Clinical Signs, Diagnosis, and Prevention of Bovine Leptospirosis

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Abstract

Bovine leptospirosis is an important cause of reproductive failure and zoonotic disease throughout the world. The disease is difficult to diagnose because of a lack of pathognomonic clinical signs and because of the inefficiency of diagnostic tests for this organism. Vaccination is the most common method chosen to prevent bovine leptospirosis. In this paper the clinical signs, diagnosis, and prevention of bovine leptospirosis are reviewed. Recent developments in the area of diagnostic tests and new data regarding the efficacy of vaccination are discussed.

Introduction

Leptospirosis is an economically important zoonotic bacterial infection of livestock that causes abortions, stillbirths, and loss of milk production. Many aspects of leptospirosis in farm animals are poorly understood, in part because of difficulty in diagnosis, complexity of the host-leptospire relationship, and changing patterns of infection.

Leptospirosis 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. 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 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, moderately warm temperatures (optimal around 28°C), and neutral or mildly stagnant water; survival is brief in dry soil or at temperatures less than 10°C or more than 34°C (1). Therefore, leptospirosis occurs most commonly in the spring, autumn, and early winter in temperate climates and during the rainy season in the tropics.

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.

Clinical signs

Clinical signs associated with leptospirosis vary and depend on the serovar and the host. In maintenance hosts, leptospirosis is generally characterized by a low serologic response, relatively mild acute clinical signs, and a prolonged renal carrier state which may be associated with chronic renal disease. In incidental hosts, leptospirosis can cause severe disease, is associ-

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ated with high titers of agglutinating antibody, and has a short or negligible renal carrier state. The clinical signs observed vary with the susceptibility of the host and with the infecting serovar. In general, young animals are more seriously affected than adult animals.

Serovars of major importance in cattle are hardjo and pomona in North America, South America, Australia, and New Zealand and hardjo in Europe. Illness due to other serovars is less common. Seroprevalence (agglutinating antibody titers ≥100) among cattle in the United States is estimated to be: 29% for serovar hardjo; 23% for pomona; 19% for icterohaemorrhagiae; and 11% for canicola (2). In recent years, infection with serovar hardjo has become increasingly recognized along with a decline in importance of serovar pomona infections.

Many leptospiral infections are subclinical, particularly in nonpregnant and nonlactating animals, and are detected only by the presence of antibodies or lesions of interstitial nephritis at slaughter. Acute or subacute leptospirosis is most commonly associated with incidental host infections and occurs during the leptospiremic phase of infection. Clinical signs associated with chronic infections are usually associated with reproductive loss through abortion and stillbirth. Chronic infection of the female genital tract also may be associated with infertility in cattle infected with serovar hardjo.

Uncommonly, severe acute disease occurs in calves infected with incidental serovars, particularly serovar pomona. Clinical signs include high fever, hemolytic anemia, hemoglobinuria, jaundice, pulmonary congestion, occasionally meningitis, and death. In lactating cows, incidental infections are often associated with agalactia with small quantities of blood-tinged milk. Recovery is prolonged.

The most common form of acute leptospirosis occurs in dairy cows as a transient pyrexia with a marked drop in milk production lasting for two to ten days. In this acute "milk drop syndrome," the milk has the consistency of colostrum, with thick clots, yellow staining, and high somatic cell count, and the udder has a uniformly soft texture. This condition occurs most commonly with serovar hardjo type hardjoprajitno infection but may be caused by other serovars. Leptospiral "milk drop syndrome" varies from an epizootic infection in a previously unexposed herd, involving over half the herd over a period of one or two months, to a more common endemic infection affecting cows in their first or second lactation. Recovery is usually in 10 days, without treatment, although cows in late lactation may dry off. A subclinical form of this "milk drop syndrome" may occur in hardjo-infected lactating cows in the absence of other clinical evidence of infection.

The chronic form of disease, most commonly associated with serovars hardjo and pomona, is associated with fetal infection in pregnant cows presenting as abortion, stillbirth, or birth of premature and weak infected calves. Infected but apparently healthy calves also may be born. Retention of fetal membranes may follow hardjo abortion. Abortion or stillbirth is commonly the only manifestation of infection but may sometimes be related to an episode of illness up to six weeks (pomona) or twelve weeks (hardjo) earlier. Serovar hardjo type hardjoprajitno appears to be more virulent than type hardjo-bovis (3,4).

