

and specific. The specific *Leptospira* serovar involved cannot be determined with this procedure. Immunohistochemistry staining is sometimes useful in identifying leptospires in bovine fetal tissues, but autolytic fragmentation of the spirochetes can make interpretation difficult. Polymerase chain reaction assays are available and used by some diagnostic laboratories to identify *Leptospira* in fetal tissues [17].

Maternal serology using the microscopic agglutination microtiter test may be useful in the diagnosis of leptospirosis, though caution must be used to distinguish among vaccination, previous exposure and recent infection. Serology for *L. hardjo* is especially difficult to interpret since infected animals often have a low or negative titer at the time of abortion. Higher serologic titers are associated with *L. pomona* infection at the time of abortion but as abortion occurs long after the initial maternal infection, seroconversion usually precedes abortion and the titer may be declining. It can be difficult to distinguish between vaccination and field exposure titers, although multivalent vaccines usually produce low- and short-lived titers compared to recent field exposures. In addition, the multivalent bacterins usually exhibit a pattern of elevated titers to multiple serovars as contrasted with elevated titers to a single serovar following field exposure. Fetal titers of 1:40 or above would be supportive of a diagnosis, but most infected fetuses have no detectable titer.

3.6. *Ureaplasma diversum*

The diagnosis of *U. diversum* abortion in cattle is infrequently reported in the United States, although in Ontario, Canada, a substantial portion of abortions have been attributed to this infection. It is not clear whether this higher incidence in this region reflects a difference in infection rate or differences in microbiologic procedures used by diagnostic laboratories to identify this fastidious organism [19,20].

Fetuses are usually aborted the last third of gestation and stillbirths or weak calves may occur. The aborted fetuses are often in fresh condition. Retained placenta is frequently reported. There is a placentitis in which the amnion is often the most affected portion of the placental membranes. The amnion is thick and opaque with multifocal to extensive areas of hemorrhage, fibrin exudation, necrosis and fibrosis. Similar changes may be present in the chorioallantois; they are usually more severe on the allantoic surface. The cotyledons may be tan to dark red, cupped with adherent caruncular material. Histologic changes in the stroma of the chorioallantois and amnion include fibrosis, necrosis

and mineralization with macrophage and plasma cell infiltrates and mononuclear vasculitis. In the fetus, the lungs may be swollen and firm. Histologically, the lung lesions consist of a non-suppurative alveolitis and periairway mononuclear infiltrate. Erosive lymphoplasmocytic conjunctivitis has been reported.

U. diversum is a common inhabitant of the upper respiratory tract and lower reproductive tract of cattle; contamination of placental and fetal tissues is a potential confounding factor in diagnosis. Diagnosis of *U. diversum* as the cause of abortion should be based on isolation of the organism from the lung, stomach contents or placenta coupled with the presence of compatible lesions in the lung and placenta [19].

3.7. *Campylobacter species*

Several *Campylobacter* species can be associated with abortion in cattle. As a group, *Campylobacter* species are associated with a substantial proportion of diagnosed abortions, ranging from less than 2% to over 10% of diagnoses (Table 1). The major *Campylobacter* species associated with bovine abortions are *Campylobacter fetus* subspecies *venerealis*, *C. fetus* serovars *fetus*, and *C. jejuni*. *C. fetus* subspecies *venerealis* is a venereal disease, whereas *C. fetus* subspecies *fetus* and *C. jejuni* infections occur as sporadic infections, presumably due to maternal hematogenous infections from the intestinal tract [21]. *C. fetus* subspecies *venerealis* is a cause of infertility due to early embryonic death with occasional abortions occurring at 4–7 mo gestation. In the placenta, a fibrinous intercotyledonary placentitis with necrosis and yellow-brown discoloration of cotyledons may be present. Aborted fetuses can be in variable post-mortem condition and may have fibrinous exudation in the pleural cavity, peritoneal cavity or pericardial sac. Splenomegaly is a variable feature and fetal serum immunoglobulin concentrations may be moderately elevated. Histologic changes include fibrinous neutrophilic placentitis, neutrophilic bronchopneumonia, fibrinous neutrophilic serositis and occasionally abomasitis. Silver stains of fetal tissues with inflammation may assist in the diagnosis by identification of curved silver stain bacteria. Immunohistochemistry has been used to detect *C. fetus* in fetal tissues and differentiate it from other *Campylobacter* species [22]. In a fresh fetus, *Campylobacter* may be identified in darkfield microscopic examination of abomasal fluid as small bacteria with darting motility. *C. fetus* subspecies *venerealis* is fastidious, requiring careful sample collection and proper transport. The bacteria can be cultured from

lung, placenta and abomasal fluid. The bacteria can also be identified in preputial samples and vaginal secretions. *C. fetus* subspecies *venerealis* must be differentiated from other intestinal *Campylobacter* species through culture which requires special media and oxygen tension. Phenotypic characterization and molecular techniques have been used to differentiate *Campylobacter* species.

