Diagnosis and Control of Bovine Leptospirosis

Carole A. Bolin, DVM, PhD, Department of Pathobiology and Diagnostic Investigation
College of Veterinary Medicine, Michigan State University, East Lansing, MI, 48824.
Phone: (517) 355-8533 Email: bolinc@msu.edu

General Introduction

Leptospirosis is an economically important zoonotic bacterial infection of livestock that causes abortions, stillbirths, infertility, and loss of milk production. The disease occurs worldwide and is caused by infection with the spirochete *Leptospira*. The pathogenic leptospires were formerly classified as members of the species *Leptospira interrogans;* the genus has recently been reorganized and pathogenic leptospires are now identified in 7 species of *Leptospira*. Leptospiral serovars are recognized and approximately 200 different serovars of pathogenic *Leptospira* have been identified throughout the world. Serovars are identified based on antigens on the surface of the organisms.

In particular regions, different leptospiral serovars are prevalent and are associated with one or more maintenance host(s), which serve as reservoirs of infection (Table 1). Maintenance hosts are often wildlife species and, sometimes, domestic animals and livestock. Transmission of the infection among maintenance hosts is efficient and the incidence of infection is relatively high. Incidental hosts are not important reservoirs of infection and the incidence of transmission is low. Transmission of the infection from one incidental host to another is relatively uncommon.

Transmission of the infections among maintenance hosts is often direct and involves contact with infected urine, placental fluids, or milk. In addition, the infection can be transmitted venereally or transplacentally. Infection of incidental hosts is more commonly indirect, by contact with areas contaminated with urine of maintenance hosts. Environmental conditions are critical in determining the frequency of indirect transmission. Survival of leptospires is favored by moisture and warm temperatures and under these conditions the organism may persist for days to weeks outside of the animal; survival is brief in dry soil or at freezing or sweltering temperatures. Therefore, leptospirosis occurs most commonly in the spring, autumn, and early winter in temperate climates.

Table 1. Maintenance hosts of leptospiral serovars commonly found in the United States

Leptospiral Serovar	Maintenance Host(s)
Hardjo	cattle
Pomona	pigs, cattle, skunks, opossum
Bratislava	pigs, horses(?)
Canicola	dogs
Icterohaemorrhagiae	rats
Grippotyphosa	raccoons, skunks, opossum

Leptospires invade the body after being deposited on mucous membranes or damaged skin. After a variable incubation period (3 to 20 days), leptospires circulate in the blood. During this period, leptospires enter and replicate in many tissues, including the liver, spleen, kidneys, reproductive tract, eyes, and central nervous system. Agglutinating antibodies can be detected in serum soon after the leptospires are in the bloodstream. Appearance of circulating antibodies coincides with the clearance of leptospires from blood and most organs. Leptospires can remain in the kidney and urinary shedding may occur for weeks to many months after infection. In maintenance hosts, leptospires also may persist in the genital tract and, less commonly, in the cerebrospinal fluid and vitreous humor of the eye.

Bovine leptospirosis in the U.S. is most commonly caused by infection with leptospires belonging to serovar Hardjo. The prevalence of infection with other serovars of *Leptospira* in cattle varies with different husbandry conditions; serovars Pomona and Grippotyphosa are the other relatively common causes of bovine leptospirosis in the U.S.

Leptospirosis caused by infection with serovar Hardjo

Prevalence—The most common cause of leptospirosis among cattle throughout much of the world is infection with leptospires belonging to serovar Hardjo. Two serologically indistinguishable but genetically distinct types of serovar Hardjo have been identified: *Leptospira interrogans* serovar Hardjo (type hardjoprajitno) and *L borgpetersenii* serovar Hardjo (type hardjo-bovis). Serovar Hardjo type hardjo-bovis is common in cattle populations throughout the world; type hardjoprajitno is isolated primarily from cattle in the United Kingdom.