Accurate data for the frequency of abortion due to hardjo and pomona are not readily available in North America. Abortion due to pomona has decreased in importance over the last decades, probably because of vaccination. Abortion and stillbirth due to hardjo are recognized more commonly. Hardjo is more important than is pomona because it causes endemic rather than more sporadic infection. In Northern Ireland, where the more virulent type hardjoprajitno occurs, hardjo was recognized as responsible for nearly half of all bovine abortions in one study (3). Type hardjoprajitno was isolated from the majority of aborted fetuses, whereas type hardjo-bovis was isolated mainly from the kidney and genital tract of carrier cows. In one large study in Canada, where type hardjo-bovis is prevalent, serovar hardjo caused about 6% of abortions; pomona abortions were not recognized (5). Hardjo infection are not commonly identified as a cause of reproductive failure in cattle in Australia and New Zealand (6). The infection is common in cattle and the incidence of human infections associated with infected cattle is relatively high.

Infertility, which has apparently responded to vaccination and treatment, has been described in hardjo-infected herds (7). Such infertility, which has not been well documented, may follow localization of leptospires in the uterus and oviduct of hardjo-infected cattle.

Diagnosis

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—Serologic assays are the most commonly used technique for diagnosing leptospirosis in animals. The microscopic agglutination test and various enzyme-immunoassays are the serologic tests most frequently used. Serology is inexpensive, reasonably sensitive, and widely available.

The microscopic agglutination test is available worldwide and 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.

Enzyme-immunoassays have been developed using a number of different antigen preparations and assay protocols. An assay which measures anti-leptospiral IgM is useful for detecting recent infection in livestock. Use of these assays is complicated in areas of the world where vaccination is common because some vaccinated animals develop IgM titers as well as IgG titers, thus giving positive results in the enzyme-immunoassays.

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 ≥1600. However, in maintenance host infections, particularly hardjo in cattle, infected animals often have a poor agglutinating antibody response to infection. Often, at the time of abortion, antibody titers may be quite low or negative in the maintenance host. In these cases, the herd serologic response to infection is often more helpful than is the individual's response in establishing the diagnosis. In abortion or stillbirth, it is often useful to do serologic testing on fetal serum, but dilutions should start at 1:10, in contrast to adult studies in which the usual starting dilution is 1:100.

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. In some cases, these patterns of cross-reactivity are predictable based on the antigenic relatedness of the various serovars of *Leptospira*. Unfortunately, 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. Paradoxical reactions may occur with the agglutination test early in the course of an acute infection, with a marked agglutinating antibody response to a serovar other than the infecting serovar.

Widespread vaccination of cattle with leptospiral vaccines in many parts of the world also complicates the interpretation of leptospiral serology. In general, cattle develop relatively low agglutinating antibody titers (100 to 400) in response to vaccination and these titers persist for one to three months after vaccination. However, some animals develop high titers after vaccination and although these high vaccination titers decrease with time, they may persist for six months or more after vaccination.

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 maintenance host infections. 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, maintenance hosts are commonly actively infected and shedding leptospires with antibody titers ≤100 (8). Therefore, a low antibody titer does not necessarily rule-out a diagnosis of leptospirosis. Antibody titers can persist for months following infection and recovery, although there is usually a gradual decline in the antibody titer with time.

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. These techniques include: darkfield microscopy, immunofluorescence, culture, histopathology with special stains, and polymerase-chain-reaction (PCR) assays. Each of these assays is useful in the diagnosis of leptospirosis and each presents special advantages and disadvantages for routine use.

Darkfield microscopy has been used as a rapid screening tool to identify leptospires in the urine of animals. The advantage of darkfield microscopy is speed; disadvantages include low specificity and sensitivity. Di-

rect visualization of the organisms is problematical, even for experienced personnel. Artifacts present in body fluids are difficult to distinguish from leptospires, even by experienced observers. The sensitivity of darkfield microscopy is low; approximately 10^5 leptospires/ml of urine must be present to be detected. It is also important to remember that leptospires are present in the urine to varying degrees with different serovars and are not usually present in urine in the early stages of acute disease. In general, darkfield microscopy, in experienced hands, can be useful to make a preliminary positive diagnosis of leptospirosis but should not be relied on to make a definitive diagnosis or to eliminate leptospirosis from the differential diagnosis.