3.8. Epizootic bovine abortion

Epizootic bovine abortion (EBA, also known as foothill abortion) is a cause of abortion and premature calving in cattle grazing foothill rangelands in California, Nevada and Oregon. The infection is transmitted to susceptible pregnant cattle by an argasid tick (*Ornithodoros coriaceus*), that feeds on deer and cattle. The disease is seen in heifers or cows exposed to endemic areas for the first time while in the first trimester of pregnancy. Abortions, either sporadic or as an outbreak, usually occur in the last trimester and premature weak calves may also occur. Following an abortion affected cattle are resistant to repeat abortion [23].

An unnamed bacterium is presumed to be the etiology of EBA based on molecular techniques on extracted 16S bacterial ribosomal DNA fragments in infected fetal tissues and ticks [24]. The DNA sequences suggest that the bacterium is a delta-proteobacteria in the Myxobacteria family. The bacterium has not been successfully grown on artificial media. It is a plump, 2–3 μm long, Gram-negative rod in smears of infected fetal tissues. Histochemical identification of the bacterium in tissues has only been successful utilizing a modified Steiner silver stain procedure. An immunohistochemistry procedure utilizing the sera from infected fetuses can readily stain the bacterium in the thymus, spleen, lymph node and in other tissues in the sites of inflammatory lesions [25].

The diagnosis of EBA is based on the identification of characteristic gross and histologic lesions which are chronic, having developed over a period of 3 mo or more. The fetus is usually fresh and may be born alive. Petechiae are common in the mucosa of the conjunctiva and oral cavity. There is enlargement of peripheral and internal lymph nodes. Enlarged superficial lymph nodes can be easily palpated through the skin. Abdominal distension due to ascites and liver enlargement is often present. Splenic enlargement and enlargement of internal lymph nodes is usual. The thymus may be reduced in size with interlobular or widespread hemorrhage and edema in the cranial

portion. Histologic examination of fetal tissues, particularly the lymphoid organs, is required to confirm the diagnosis. Thymic lesions, which are unique in EBA, develop late in the course of the disease and consist of a loss of cortical thymocytes and infiltration of the medullary region with macrophages. The thymic interlobular septa are distended with edema, fibrin, hemorrhage and cellular infiltrates consisting of macrophages and other mixed inflammatory cells. The gross enlargement of the lymph nodes is associated with lymphoid hyperplasia and widespread macrophage infiltration in the sinuses and medulla. There is lymphoid hyperplasia and histiocytic infiltration in the spleen. Late in the course of disease, following the proliferative response, acute necrotic foci develop in lymphoid organs. There are widespread inflammatory lesions with a vascular orientation in most organs, including the brain, lung, heart, liver, kidney, skeletal muscle and other organs. Fetal serum immunoglobulin levels are usually markedly elevated (100–1000 mg/dL).

3.9. Mycotic abortion

Mycotic abortion is reported worldwide; in some regions, it is the major identified cause of abortion [26]. In the United States, the incidence appears to be lower in that the proportion of bovine abortion diagnoses attributed to mycotic infections varies from less than 1% to 10% (Table 1). The majority of mycotic abortions in cattle in the United States are associated with *Aspergillus fumigatus* infections [27,28]. Infections by other *Aspergillus* spp., *Absidia* spp., *Mucor* spp., *Rhizopus* spp., *Candida* spp. and other fungi occur less commonly [29]. The fungi responsible are ubiquitous saprophytes in the environment. The concentration of fungal conidia in the environment may increase the risk for fetal infection; more cases occur in the winter where cattle are housed and fed. Injury to the respiratory or digestive tract of the dam may also enhance the entry of fungi into the bloodstream. Localization in the uterus probably occurs by hematogenous spread from these sites of entry. Experimentally, intravenous injection of *A. fumigatus* causes abortion 23–35 d post-infection [30]. *Mortierella wolffi*, a common abortifacient in the southern hemisphere, is rare in North America.

