

A clinical, evidence-based approach to infectious causes of infertility in beef cattle

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Abstract

Infertility is the diminished or absent capacity to produce viable offspring. Infections that reduce ovulation rates, fertilization rates, embryonic survival rates, fetal survival rates or perinatal survival rates result in observed infertility in beef cows. Reproductive pathogens include *Leptospira*, *Campylobacter*, *Hemophilus*, *Brucella*, bovine herpesvirus-1, bovine viral diarrhea virus, *Trichomonas foetus*, and *Neospora caninum*. Infectious infertility can be prevented or controlled with appropriate surveillance, biosecurity, and/or vaccination. The objective of this review is to briefly summarize current scientific information to assist with adoption of surveillance methods, implementation of biosecurity and selection of appropriate commercially available vaccines. © 2006 Elsevier Inc. All rights reserved.

Keywords: Infertility; Reproduction; Immunization; Vaccination; Cattle

1. Introduction

Infectious agents can be responsible for reduced ovulation rates, fertilization rates, embryonic survival rates, fetal survival rates, or perinatal survival rates. Reduction of any of these may result in observed infertility in beef herds. Notably, infection of bulls could reduce fertilization rates within herds, yet research regarding infectious infertility in bulls is extremely limited. Signs of reproductive disease may also be attributed to non-infectious causes such as genetic abnormalities, environmental toxins and physical trauma (Table 1). As diagnosis of an etiologic agent occurs in less than a majority of reproductive losses; consequently, vaccination practices are often instituted without a supporting laboratory diagnosis [1]. Unfortunately, this tendency to vaccinate without

diagnosis of reproductive pathogens can produce a waste of economic resources, an unrealistic sense of protection and a means of pathogen transmission [2]. Therefore, the objective of this manuscript is to briefly summarize current scientific information to assist with adoption of surveillance methods, implementation of biosecurity, and selection of appropriate commercially available vaccines.

2. Reproductive pathogens

2.1. *Leptospira*

In cattle, hardjo-bovis and -prajitno genotypes (i.e. sub-serovars) are responsible for host-adapted persistent infections, whereas other serovars cause sporadic infections. These host-adapted serovars cause insidious reproductive losses throughout gestation (abortion rates of 3–10%), yet are much more economically important than are incidental serovars. Moreover, infected cattle serve as a source of zoonotic infections for humans,

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Table 1
Causes of bovine abortion, categorized by etiologic classification

Bacterial causes
1. <i>Leptospira</i>
2. <i>Brucella abortus</i>
3. <i>Listeria</i>
4. <i>Campylobacter</i>
5. <i>Hemophilus</i>
6. <i>Ureaplasma</i> epizootic bovine abortion
7. <i>Mycoplasma</i>
8. <i>Chlamydia</i>
9. <i>Anaplasma</i>
10. <i>Salmonella</i>
Viral causes
1. IBR (Bovine herpes)
2. BVD
3. Bluetongue
4. Vesiviruses
5. Cache Valley virus
Protozoal causes
1. <i>Tritrichomonas</i>
2. <i>Neospora</i> spp.
3. <i>Sarcocystis neuroni</i>
Fungal causes
1. <i>Aspergillus fumigatus</i>
2. <i>Mucor</i> spp.
3. <i>Mortierella wolffii</i>
Toxic causes
1. Nitrates
2. Poison hemlock
3. Lupines
4. Tobacco
5. Ponderosa pine
6. Snakeweed
7. Locoweed
Other causes
1. Epizootic bovine abortion
2. Genetic
3. Environmental
4. Physical trauma

since cattle shed organisms in urine. Hardjo-bovis is maintained as a persistent infection in the proximal renal tubules of the kidneys and is associated with extensive shedding in urine. Hardjo-prajitno exhibits a predilection for the genital tract and is considered an important cause of infertility in the United Kingdom. Venereal transmission of hardjo-prajitno commonly occurs [3]. Hardjo-prajitno is also associated with clinical mastitis and a transient but significant decrease in milk production. Appropriate diagnostic samples include maternal serum (serology by macroscopic agglutination test [MAT]), fetal serum (serology by MAT), fetal kidney and fluids (immunofluorescence testing), maternal urine (culture, fluorescent antibody

testing, and PCR) and paired serum samples from at least 10 herdmates in contact with aborted material [4].