Reliable estimates of the prevalence of serovar Hardjo infections have not been available in the US because of the difficulty in establishing the diagnosis. In a recent study, we tested urine and serum from 15 cows in each of 44 dairy herds from four different regions of the U.S. Overall, at least one infected cow was detected in 59% of the herds tested and, in most cases, serologic results indicated that the likely infecting serovar was Hardjo. When serovar Hardjo infection becomes endemic within a herd or region, it is common to have 30 to 40% of the animals infected and shedding the organisms in their urine at any one time.

Clinical Signs—Infection by serovar Hardjo generally results in no or relatively mild acute clinical signs but produces a renal carrier state associated with long-term urinary shedding. Persistent infection of the male and female genital tract is also a prominent feature of serovar Hardjo infections. Clinical signs of serovar Hardjo infection in dairy cattle are subtle and generally involve decreased reproductive efficiency and milk production.

Abortions, stillbirths, or birth of weak calves occur as a result of serovar Hardjo infection but generally, are only seen when a cow is infected for the first time when she is pregnant. Abortions may occur many weeks after infection of the dam and are usually not associated with any obvious illness in the cow. Infected, but apparently healthy, calves also may be born and retention of fetal membranes may follow Hardjo abortion. Abortions due to serovar Hardjo infection tend to occur sporadically as opposed to abortion 'storms' which may occur as a result of infection with serovars Pomona or Grippotyphosa, for example.

Perhaps the most economically significant manifestation of serovar Hardjo infection is the result of persistent infection of the reproductive tract. Infertility which results in increased services per conception and prolonged calving intervals is associated with this infection. The precise pathogenesis of these events is not clearly understood but presumably the presence of leptospires in the uterus and oviducts of infected cows interferes with implantation of the embryo or other early pregnancy events.

Diagnosis of leptospirosis

Diagnosis of leptospirosis is dependant on a good clinical and vaccination history and the availability of diagnostic testing at a laboratory with experience in the diagnosis of leptospirosis. Coordination between the diagnostic laboratory and the veterinarian is required to maximize the chances of making an accurate diagnosis. It is advisable to contact the diagnostic laboratory prior to submission of samples to assure that appropriate samples are collected and that the samples arrive at the diagnostic laboratory in suitable condition. In addition, in problem situations, it may be necessary to consult reference or regional diagnostic laboratories, which have expertise in the diagnosis of this infection.

Diagnostic tests for leptospirosis can be separated into those designed to detect antibodies against the organism and those designed to detect the organism or its' DNA in tissues or body fluids of animals. Each of the diagnostic procedures, for detection of the organism or for antibodies directed against the organisms, has a number of advantages and disadvantages. Some of the assays suffer from a lack of sensitivity and others are prone to specificity problems. Therefore, no single technique can be recommended for use in each clinical situation. Use of a combination of tests allows maximum sensitivity and specificity in establishing the diagnosis. Serological testing is recommended in each case, combined with one or more techniques to identify the organism in tissue or body fluids.

***Serologic tests*—** The microscopic agglutination test (MAT) is the most commonly used technique for diagnosing leptospirosis in animals. Serology is inexpensive, reasonably sensitive, and widely available. The MAT involves mixing appropriate dilutions of serum with live leptospires of serovars prevalent within the region. The presence of antibodies is indicated by the agglutination of the leptospires.

Detection of high titers of antibody in animals with a disease consistent with leptospirosis may be sufficient to establish the diagnosis. This is particularly true in the investigation of abortions caused by incidental host infections in which the dam's agglutinating antibody titer is $\geq$800-1600. However, in maintenance host infections, particularly serovar Hardjo infection, infected animals often have a poor antibody response to infection. Often at the time of abortion, antibody titers may be quite low or negative against serovar Hardjo. In these cases, the herd serologic response to infection or detection of the organism in tissues or fluids are often more helpful than is the individual's antibody titer in establishing the diagnosis.

Interpretation of leptospiral serologic results is complicated by a number of factors. These factors include: cross-reactivity of antibodies, antibody titers induced by vaccination, and lack of consensus about what antibody titers are indicative of active infection. Antibodies produced in an animal in response to infection with a given serovar of *Leptospira* often cross-react with other serovars of leptospires. Therefore, a cow infected with a single serovar is likely to have antibodies against more than one serovar in an agglutination test. Patterns of cross-reactive antibodies vary widely between species of animals and between individuals within a species. However, in general, the infecting serovar is assumed to be the serovar to which that animal develops the highest titer.