Mycotic abortions usually occur as sporadic third trimester abortions. Clinical signs in the dam are infrequent, with the exception of retained placentas. Placentitis is the primary lesion so submission of the placenta is critical to the diagnosis. Grossly there is

often a severe placentitis involving both the cotyledons and intercotyledonary placenta results in a diffusely thickened, leathery placenta. Cotyledons may have necrotic, hemorrhagic infarcts with adherent caruncular tissue. Histologically the placenta may have suppurative inflammation, necrosis and vasculitis with thrombosis associated with fungal invasion. Fetal autolysis may be minimal, especially with *A. fumigatus* infections. In the fetus lesions are variable and may be absent. In a minority of affected fetuses there may be raised circumscribed plaques on the skin. Internally fetal lesions associated with mycotic infection may include bronchopneumonia or focal digestive tract inflammation associated with fungal invasion. A rapid presumptive diagnosis may be obtained by direct microscopic examinations of scrapings of placental or skin lesions after digestion with 10% potassium hydroxide (KOH) solution. Samples of the abomasal fluid can be examined in a similar manner. The fungi are not digested by the KOH solution and can be visualized microscopically in the wet mount. Histologic identification of fungi in tissue lesions utilizing histochemical stains is also effective. For isolation cultures of the placenta, abomasal fluid, or lung on fungal media with antibiotics to suppress bacterial growth can be employed [27]. As fungi are ubiquitous in the environment their presence could be the result of contamination so the diagnosis of mycotic abortion requires compatible lesions in the placenta or fetus in addition to the microscopic demonstration or isolation of fungi.

3.10. Infectious bovine rhinotracheitis (IBR, bovine herpesvirus type 1)

Bovine herpesvirus type 1 is found worldwide, although in some European countries eradication efforts have been successful [31]. In the United States, IBR remains a significant cause of abortion which is frequently epizootic (Table 1). In addition to abortions, disease associated with infection includes vulvovaginitis, balanoposthitis, respiratory disease, conjunctivitis, encephalomyelitis and fatal systemic infections in neonatal cattle. Infection at breeding can cause infertility. Embryonic death may result from infections early in pregnancy. The virus may persist as a latent infection following acute infection. Infection occurs through contact with infected cattle shedding virus from respiratory, ocular and reproductive secretions. Exposure of previously unexposed, non-vaccinated 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. Most abortions occur several weeks (20–52 d) following initial infection of the dam. Cows may exhibit a range of signs reflecting the various clinical disease forms, including subclinical infection. Because of the delay from maternal infection to abortion, aborting cows may exhibit signs of illness other than abortion [32,33].

Aborted fetuses are usually 5 mo to term and are autolyzed with red-tinged fluid in body cavities and fascia. There are usually no gross lesions other than placental edema, although rarely indistinct pinpoint white foci may be present in the liver. A presumptive diagnosis can be made from histopathology of the liver which despite considerable autolysis usually has striking multifocal necrosis, best appreciated at low magnification. Focal necrotizing lesions are usually present in other fetal tissues, especially lung, adrenal, spleen and lymph node. Eosinophilic intranuclear inclusions are often difficult to identify but may be identified in the adrenal cortical lesions. Placentitis with necrosis and vasculitis in placenta villi is usually present associated with abundant viral antigen as detected with immunohistochemistry. Diagnosis is confirmed by viral isolation, by detection of viral antigen in fetal tissues by immunofluorescent staining on frozen tissue sections (especially kidney) or by immunohistochemistry on formalin-fixed tissues (especially liver, lung, kidney, adrenal, placenta) using monoclonal antibodies [34,14].

3.11. Bovine virus diarrhea (BVD) virus

BVD virus infection is widespread in the cattle population and in susceptible pregnant animals fetal infection is likely to occur. Fetal infections have a variable outcome depending on the timing of the infection, the biotype and other properties of the virus. Proof that BVD virus infection is the cause for an abortion is confounded by the fact that the virus may infect the fetus without causing abortion and there are diverse fetal lesions attributed to infection. An important aspect of fetal BVD virus infection is that non-cytopathic BVD virus infections in fetuses prior to 4 mo of gestation can result in persistently infected live calves that are a major source of infection for other cattle. Contact with infected animals shedding the virus and contaminated biologics are additional sources of infection [14,35].

There are often no obvious clinical signs seen in herds with fetal losses due to BVD virus and abortions occur a few days or several weeks following maternal

infection. Fetal BVD virus infection can have variable outcomes depending on the gestational age of the fetus infected and other factors. First trimester infections can cause infertility, embryonic death, fetal resorption, mummification, or abortion. However, infections with non-cytopathic BVD virus from 18 to 125 d gestation may result in persistently infected live calves. Fetal infections beyond approximately 4 mo 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 (from approximately 100–150 d gestation), can also result in the birth of term calves with congenital anomalies [35,36]. In one survey, BVD virus infection were frequently present in abortions with other bacterial or mycotic infections implying that BVD virus infection may contribute to abortion by affecting the dam's immune response during pregnancy [14].