The MAT for antibodies has been the most common test for diagnosis of leptospirosis [5]. Antibody titers detected by MAT could not be correlated with leptospiuria [5]. In general terms, although antibody titers in the absence of vaccination can be useful in diagnosing leptospirosis, antibody titer peaks after infection also may not be detectable by MAT [5]. Research indicates that there is no value in examining paired serum samples from individual cows after abortion because titers are either falling or static at the time of abortion [6]. However, Ellis et al. reported that if serum from an unvaccinated aborting cow had a titer of >1000, then there was an 80% probability of fetal infection [6]. Up to 17% of infected fetuses may have MAT antibodies and titers as low as 10 may be significant [7].

Although darkfield microscopy (DFM) has been used for more than 50 years to demonstrate leptospires in urine and tissues, it requires a skilled microscopist and lacks sensitivity and specificity. Examination of fluid and tissues for leptospires has been significantly improved by fluorescent antibody tests (FAT) [5]. Unlike DFM, the FAT detects degenerated as well as intact leptospires with greater sensitivity and specificity [5]. However, FAT is currently unable to definitively identify the infecting serovar. Urine samples to be evaluated by FAT should be collected after furosemide administration, chilled (but not frozen) and shipped overnight to the diagnostic laboratory.

Testing of urine with PCR is more reliable than testing of tissues with PCR for leptospires. Most currently available PCR assays are not able to determine the infecting serovar [8]. Unfortunately, complex PCR assays may be prone to false-positive reactions due to their exquisite sensitivity. Thus, PCR results should be interpreted with full knowledge of the quality-control procedures used in the laboratory.

While whole-cell pentavalent bacterins historically contain hardjo-prajitno, pomona, grippotyphosa, canicola, and icterohaemorrhagiae (Table 2), hardjo-bovis is considered the most common isolate from dairy and beef herds in the United States. Pentavalent leptospiral vaccine provides limited protection from infection and reproductive losses in cattle due to hardjo-bovis [9]. Administration of common pentavalent leptospiral bacterins more than twice a year is unlikely to improve protection from reproductive loss due to hardjo [9]. Immunity is serovar specific, but not necessarily sub-serovar specific as a monovalent hardjo-bovis vaccine (Spirovac[®], Pfizer Animal Health, New York, NY,

Table 2

Nomenclature of pathogenic *Leptospira* in traditional pentavalent vaccines for cattle

Scientific name	Species	Serovar	Type	Common name	Found in traditional pentavalent vaccines
<i>L. borgpetersenii</i> serovar hardjo (type: hardjo-bovis)	<i>borgpetersenii</i>	Hardjo	Bovis	Hardjo-bovis	No
<i>L. interrogans</i> serovar hardjo (type: hardjo-prajitno)	<i>interrogans</i>	Hardjo	Prajitno	Hardjo-prajitno	Yes
<i>L. interrogans</i> serovar pomona	<i>interrogans</i>	Pomona		Pomona	Yes
<i>L. interrogans</i> serovar canicola	<i>interrogans</i>	Canicola		Canicola	Yes
<i>L. interrogans</i> serovar icterohaemorrhagiae	<i>interrogans</i>	Icterohaemorrhagiae		Icterohaemorrhagiae	Yes
<i>L. kirschneri</i> serovar grippityphosa	<i>kirschneri</i>	Grippityphosa		Grippityphosa	Yes

USA) and a monovalent hardjo-prajitno vaccine (Leptavoid[®], Schering-Plough Animal Health, Uxbridge, Middlesex, UK) have both conferred protective immunity in field studies with hardjo [10,11]. The reason that these two monovalent vaccines produce protective immunity while traditional pentavalent bacterins and other monovalent vaccines do not may be due to the strong cell-mediated immunity conferred by these vaccines [12]. A monovalent hardjo-bovis vaccine (Spirovac[®]) prevented renal colonization of hardjo-bovis for up to 1 year after vaccination (data on file, APHIS, USDA) [10]. Immunization will not eliminate urinary shedding in cattle with an ongoing, persistent, renal infection with hardjo-bovis. Injectable, long-acting oxytetracycline administered intramuscularly at 20 mg/kg, tilmicosin administered subcutaneously at 10 mg/kg, and multiple injections of ceftiofur administered intramuscularly are effective in eliminating persistent renal infection with hardjo-bovis in cattle [13]. Bulls that are persistently infected with hardjo-bovis may be difficult to cure as the organism persists in the seminal vesicles as well as the kidneys [13,14].