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 can be used on frozen samples. Interpretation of immunofluorescence tests may be difficult and requires a skilled laboratory technician. The fluorescent antibody conjugate currently available for general use is not serovar-specific; serologic examination of the animal is still required to identify the infecting serovar. Serovar-specific fluorescent antibody conjugates have been prepared and are in use in Canada and some research laboratories.

Bacteriologic culture of blood, urine, or tissue specimens is the definitive method for the diagnosis of leptospirosis. Leptospiremia occurs early in the clinical course of leptospirosis and is usually of short duration and low level. Therefore, blood is only useful for culture in the first few days of clinical illness and prior to antibiotic therapy. Leptospires are usually present in the urine of animals ≥10 days after the onset of clinical signs. Urine for culture should be collected after injection of furosemide (9). Furosemide increases the glomerular filtration rate and "flushes" more leptospires into the urine and produces dilute urine, which enhances survival of the leptospires. Urine, blood, and tissue samples for culture should be diluted in 1% bovine serum albumin transport medium (10) as soon as possible after collection. Culture of leptospires is difficult, time-consuming, and requires specialized culture medium. However, isolation of the organism from the animal allows definitive identification of the infecting serovar. Diagnostic laboratories rarely culture specimens for the presence of leptospires. However, a few laboratories with a particular interest in leptospirosis can conduct such testing and may be consulted if leptospiral culture is required.

The use of special stains in histopathology can be effective for identification of leptospires in animal tissues. This common diagnostic technique is the only one that can be used on formalin-fixed tissues. Tissues to be examined include kidney in adults and placenta, lung, liver, and kidney in the case of abortions. Leptospires are not visible in tissues using routine stains, but characteristic inflammation can be observed in affected kidneys; hepatic lesions are less specific. Application of silver stains or immunohistochemical stains to tissue sections will allow detection of leptospires or leptospiral antigens in the renal tubules and interstitium of the kidney, liver, lung, or placenta. Low sensitivity is a disadvantage of this diagnostic technique. Leptospires are often present in small numbers in affected tissues, particularly in chronic leptospirosis. The infecting serovar cannot be determined by histopathology; serologic studies must also be conducted.

Techniques have been developed recently that allow detection of leptospiral DNA in clinical samples. These tests include DNA probe tests which detect leptospiral DNA directly and tests which rely on the PCR amplification of DNA in tissues or body fluids. In general, DNA probes are not used because of a lack of sensitivity and technical difficulties in their use. PCR tests, however, are being used for the diagnosis of leptospirosis in animals (11, 12). 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. Processing of tissue samples is more difficult and tissues often contain inhibitors to the amplification reaction and, therefore, may cause false-negative results. Most 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 complex and 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.

Treatment

Animals with acute leptospirosis can be treated with streptomycin (12.5 mg/kg twice daily for three days) or tetracycline (10 to 15 mg/kg twice daily for three to five days). Streptomycin treatment can be combined with ampicillin or large doses of penicillin G. Leptospires also are highly susceptible to erythromycin, tiamulin, and tylosin, although these antibiotics cannot be relied on to remove the renal carrier state. A single dose of streptomycin (25 mg/kg) will usually remove the chronic renal carrier state caused by pomona or other serovars; chronic hardjo type hardjoprajitno infections may resist this treatment regimen (13). **Streptomycin is no longer available for use in the United States.** Injectable, long-acting oxytetra-

cycline at a dose of 20 mg/kg or amoxycillin (14) with two injections (48 h apart) at a dose of 15 mg/kg may be substituted for streptomycin to treat chronic infections.

Control

The goals of programs to control leptospirosis vary in different parts of the world. In some areas, leptospirosis is a significant cause of morbidity and losses within the cattle population and control programs are instituted to reduce these losses with an emphasis on prevention of clinical disease. In other regions, e.g. New Zealand and The Netherlands, animal disease caused by infection with serovar hardjo is less of a problem, but the incidence of human infections with this agent is unacceptably high. In these circumstances, control programs are initiated in cattle to control leptospirosis in human beings with an emphasis on preventing cattle from shedding the organism in urine. Clearly, institution of an optimal program to control bovine leptospirosis will accomplish both major goals of preventing urinary shedding and preventing clinical disease.

