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LEPTOSPIROSIS IN CATTLE: DISEASE REVIEW AND UPDATE

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EPIDEMIOLOGY AND CLINICAL SIGNS

Leptospirosis is an economically important zoonotic bacterial infection of livestock that causes abortions, stillbirths, infertility, 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. 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.

Leptospiral 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. The most common cause of leptospirosis among cattle in the United States and throughout much of the world is infection with leptospires belonging to serovar Hardjo for which they are the maintenance host. 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.

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 associated 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.

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 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 infection with serovar Hardjo type hardjo-bovis as well. 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 serovar Hardjo, is associated with fetal infection in pregnant cows presenting as abortion, stillbirth, or birth of premature and weak infected calves. Infected but healthy calves also may be born. 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.

Infertility, which has responded to vaccination and treatment, has been described in Hardjo-infected herds. Such infertility which is characterized by increased services per conception and prolonged calving intervals follows localization of leptospires in the uterus and oviduct of Hardjo-infected cattle.

DIAGNOSIS

Diagnosis of leptospirosis is dependent 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 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. These assays are sensitive and specific and have been used extensively in Europe on both serum and milk. However, ELISA tests are of little value in areas of the world (such as the U.S.) where vaccination is common. In a recent study in the U.S., virtually all milk and serum samples from vaccinated cattle were positive by the ELISA test.

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 Hardjo infections. Therefore, diagnosis of leptospirosis based on a single serum sample must be made with caution and with consideration of the clinical picture and vaccination history. 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 . 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. Direct 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 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. 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.

SUMMARY

The most common cause of bovine leptospirosis in the U.S. is serovar Hardjo. Recent prevalence studies indicate that as many as 50% of dairy herds in the U.S. contain animals infected with this organism. Prevalence studies in beef herds are underway. Cattle are the maintenance host for serovar Hardjo and the organism colonizes the renal and genital tracts of infected animals commonly resulting in urinary shedding of the organism and reproductive sequelae. Diagnosis of serovar Hardjo infection is difficult and requires a combination of approaches. Serology alone often fails to identify animals infected with serovar Hardjo as seronegative shedders are common in infected cattle herds. The recommended diagnostic testing strategy includes the primary use of a test (FA or PCR) to detect the organism in the urine of a sample of cattle in the herd followed by serological testing to provide insight into the likely infecting serovar of *Leptospira*.