2.2. *Campylobacter fetus* subsp. *venerealis*

Infection of cattle with *Campylobacter fetus* subsp. *venerealis* usually causes temporary infertility or death of the late embryo/early fetus (30–70 days of gestation), but may cause sporadic abortions [14,15]. Abortions due to *C. fetus* subsp. *venerealis* have been recognized between the fourth and eighth month of gestation. Campylobacteriosis is characterized by repeat breeding and irregular estrous cycles in dairy cattle and small calf crops and prolonged calving seasons in beef cattle [16]. The agent is transmitted venereally in cattle and among bulls by contact with contaminated bedding [17]. Fertility usually returns 4–8 months after the initial infection. The organism may survive in the cervix

through a full gestation, but this is rather rare (<1%) [17]. When herd history reveals infertility, preputial smegma, semen, fetal fluid, placenta and vaginal discharge can provide a definitive diagnosis. Appropriate samples should be submitted in anaerobic transport-enrichment media (Weybridge, Cary-Blair, Clark's, etc.) as selected by the laboratory.

Several different *C. fetus* vaccines are available, including oil-adjuvanted and aluminum hydroxide-absorbed types. Bacterins in oil adjuvants have proven to be more effective and to provide longer lasting protection after a single dose [18]. In recent field trials in Argentina, two commercial aluminum hydroxide-absorbed *C. fetus* bacterins provided no significant protection against reproductive loss due to *C. fetus* [19]. Subcutaneous administration of a single dose of oil-adjuvanted vaccine for *Campylobacter* has proven efficacious in protecting heifers from reproductive losses associated with this bacterium. Unfortunately, oil-adjuvanted vaccines cause localized granuloma formation and fibrosis at the site of injection; these lesions may be objectionable in registered stock or show cattle.

Vaccination programs to prevent campylobacteriosis should include bulls as well as heifers and cows. Vibrin[®] (Pfizer Animal Health) is the only oil-adjuvanted *C. fetus* bacterin available in the United States that has been evaluated in bulls. Two 5-mL doses (2.5 times the dosage recommended for vaccination of cows) should be subcutaneously administered to breeding bulls at 4-week intervals, beginning 8 weeks before the start of the initial breeding season. Annual revaccination with this dose is recommended 4 weeks prior to breeding [20].

2.3. *Hemophilus somnus*

In cattle, *Hemophilus somnus* is associated with thromboembolic meningoencephalitis, polyarthritis,

respiratory disease, and reproductive infections [3]. This agent is thought to cause weak-calf syndrome or stillbirths more often than abortions. Lesions are associated with a necrotizing to necrosuppurative placentitis and changes in the fetal brain. This agent also plays a controversial role in causing infertility [3]. *H. somnus* is part of the normal bacterial flora of the male and female bovine genital tract. Therefore, pure cultures of this organism are considered necessary for incrimination as the etiology of abortion in the cow. Appropriate diagnostic samples include fetal fluid, placenta and vaginal discharge. Criteria for diagnosis include: (a) nearly pure culture in stomach content or tissues; (b) inflammatory lesions present; and (c) no other more likely cause for the abortion is found. While vaccination may improve fertility and decrease the number of abortions when pathology is appropriately attributed to this agent, no controlled research could be found to support anecdotal clinical reports.

2.4. *Brucella abortus*

Brucella abortus causes abortion in cattle, generally in the last half of gestation [3]. The Cooperative State Federal Brucellosis Eradication Program was launched in the United States in 1934. As of September, 2005, control programs have resulted in 48 states (plus Puerto Rico and the Virgin Islands) being free of brucellosis, whereas the remaining states, Wyoming and Texas, are certified as class A (less than 0.25% infected herds; USDA statistics). Thus, the disease is now of immediate practical importance in only a few areas of the United States.

Historically, a main component of the eradication program was calfhood vaccination with *B. abortus* strain 19 vaccine [21]. However, with that vaccine, differentiation between vaccinated and field strain-infected cattle was difficult. Therefore, *B. abortus* vaccine strain RB51 was developed to overcome the serologic problems associated with the strain 19 vaccine [21]. Animals vaccinated with RB51 easily can be distinguished from field-infected cattle due to lack of antibodies against the *O*-polysaccharide chain. The USDA-Animal, Plant and Health Inspection Service has designated strain RB51 as the official vaccine for cattle in the United States. Although strain RB51 is less abortifacient for cattle than strain 19, vaccination of pregnant heifers may result in abortion and subsequent zoonotic exposure if obstetrical assistance is necessary (Centers for Disease Control, Morbidity and Mortality Weekly Report, 13 March 1998) [21,22]. Thus, the vaccine is only to be administered to young heifers prior to pregnancy.