The fetal lesions associated with abortions attributed to BVD virus infection are quite variable. The fetuses can be fresh or autolyzed and mummification can occur. The aborted fetuses may be small for their gestational age or premature small calves may be born. Gross lesions that may suggest BVD virus infection in the nervous system includes microencephaly, cerebellar hypoplasia, hydranencephaly and hydrocephalus. Ocular lesions including microphthalmia, cataracts, retinal dysplasia and optic neuritis have been described. Alopecia may be present. Thymic hypoplasia with histologic evidence of thymic cortical atrophy is associated with BVD virus infection. Pulmonary and renal hypoplasia or dysplasia has been reported. BVD virus infection has been associated with a necrotizing myocarditis with non-suppurative vasculitis in the heart and other organs. Affected fetuses may exhibit marked anasarca with a round dilated heart and chronic passive congestion of the liver [14,35].

To attribute BVD virus infection as the cause of abortion, evidence of infection needs to be combined with compatible fetal pathology and/or herd history. Fetal infection can be determined by detection of the virus in fetal tissues by various methods [36,37]. Virus isolation on a pool of fetal tissues (most often lung, liver, kidney and/or lymphoid organs) is widely used in diagnostic laboratories although it is a relatively expensive and time consuming procedure. Virus isolation is specific but the sensitivity in diagnostic submissions is estimated to be reduced due to autolysis and other factors. Fluorescent antibody staining for BVD virus on frozen sections of either lung or liver and

kidney is often used as it is a convenient and rapid procedure. However, there are numerous false positives and false negative results with this procedure compared to either virus isolation or immunohistochemistry. BVD virus immunohistochemistry using monoclonal antibody appears to be a sensitive and specific procedure capable of detecting a variety of BVD virus isolates [38,39]. Useful tissues for BVD immunohistochemistry staining include kidney, lung and placenta. In fetuses with vasculitis and necrotizing myocarditis, immunohistochemistry may demonstrate the presence of BVD viral antigens in the vessel wall and/or muscle. A number of reverse transcription polymerase chain reaction procedures have been evaluated which appear to be highly sensitive in detecting BVD virus [36].

After 4 mo gestation the fetus may respond immunologically to BVD virus infection so a positive BVD virus titer in the fetal fluids is an indication of fetal infection. However, if earlier infection by non-cytopathic BVD virus induces persistent infection, the fetus may be immunotolerant so a negative fetal serology does not rule out infection. Serology on the dam is of limited value in diagnosing BVD abortion, particularly if animals are vaccinated.

An important outcome of fetal infection with non-cytopathic BVD virus is the birth of persistently infected calves which are a significant source of infection to the herd. Virus isolation, serology, immunohistochemistry, polymerase chain reaction and antigen capture ELISA procedures have been used to screen for the presence of persistently infected animals as a means to control this infection. The choice of the optimal screening procedure for a specific herd will depend on a number of factors which have been recently discussed [36,40]. Skin ear notch biopsy procedures utilizing BVD virus immunohistochemistry or antigen capture ELISA to identify individual persistently infected animals have been evaluated [41,42].

3.12. *T. foetus*

T. foetus is a venereal disease that is primarily associated with early embryonic loss rather than mid- to late-term abortions. However, occasional abortions may occur and submission of these may offer the first opportunity to identify this disease in some infected herds. Abortions can occur from 2 mo to late gestation, with variable autolysis. In most cases, there are no gross lesions other than placental edema. Histologic lesions consist of pleocellular placentitis and fetal bronchopneumonia occasionally with multinucleated giant cells

in the lung airways [43]. In fresh samples, trichomonads may be identified in abomasal fluid samples by dark field microscopy followed by confirmation by culture of the fluid utilizing appropriate media. The trichomonads may be visualized in the placenta, lung and other tissues in routine hematoxylin–eosin stained sections. Histochemical stains such as Giemsa and Bodian's silver protargol are useful in identifying trichomonads in tissue sections. Immunohistochemistry utilizing polyclonal and monoclonal antibodies is an effective method to detect *T. foetus* in fetal and placental sections [44].

3.13. *Neospora caninum*

Neosporosis is a common infection of cattle and is a major cause of abortion worldwide [45–49]. The protozoal parasite can be maintained in the cow as a chronic infection which can be vertically transplacentally transmitted to her fetus during pregnancy. Although some infected cows abort, many fetal infections produce a congenitally infected calf. A congenitally infected heifer calf is capable of transmitting the infection onto the next generation when she becomes pregnant, thus maintaining the infection in the herd [50–53]. Cattle may also acquire *N. caninum* infection by horizontal (postnatal) infection through the ingestion of oocysts shed in the feces of the definitive hosts. Dogs and coyotes have been identified as definitive hosts [47,48,54].

Endemic and epidemic abortion patterns have been described [55]. In the endemic pattern of abortion, the herd experiences an elevated abortion rate of greater than 5%/yr which persists for years. The epidemic pattern of abortion is less common and is characterized by abortions in a high proportion of pregnant cattle over a relatively brief period of time. Cows that abort a *Neospora* infected fetus may have additional abortions or infected fetuses in subsequent pregnancies. Cows and heifers that are seropositive are at an increased risk of abortion.