Widespread vaccination of cattle with leptospiral vaccines also complicates the interpretation of leptospiral serology. In general, vaccinated cattle develop relatively low agglutinating antibody titers (100 to 400) to the serovars in the vaccine and these titers persist for one to three months after vaccination. However, some animals develop high titers after vaccination (particularly those vaccinated several times each year) and although these high vaccination titers decrease with time, they may persist for six months or more after vaccination. Introduction of new vaccines may also change the typical pattern of post-vaccination antibody titers.

The third complication of interpretation of leptospiral serological testing is caused by a lack of consensus as to what titer is "significant" for the diagnosis of leptospiral infection. An agglutinating

antibody titer of >100 is considered significant by many. However, this cut-off level may be exceeded in vaccinated animals and may not be reached in cattle infected with serovar Hardjo. Therefore, diagnosis of leptospirosis based on a single serum sample must be made with caution and with full consideration of the clinical picture and vaccination history of the animal. In cases of acute leptospirosis, a fourfold rise in antibody titer is often observed in paired serum samples. However, cattle are commonly actively infected and shedding serovar Hardjo with antibody titers $\leq$ 100. Leptospiral antibody titers are often steady or decreasing at the time of abortion and up to 50% of cows aborting due to serovar Hardjo will be seronegative at the time of abortion. Therefore, a low antibody titer does not necessarily rule-out a diagnosis of leptospirosis.

***Detection of leptospires*—**Other techniques available for the diagnosis of leptospirosis in livestock involve procedures to detect leptospires or leptospiral DNA in tissues or body fluids. Commonly used techniques include: Immunofluorescence (fluorescent antibody tests or FA) and polymerase-chain-reaction (PCR) assays. Organisms can also be cultured from infected animals but culture is expensive, takes many weeks, and is generally only available in reference laboratories.

Immunofluorescence can be used to identify leptospires in tissues, blood, or urine sediment. The availability of this test is increasing, and the test is rapid, has good sensitivity, and is very useful for screening urine samples or fetal tissues for leptospires. Interpretation of immunofluorescence tests may be difficult and some diagnostic labs may be more experienced with this test than others. The fluorescent antibody conjugate currently in general use is not serovar-specific; serologic examination of the animal is still required to identify the infecting serovar.

Polymerase chain reaction (PCR) tests can be used to detect leptospiral DNA in clinical samples. These tests rely on the PCR amplification of DNA in tissues or body fluids. A number of PCR procedures are available and each laboratory running the test may select a slightly different procedure that works well for them. In general, PCR testing of urine is more reliable than testing of tissues. PCR assays are able to detect the presence of leptospires but are not able to determine the infecting serovar. PCR can be a sensitive and specific technique for the diagnosis of leptospirosis. Unfortunately, the process is exquisitely sensitive to contamination with exogenous leptospiral DNA and, therefore, may be prone to false-positive reactions. It is very important that PCR results be interpreted with full knowledge of the quality control procedures used in the laboratory.

Control of leptospirosis

An optimal program to control bovine leptospirosis will prevent clinical disease and urinary shedding in animals exposed to a variety of leptospiral serovars. The most common approaches to the control of leptospirosis in cattle are based on prevention of exposure, vaccination, and selective treatment.

In all cases, efforts should be made to limit direct and indirect contact between cattle and carriers of leptospirosis (for example, by rodent control around buildings, fencing swampy ground or streams). In addition, adequate quarantine procedures should be undertaken to prevent introduction of Hardjo into a herd through purchase of infected animals. However, given the ubiquitous nature of wildlife that may be carriers of leptospirosis and the prevalence of serovar Hardjo infection in cattle, prevention of all exposure to leptospirosis is virtually impossible in most dairy and beef operations. Therefore, vaccination is relied upon to enhance resistance of the animals to infection with the serovars of *Leptospira* in the region.