2.5. *Infectious bovine rhinotracheitis (Bovine herpesvirus-1)*

Bovine herpesvirus-1 is the most frequently diagnosed viral cause of abortion in North American cattle [1,23]. In an abortion storm, up to 60% of the herd may abort due to this virus. Abortions commonly follow the respiratory and conjunctival forms of the disease. Bovine herpesvirus-1 (BHV-1) carried in blood leukocytes can become localized in placental vessels. Subsequently, BHV-1 kills the fetus within 24 h after entry. The aborted tissues are uniformly dark red as a result of hemoglobin imbibition. Lesions within the fetus include foci of necrosis in the liver, spleen, adrenal glands, lung, and kidneys. Expelled fetuses can transmit virus to naïve cattle.

This virus may remain latent in the trigeminal and sacral dorsal root ganglia of infected animals [24]. Duration of the carrier state is probably for the life of the animal. This virus may also be associated with a necrotizing oophoritis with localized inflammation in the corpus luteum [25]. Pustular vulvovaginitis and balanoposthitis are clinical manifestations of BHV-1 infection that tend to remain localized in the genital tract [26]. Abortions due to infectious pustular vulvovaginitis-like virus subtypes of BHV-1 have been reported in some instances [27]. Bovine herpesvirus-1 may be transmitted via contaminated, cryopreserved semen.

The virus may be diagnosed with a fluorescent antibody test on fetal kidney or lesions in the fetal liver and adrenal. A four-fold rise in titer may be noted after abortion, but viral titers are seldom of help in diagnosis because the dam may be infected for months prior to aborting and may actually have a decreasing titer following abortion.

Vaccination can be effective in preventing disease but may not prevent infection or viral latency. Modified live-virus (MLV) vaccines containing BHV-1 were introduced in the United States in 1956 [16]. In spite of mounting evidence from the field, it was not until 1964 that manufacturers conceded that the MLV vaccine was not safe for use in cattle between the third and eighth month of gestation [16]. A MLV vaccine (Bovishield™, Pfizer Animal Health) is currently approved in the United States for administration to pregnant cattle provided they previously were inoculated with the vaccine prior to breeding [28]. Unfortunately, abortions may occur due to BHV-1 after administration of the licensed vaccine to previously vaccinated cows [28]. Use of modified live vaccines may also cause a temporary infertility due to follicular necrosis in seronegative cows. Administration of a modified live BHV-1 vaccine prior to breeding has

been shown to protect against abortions. In 1971, an intranasal MLV-IBR vaccine was developed that could be safely administered to cattle at any stage of reproduction [16]. Vaccination with a killed BHV-1 vaccine that includes a unique adjuvant also has been shown to protect against abortions due to BHV-1.

2.6. Bovine viral diarrhea virus (BVDV)

Thorough review articles and book chapters focus on reproductive effects of bovine viral diarrhea virus (BVDV) [29–32]. Timing of infection during gestation is a key determinant in whether pestiviral infection will cause birth defects or persistently infected animals. Eye, hair and neurologic abnormalities have been noted in infected fetuses. Virus usually is not present in fetuses or newborns that have sustained teratogenic viral damage. However, these calves do exhibit precolostral anti-BVDV antibodies. Validated assays (immunohistochemistry, antigen capture ELISA, virus isolation, and PCR) are available to diagnose BVDV in adult cattle, young calves and aborted fetuses [33].

To a degree, the importance of BVDV as a pathogen and the costs of living with it are manifested by the large number of licensed vaccines (>180) that have been available in the United States since 1964 [16,34]. While vaccination is an integral part of BVDV control programs in North America, vaccination has not provided complete protection of animals [35,36]. Vaccination of cows has been particularly inconsistent in protecting the developing fetus from infection, and the result can be the birth of offspring that serve as reservoirs and shed BVDV for life [31,37,38]. Other challenges for development of protective and safe vaccines are the wide antigenic diversity among field strains and the immune suppression associated with modified live vaccines [31]. Modified live vaccines also have resulted in infection of ovarian tissue and thus might negatively impact fertility if administration is inappropriately timed during the cycle of reproduction [39]. When using a MLV vaccine containing BVDV, one should realize that a normally avirulent MLV strain can cause notable disease when administered to immunosuppressed calves [40]. Unfortunately, vaccines can be inadvertently contaminated with BVDV during production or use on the farm [2,41,42] and undetected contamination of vaccines can cause severe disease [2,43].