Leptospirosis can be eradicated from a herd or a region by a combination of progressive identification of carriers and antibiotic treatment. However, this approach depends on tight controls regarding the introduction of new animals and is often not possible because of husbandry conditions. The disadvantage of eradication is that it leaves the herd open to infection by leptospires introduced by livestock or wildlife maintenance hosts.

Control is 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 incidental infections (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 or pomona into a herd through purchase of infected animals.

Vaccines—Immunity is serovar specific. Polyvalent vaccines containing common serovars endemic to the host and region are generally available. Sometimes, vaccines are used that are manufactured in another region of the world and may not provide protection against serovars prevalent locally. In addition, the vaccines may contain serovars not commonly associated with disease in the region. Different vaccines vary in efficacy and vaccine failures may occur.

Annual vaccination of all cattle in a closed herd with appropriate bacterins, or twice yearly vaccination in an open herd, is the most effective approach to control. Newly introduced cattle should be treated with dihydrostreptomycin (25 mg/kg IM, two doses 10 days apart), long-acting oxytetracycline (20 mg/kg, IM, two doses 10 days apart), or amoxycillin (15 mg/kg, two doses 48 hours apart) for elimination of most chronic renal infection and vaccinated before they enter the herd. Vaccination can be combined with antibiotic treatment in the face of an outbreak. Calves should be four to six months or older before vaccination and should be vaccinated twice with four weeks between vaccinations. Vaccination thereafter is with a single dose annually. Because of the short-lasting, low-titer, agglutinating antibody response, annual vaccination with most available vaccines will progressively reduce and eventually abolish the herd seroprevalence of leptospirosis. In an infected animal, vaccination will not reduce urinary shedding but often considerably increases the antibody titer. Persistent low-titer reactions, which may last for years, may prevent bulls from entering studs or cattle from being exported. Treatment often does not abolish these titers. Because of the low sensitivity of the agglutination test for detecting hardjo carriers, international recommendations (Internal Zoo-Sanitary Code, Office International des Epizooties) for importation of livestock suggest reliance on antibiotic treatment before any movement, rather than on serologic testing. Regulations generally should be changed for bull studs or export requirements to allow control by the combination of vaccination and antibiotic treatment rather than by the use of serologic tests to detect carriers.

Vaccination with incidental serovars usually gives excellent protection against challenge. Field evidence has shown that hardjo vaccination reduces reproductive losses due to hardjo infection as well as leptospiruria (15, 16, 17). However, a series of experimental studies and field data in the United States has shown that vaccination with leptospiral vaccines typical of those available in the United States does not prevent renal infection, urinary shedding, or fetal infection with hardjobovis (18, 19, 20). However, a similar study conducted with a serovar hardjo vaccine manufactured in Australia (CSL Limited) showed good protection against infection and urinary shedding following challenge with U.S. isolates of serovar hardjo. The reasons for differences in efficacy of serovar hardjo vaccines may vary as a result of vaccine composition, husbandry conditions, and type and pathogenicity of serovar hardjo strains prevalent in the region.

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Abstract

Human exposure to *Mycobacterium paratuberculosis* via pasteurised milk: A modelling approach

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Paratuberculosis is a disease of cattle caused by infection with *Mycobacterium paratuberculosis*, and it has been suggested that this bacterium may also play a role in the aetiology of Crohn's disease in humans. *M paratuberculosis* is shed in the milk and may be able to survive pasteurisation. Therefore, people may be exposed to it by the consumption of pasteurised milk. The risk of such exposure has been analysed using a modelling approach and the model has been used to evaluate the effects of intervention measures at different points in the potential route of transmission. On the basis of data from the literature and expert opinion, an initial point estimate of the exposure level of about 0·5 cfu/litre pasteurised milk was derived, mainly due to milk from clinically affected animals. The model indicates the need for quantitative data on variations in the shedddding rates of *M paratuberculosis* in faeces and milk, and the levels of faecal contamination of milk. Such data are essential for a proper analysis of potential exposure, and may result in a 100-fold increase in the estimated median level of exposure.