N. caninum abortions occur throughout the year and have been reported in both dairy and beef cattle. There are no signs of clinical illness in cows that abort. The aborted fetuses are usually autolyzed and placentas are not retained. Abortions have been diagnosed in both heifers and cows from 3 mo gestation to term, but the majority of abortions occur in the second trimester of pregnancy which is a distinctive feature of this disease. Fetal mummification has been associated with *Neospora* outbreaks. A rare outcome of fetal *Neospora* infection is the birth of a full-term calf with central nervous system signs. However, the majority of calves

that acquire a *Neospora* infection during gestation are born clinically normal. These calves have a high precolostral antibody titer to *N. caninum* which is useful in diagnosing in utero infection.

Fetuses usually abort in mid-gestation, 4–6 mo gestation, and are autolyzed with serosanguinous fluid accumulation in body cavities. Subtle gross lesions, consisting of pale white foci or streaks in the skeletal muscles or the heart may be observed. There are widespread histologic lesions in many organs, the most diagnostically important in the brain consisting of scattered foci of cellular infiltrates and/or foci of necrosis. Other routinely identified lesions include non-suppurative epicarditis and/or myocarditis and myositis. Liver lesions consist of portal hepatitis with foci of paracentral hepatic necrosis. Lung and kidney often have scattered non-suppurative interstitial infiltrates. Placentitis varies in severity but may be the primary cause of fetal death and abortion.

Immunohistochemistry using antibodies raised against *N. caninum* antigens is an effective method to identify the tachyzoite and tissue cyst stages of the parasite in fetal tissues. *Neospora* immunohistochemistry is most successful in sections of brain, lung, kidney, skeletal muscle and placenta [5,45]. The use of pathology and immunohistochemistry on aborted fetuses to establish a diagnosis has been questioned because the fetus can be infected and not abort due to the infection [56]. The presumption is that in early gestation there is little resistance to disseminated infection which results in abortion or mummification, whereas latter in gestation the fetus can survive this infection. A diagnosis of *Neospora* infection as the cause of abortion should take in consideration the gestational age, autolyzed condition, compatible disseminated inflammatory lesions, detectable parasites and no other identified causes. A fetus with mild lesions, often limited to focal encephalitis in late term fetuses, may likely have an incidental *Neospora* infection so other causes for the abortion should be investigated.

Neospora PCR techniques have been used to diagnose fetal *N. caninum* infection and are reported to be more sensitive than immunohistochemistry in identification of fetal infection [57]. However, establishing *Neospora* infection as the cause for the abortion on the basis of a positive *Neospora* PCR should employ the same criteria for diagnostic significance as for a positive *Neospora* immunohistochemistry test.

A variety of serologic tests are available to assist in the diagnosis of neosporosis. These include the indirect fluorescent antibody test, the modified agglutination test, and a number of enzyme-linked immunosorbent

assays [58–61]. The assays utilize *N. caninum* tachyzoites or specific derived antigens. The 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 non-infected cattle. In some tests, the positive cut-off titer has been selected based on the antibody titer in a cow that has aborted an infected fetus so this cut-off may not be the most appropriate for the serologic diagnosis of a chronic infection in cattle which vary in age and pregnancy status. A single serum sample from an individual cow may not accurately reflect her infection status since titers in known positive cattle fluctuate and may fall below the cut-off value for some period of time. In rare instances, cows that abort a *Neospora* infected fetus may not have a significantly elevated titer. Also, previously elevated titers at abortion may decline over several months following abortion. In newborn calves, *Neospora* serology on precolostral serum is an effective method to determine fetal exposure. In aborted fetuses, *Neospora* serology is less useful in diagnosis. An infected fetus may have a negative titer because of the gestational age, duration of infection prior to death, or autolysis. A negative fetal titer does not rule-out infection nor does a positive titer prove that this infection caused the abortion. In the individual aborting cow, a positive serology result does not prove that the abortion was due to neosporosis but it can assist the diagnosis. Serology can be used on a herd basis to investigate the association between seropositivity and abortion by comparing results among aborting and non-aborting cattle to estimate the extent that the abortions can be attributed to *Neospora* infection [49,55].