Leptospiral vaccines currently available for use in cattle in the U.S. are all 5-way, killed, whole-cell vaccines containing serovars Pomona, Canicola, Icterohaemorrhagiae, Grippotyphosa, and Hardjo. These antigens are also available in combination with various other viral and bacterial vaccines. In

general, these leptospiral vaccines provide good protection against disease induced by each of the serovars except serovar Hardjo.

A series of experimental studies and field data from the United States showed that vaccination with leptospiral vaccines typical of those available in the United States, does not prevent renal infection, urinary shedding, or fetal infection with serovar Hardjo isolates from the U.S. (type hardjo-bovis). Field data on the prevalence of serovar Hardjo infection, even in well vaccinated herds, provides further evidence that the currently available vaccines are not providing good protection against serovar Hardjo. Many of the Hardjo vaccines available were licensed many years ago and were not subjected to rigorous efficacy trials using virulent strains of serovar Hardjo, routes of challenge which mimic natural exposure, and modern methods of determining if challenged cattle became infected with serovar Hardjo.

Recently, however, two serovar Hardjo vaccines have been extensively investigated using appropriate challenge strains and methods. In contrast to many other serovar Hardjo vaccines, these two products were shown to provide excellent protection against infection and shedding of serovar Hardjo. The studies establishing the efficacy of Leptavoid (Schering-Plough) were reported by Dr. Bill Ellis (Veterinary Science Division, Stormont, Northern Ireland) and those regarding Spirovac (CSL, Inc) were reported from my laboratory. One of these vaccines will soon be available for use in the United States (Spirovac, marketed by Pfizer) and will provide additional options for veterinarians and dairy producers in the U. S.

Why these two vaccines protect so well against serovar Hardjo and others do not is not entirely clear. Based on recent studies, it appears that Hardjo vaccines that do protect cattle from serovar Hardjo induce a strong and long lasting cell-mediated immune response in vaccinated animals. The cell-mediated immune response in vaccinated cattle is demonstrated by high levels of production of gamma-interferon by lymphocytes exposed to serovar Hardjo antigen in culture. Cattle naturally infected with serovar hardjo and cattle vaccinated with non-protective serovar hardjo vaccines do not demonstrate this cell-mediated immune response. These data suggest, but do not prove, that it is the cell-mediated immune response induced by these efficacious serovar Hardjo vaccines that is responsible for protection of cattle from serovar Hardjo. Clearly, more studies are needed to prove this hypothesis and to determine which antigens induce the cell-mediate immune response.

Traditional vaccination regimens for leptospirosis include 2 initial doses of vaccine, followed by annual vaccination of all cattle in a closed herd with 5-way vaccines, or twice yearly vaccination in an open herd. This regimen provides good protection against serovars Pomona, Grippotyphosa, Icterohaemorrhagiae, and Canicola—but not serovar Hardjo. At this time there is little evidence to indicate that use of the 5-way vaccines multiple times per year increases the protection against serovar Hardjo and vaccination 3 -5 times a year for leptospirosis has the potential to cause hypersensitivity problems in some animals. If the veterinarian and producer elect to use the new serovar Hardjo vaccine, it will still be necessary to use a 5-way vaccine as before. The new serovar Hardjo vaccine will require two initial doses followed by yearly boosters.

Antibiotics can be used to treat individual animals and will, in general, eliminate persistent Hardjo infections. Antibiotic treatment stops urinary shedding and is likely to improve clinical signs associated with persistent colonization of the reproductive tract with serovar Hardjo. However, it is not clear how long a 'cured' animal resists reinfection. Therefore, antibiotic therapy should not be relied upon as an overall control program for leptospirosis—usually antibiotic treatment is matched with a good vaccination program. Likewise, there is no evidence that vaccination (even with the new vaccines), will cure an already infected animal. Therefore a combination of approaches should be considered to efficiently bring the infection under control in a herd infected with serovar Hardjo. Long-acting oxytetracycline (20 mg/kg, IM, two doses 10 days apart) has been shown to be effective in the treatment of serovar Hardjo infections and is recommended.