2.7. *Tritrichomonas foetus*

Trichomonosis is an important disease of cows naturally bred by bulls [44]. *Tritrichomonas foetus*

colonizes the stratified squamous epithelial surfaces of the vagina, glans penis, and proximal portion of the prepuce and the mucosal surface of the uterus. Resulting inflammation of the vagina, cervix and endometrium produces a hostile intrauterine environment. This protozoan may cause early embryonic death or abortion from breeding up to 7 months of gestation, with the majority of reproductive loss at 50–70 days of gestation [15]. Abortions may be most common in older cows with partial immunity. The organism may be found in pyometral discharge, placental fluids or fetal stomach contents. Cows may remain infected with the organism for >150 days.

Control of trichomonosis has been based on testing all bulls prior to the breeding season [44]. Testing consists of microbial culture of aspirated preputial smegma that is immediately inoculated into a transport growth medium. Two or three culture attempts are necessary to ensure negative results [14]. Within infected herds, control of this pathogen may be achieved by using only 1–3 year-old-bulls. By 1 year after recovery, cows are susceptible to reinfection. There is a vaccine available for *T. foetus* (Trichguard[®] or Trichguard Plus[®], Fort Dodge Animal Health, Madison, NJ, USA). The vaccine stimulates cows to clear the infection in a matter of weeks (versus months in unvaccinated cows) [45]. No vaccine efficacy has been shown in bulls.

2.8. *Neospora caninum*

Accurate assessments of *Neospora*-induced reproductive losses in beef cattle are lacking [46]. Abortions due to *Neospora caninum* typically occur during the early second trimester, but may occur throughout gestation [47]. Transplacental transmission is common in endemic herds and the resulting vertical transmission is considered the major mode of bovine infection [46]. There are indications that younger cows are more efficient than older cows at vertically transmitting this pathogen [48].

Canids are the definitive host for this coccidian parasite which is not spread horizontally cow-to-cow. In 1998, domestic dogs were confirmed to be definitive hosts when they shed oocysts in their feces after consuming tissues of infected mice [49]. Environmentally resistant oocysts shed by a single dog (usually up to 500,000 oocysts) after consuming infected tissues from a single calf are potentially capable of infecting hundreds to thousands of cattle [50]. Oocyst excretion has also been documented from dogs that have consumed naturally infected bovine placenta [51]. Recently, coyotes were identified as definitive hosts of *N. caninum* [52]. In North American studies, the

seroprevalence in coyotes (10 and 11%) exceeded the seroprevalence in domestic dogs (7%) [49]. A spatial study in Texas showed that the risk of exposure to *N. caninum* in beef cattle increased in association with higher concentrations of coyotes and gray foxes and greater densities of beef cattle [53]. White tailed deer and wild brown rats have recently been identified as natural intermediate hosts of this coccidian parasite [49]. The sylvatic cycle of this pathogen among coyotes and white-tailed deer complicates measures for controlling *N. caninum* in beef cattle [54].

Lesions considered to be virtually diagnostic for *N. caninum* are non-suppurative encephalitis with foci of necrosis and gliosis, non-suppurative myositis, hepatitis, and most consistently myocarditis. Aborted fetuses usually are autolyzed or mummified. Protozoal tachyzoite cysts with a thick wall or free zoites in the brain parenchyma may be noted. In the California diagnostic lab, 18–19% of all submitted fetuses are diagnosed with *Neospora* infection [55]. Abortions due to this pathogen are more common in drylot dairies. There is a vaccine based on whole killed tachyzoites (Neoguard[®], Intervet, Millsboro, DE, USA) available for *N. caninum* [47]. Although maternal antibodies to *N. caninum* do not prevent fetal infection, administration of the vaccine has been associated with a statistical reduction in bovine abortions in areas where *N. caninum* is prevalent [47]. However, use of the vaccine does prevent the use of serology to detect infected animals.

3. Summary

The decision to vaccinate cattle to prevent infectious diseases of the reproductive tract should be based on: (a) appropriate diagnosis of reproductive pathogens; (b) expected outcome of natural infection; (c) safety of available vaccines; (d) analysis of the risk tolerance of animal health decision-makers; and (e) protection afforded by appropriate administration of the selected vaccine. This review has attempted to briefly summarize available information to assist in decision-making processes regarding pathogen-surveillance, biosecurity implementation, and vaccination. Optimal reproductive performance of beef cattle will result from thoughtful development and careful application of animal health programs.

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