4. Summary

Laboratory results obtained from the abortion submissions should be interpreted by the veterinarian to determine whether they provide a sufficient answer to the herd abortion problem. On occasion, aborted fetuses may present with incidental infections, have multiple infections, or the fetus submitted may not be representative of the herd problem. When laboratory testing is completed, the clinical situation and laboratory diagnosis can be compared to assess whether the abortion submission is a representative sample and if the identified cause is a significant factor in the herd abortion problem. Identification of an infectious cause in abortion submissions is frequently unsuccessful as

illustrated by the surveys listed in the table where a specific etiology is identified for less than half of aborted fetuses submitted (Table 1). The failure to find an infectious cause may be a correct interpretation in cases in which there are abnormal genetic, hormonal, metabolic, developmental or other factors responsible which are often difficult or impossible to confirm in diagnostic laboratory submissions. The lack of relevant findings in multiple fetuses submitted from a herd can be used as evidence by the veterinarian to investigate other possible non-infectious factors. However, among the fetuses submitted to veterinary diagnostic laboratories for which no etiology is identified, many appear to have an infectious etiology. In large Midwest survey [6], the author identified lesions suggestive of an infectious cause but for which no etiologic agent could be identified in 1554 of 8995 fetuses submitted (17.34%). Similar findings were obtained in two surveys from California; in a small survey, 24.6% of submitted fetuses had undiagnosed inflammatory lesions [5] and in more recent unpublished data, 17% of nearly 2300 fetal abortion submissions had inflammatory changes for which no agent was identified. These findings are encouraging to the veterinary laboratory diagnostician, suggesting that there is upward potential for bovine abortion diagnoses. Improvements in our knowledge and in detection methods for infectious agents should allow us to increase the diagnosis rate on abortion submissions and perhaps identify new causes of bovine abortion.

References

- [1] Hubbert WT, Booth GD, Bolton WD, Dunne HW, McEntee K, Smith RE, et al. Bovine abortions in five northern states, 1960 to 1970: evaluation of diagnostic laboratory data. *Cornell Vet* 1973;63:291–316.
- [2] Kirkbride CA, Bicknell EJ, Reed DE, Robl MG, Knudtson WU, Wohlgenuth K. A diagnostic survey of bovine abortion and stillbirth in the northern plains states. *J Am Vet Med Assoc* 1973;162:556–60.
- [3] Bolton WD, Durrell WB, Wadsworth JR, Murray RW. A survey of abortions in Vermont dairy cattle. *J Am Vet Med Assoc* 1969;155:500–3.
- [4] Anderson ML, Blanchard PC, Barr BC, Hoffman R. A survey of causes of bovine abortion occurring in the San Joaquin Valley, California. *J Vet Diag Invest* 1990;2:283–7.
- [5] Anderson ML, Blanchard PC, Barr BC, et al. *Neospora*-like protozoan infection as a major cause of abortion in California dairy cattle. *J Am Vet Med Assoc* 1991;198:241–4.
- [6] Kirkbride CA. Etiologic agents detected in a 10-year study of bovine abortions and stillbirths. *J Vet Diagn Invest* 1992;4:175–80.
- [7] Roberts SJ. *Veterinary obstetrics and genital diseases theriogenology*, 3rd ed., Woodstock, VT: Published by the Author; 1986. p. 19.

- [8] Barr BA, Anderson ML. Infectious disease causing bovine abortion and fetal loss. *Vet Clin N Am Food Anim Pract* 1993;9:343–68.
- [9] Kirkbride CA, editor. Laboratory diagnosis of abortion in food animals. 3rd ed., Iowa State University Press; 1990.
- [10] Miller RB, Quinn PJ. Observations on abortions in cattle: a comparison of pathological, microbiological and immunological findings in aborted fetuses and fetuses collected at abattoirs. *Can J Comp Med* 1975;39:270–90.
- [11] Miller RB. A summary of some of the pathogenic mechanisms involved in bovine abortion. *Can Vet J* 1977;18:87–95.
- [12] Kirkbride CA. Bacterial agents detected in a 10-year study of bovine abortions and stillbirth. *J Vet Diagn Invest* 1993;5:64–8.
- [13] Yaeger MJ, Holler LD. Bacterial causes of bovine infertility and abortion. In: Youngquist RS, Threlfall WR, editors. *Current therapy in large animal theriogenology*. 2nd ed., WB Saunders Co.; 2007. p. 389–98.
- [14] Kirkbride CA. Viral agents and associated lesions detected in a 10-year study of bovine abortions and stillbirths. *J Vet Diagn Invest* 1992;4:374–9.
- [15] Nicoletti P. Brucellosis. In: Howard JL, editor, Smith RA, Assoc. editor, *Current veterinary therapy food animal practice*, 4th ed. WB Saunders Co.; 1999. p. 364–8.
- [16] Hinton M. The diagnosis of salmonella abortion in cattle with particular reference to *Salmonella Dublin*. A review. *J Hyg (Lond)* 1977;79:25–38.
- [17] Bolin CA, Prescott JF. Leptospirosis. In: Howard JL, editor, Smith RA, Assoc. editor, *Current veterinary therapy food animal practice*, 4th ed. WB Saunders Co.; 1999. p. 352–8.
- [18] Ellis WA. Leptospirosis as a cause of reproductive failure. *Vet Clin N Am Food Anim Pract* 1994;10:463–78.
- [19] Miller R, Chelmonska-Soyta A, Smits B, Foster R, Rosendal S. *Ureaplasma diversum* as a cause of reproductive disease in cattle. *Vet Clin N Am Food Anim Pract* 1994;10:479–90.
- [20] Cardoso MV, Blanchard A, Ferris S, Verlengia R, Timenetsky J, Cunha RAF. Detection of *Ureaplasma diversum* in cattle using a newly developed PCR-based detection assay. *Vet Micro* 2000;72:241–50.
- [21] Walker RL. Bovine venereal Campylobacteriosis. In: Howard JL, editor, Smith RA, Assoc. editor, *Current veterinary therapy food animal practice*, 4th ed. WB Saunders Co.; 1999. p. 323–6.
- [22] Campero CM, Anderson ML, Walker RL, Blanchard PC, Barbano L, Chiu P, et al. Immunohistochemical identification of *Campylobacter fetus* in natural cases of bovine and ovine abortions. *J Vet Med B Infect Dis Vet Public Health* 2005;52: 138–41.
- [23] BonDurant BH, Anderson ML, Stott JL, Kennedy PC. Epizootic bovine abortion (foothill abortion). In: Threlfall WR, Youngquist RS, editors. *Current therapy in large animal theriogenology*. 2nd ed., WB Saunders Co.; 2007. p. 413–6.
- [24] King DP, Chen CI, Blanchard MT, Aldridge BM, Anderson M, Walker R, et al. Molecular identification of a novel deltaproteobacterium as the etiologic agent of epizootic bovine abortion (foothill abortion). *J Clin Microbiol* 2005;43:604–9.
- [25] Anderson ML, Kennedy PC, Blanchard MT, Barbano L, Chin P, Walker RL, et al. Histochemical and immunohistochemical evidence of a bacterium associated with lesions of epizootic bovine abortion. *J Vet Diagn Invest* 2006;18:76–80.
- [26] McClausland IP, Slee KJ, Hirst FS. Mycotic abortion in cattle. *Aust Vet J* 1987;64:129–32.
- [27] Walker RL. Mycotic bovine abortion. In: Youngquist RS, Threlfall WR, editors. *Current therapy in large animal theriogenology*. 2nd ed., WB Saunders Co.; 2007. p. 417–9.
- [28] Knudtson WU, Kirkbride CA. Fungi associated with bovine abortion in the northern plains states (USA). *J Vet Diagn Invest* 1992;4:181–5.
- [29] Foley GL, Schlafer DH. Candida abortion in cattle. *Vet Pathol* 1987;24:532–6.
- [30] Hill MWM, Whiteman CE, Benjamin MM, Ball L. Pathogenesis of experimental bovine mycotic placentitis produced by *Aspergillus fumigatus*. *Vet Pathol* 1971;8:175–92.
- [31] Ackermann M, Engels M. Pro and contra IBR-eradication. *Vet Microbiol* 2006;113:293–302.
- [32] Crandell RA. Infectious bovine rhinotracheitis. In: Howard JL, editor. *Current veterinary therapy food animal practice*. 2nd ed., WB Saunders Co.; 1986. p. 470–2.
- [33] Kelling CL. Viral diseases of the fetus. In: Youngquist RS, Threlfall WR, editors. *Current therapy in large animal theriogenology*. 2nd ed., WB Saunders Co.; 2007. p. 399–408.
- [34] Smith GH, Collins JK, Carman J. Use of an immunoperoxidase test for the detection of bovine herpesvirus-1 in aborted fetal tissue. *J Vet Diagn Invest* 1989;1:39–44.
- [35] Grooms DL. Reproductive consequences of infection with bovine viral diarrhea virus. *Vet Clin N Am Food Anim Pract* 2004;20:5–19.
- [36] Saliki JT, Dubovi EJ. Laboratory diagnosis of bovine viral diarrhea virus infections. *Vet Clin N Am Food Anim Pract* 2004;20:69–83.
- [37] Ellis JA, Martin K, Norman GR, Haines DM. Comparison of detection methods for bovine viral diarrhea virus in bovine abortions and neonatal death. *J Vet Diagn Invest* 1995;7:433–6.
- [38] Haines DM, Clark EG, Dubovi EJ. Monoclonal antibody-based immunohistochemical detection of bovine virus diarrhea virus in formalin-fixed, paraffin embedded tissues. *Vet Pathol* 1992;29: 27–32.
- [39] Baszler TV, Evermann JF, Kaylor PS, Byington TC, Dilbeck PM. Diagnosis of naturally occurring bovine viral diarrhea virus infections in ruminants using monoclonal antibody-based immunohistochemistry. *Vet Pathol* 1995;32:609–18.
- [40] Fulton RW, Hessman B, Johnson BJ, Ridpath JF, Saliki JT, Burge LJ, et al. Evaluation of diagnostic tests used for detection of bovine viral diarrhea virus and prevalence of subtypes 1a, 1b, and 2a in persistently infected cattle entering a feedlot. *J Am Vet Med Assoc* 2006;228:578–84.
- [41] Cornish TE, van Olphen AI, Cavender JL, Edwards JM, Jaeger PT, Vieyra LL, et al. Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. *J Vet Diagn Invest* 2005;17:107–10.
- [42] Kennedy JA, Mortimer RG, Powers B. Reverse transcription-polymerase chain reaction on pooled samples to detect bovine viral diarrhea virus by using fresh ear-notch-sample supernatants. *J Vet Diagn Invest* 2006;18:89–93.
- [43] Rhyan JC, Stackhouse LL, Quinn WJ. Fetal and placental lesions in bovine abortion due to *Tritrichomonas foetus*. *Vet Pathol* 1988;25:350–5.
- [44] Rhyan JC, Wilson KL, Burgess DE, Stackhouse LL, Quinn WJ. Immunohistochemical detection of *Tritrichomonas foetus* in formalin-fixed, paraffin-embedded sections of bovine placenta and fetal lung. *J Vet Diagn Invest* 1995;7:98–101.
- [45] Anderson ML, Barr BC, Conrad PA. Protozoal causes of reproductive failure in domestic ruminants. *Vet Clin N Am Food Anim Pract* 1994;10:439–61.
- [46] Anderson ML, Andrianarivo AG, Conrad PA. Neosporosis in cattle. *Anim Reprod Sci* 2000;60–61:417–31.

- [47] Dubey JP. Neosporosis in cattle. *J Parasitol* 2003;89:S42–56.
- [48] Dubey JP, Schares G. Diagnosis of bovine neosporosis (review). *Vet Parasitol* 2006;140:1–34.
- [49] Thurmond MC, Hietala SK. *Neospora caninum* infection and abortion in cattle. Howard JL, editor, Smith RA, Assoc. editor, Current veterinary therapy: food animal practice. WB Saunders Co.; 1999. p. 425–31.
- [50] Anderson ML, Reynolds JP, Rowe JD, Sverlow KW, Packham AE, Barr BC, et al. Evidence of vertical transmission of *Neospora* sp. in dairy cattle. *J Am Vet Med Assoc* 1997;210:1169–72.
- [51] Pare' J, Thurmond MC, Hietala SK. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. *J Parasitol* 1997;83:82–7.
- [52] Pare' J, Fecteau G, Fortin M, Marsolais G. Seroepidemiologic study of *Neospora caninum* in dairy herds. *J Am Vet Med Assoc* 1998;213:1595–8.
- [53] Schares G, Peters M, Wurm R, Bearwald A, Conraths FJ. The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analyzed by serologic techniques. *Vet Parasitol* 1998;80: 87–98.
- [54] Gay JM. Neosporosis in dairy cattle. An update from an epidemiological perspective. *Theriogenology* 2006;66:629–32.
- [55] Thurmond MC, Hietala SK, Blanchard PC. Herd-based diagnosis of *Neospora caninum*-induced endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. *J Vet Diagn Invest* 1997;9:44–9.
- [56] Thurmond MC, Hietala SK, Blanchard PC. Predictive values of fetal histopathology and immunoperoxidase staining in diagnosing bovine abortion caused by *Neospora caninum* in a dairy herd. *J Vet Diagn Invest* 1999;11:90–4.
- [57] Baszler TV, Gay LJC, Long MT, Mathison BA. Detection by PCR of *Neospora caninum* in fetal tissues from spontaneous bovine abortions. *J Clin Microbiol* 1999;37:4059–64.
- [58] Pare' J, Hietala SK, Thurmond MC. An enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of *Neospora* infection in cattle. *J Vet Diagn Invest* 1995;7:352–9.
- [59] Packham AE, Sverlow KW, Conrad PA, Loomis EF, Rowe JD, Anderson ML, et al. A modified agglutination test for *Neospora caninum*: development, optimization, and comparison to the indirect fluorescent-antibody test and enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol* 1998;5: 467–73.
- [60] Wouda W, Brinkhof J, van Maanen C, de Gee AL, Moen AR. Serodiagnosis of neosporosis in individual cows and dairy herds: a comparative study of three enzyme-linked immunosorbent assays. *Clin Diagn Lab Immunol* 1998;5:711–7116.
- [61] Bjorkman C, Uggla A. Serological diagnosis of *Neospora caninum* infection. *Int J Parasitol* 1999;29:1497–507.