Leptospira interrogans Serovar *hardjo* Infection of Cattle

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Leptospirosis is a worldwide zoonotic disease caused by infection with the spirochete bacterium *Leptospira interrogans. L. interrogans* is further divided on the basis of antigenic relatedness into serovars. Approximately 200 different serovars of *L. interrogans* have been identified and within a geographic area, certain serovars are prevalent and become adapted to a particular maintenance host (Table 1). In the United States the serovars of *L. interrogans* most commonly associated with bovine leptospirosis are *hardjo, pomona* and *grippotyphosa.* Serovars *icterohaemorrhagiae, canicola,* and *bratislava* are also occasionally associated with leptospirosis in cattle.

Table 1. Serovars and maintenance hosts of *L. interrogans* found in U.S. swine.

Serovar	Maintenance Host
hardjo	cattle
pomona	swine, cattle, skunks
grippotyphosa	raccoons, opossums
icterohaemorrhagiae	rats
canicola	dogs
bratislava	swine, horses(?)
ballum	mice

The clinical signs associated with leptospirosis vary with the host and infecting serovar. In general, a disease associated with infection of the maintenance host is characterized by a low serological response, rapid transmission from animal to animal, relatively mild clinical signs which are usually associated with transplacental infection in pregnant animals, and a prolonged renal carrier state (Table 2). In incidental hosts, leptospirosis can be a severe disease, is associated with high titers of agglutinating antibody, and has a short or nonexistent renal carrier state. The major source of economic loss to the livestock industry associated with leptospirosis is caused by abortions, stillbirths, birth of weak neonates, agalactia, and infertility.

Leptospires invade the host after being deposited on mucous membranes or damaged skin. After an incubation period of variable length (3 to 20 days), leptospires circulate in the blood. During the period of leptospiremia, leptospires enter and replicate in many tissues including the liver, reproductive tract, spleen, kidneys, eye, and nervous system. Agglutinating antibodies can be detected in serum soon after the period of leptospiremia. Appearance of circulating antibodies coincides with clearance of leptospires from blood and most organs. Leptospires may remain in the kidneys and reproductive tract. In maintenance hosts, chronic infection of the kidney and reproductive tract occurs and leptospires are shed in urine for months after infection.

Table 2. Features of leptospirosis in maintenance and incidental hosts.

Maintenance Host	Incidental Host
often subclinical	acute, severe disease
young and pregnant affected	all ages affected
long term shedding	short term shedding
low titers of antibody	high titers of antibody
difficult to diagnose	easily diagnosed

The mechanisms whereby leptospires cause disease and tissue damage are not fully known. Ability to actively penetrate intact mucous membranes, tissues, and blood vessels is likely important for virulence of this organism. A hemolysin has been described and genes coding for a hemolysin have been cloned from serovars *pomona* and *hardjo.* The exact role of hemolysin in the pathogenesis of leptospirosis is not clear. A cytotoxin has been identified in association with leptospires. Ability to evade the host immune response is another potential virulence factor for leptospires. In maintenance hosts, leptospires colonize the kidneys and often the genital tract and can persist in these locations for months to years, despite the presence of agglutinating antibody. Why this occurs is not clear, but may be related to the immunologically privileged nature of these sites or antigenic variation of the infecting leptospires.

Infection with serovar *hardjo* is the most common cause of bovine leptospirosis in the U.S. Estimates of the prevalence of serovar *hardjo* infection in cattle vary depending on the area of the country and the methods used to identify infection. On the basis of microbiologic and serologic data, the prevalence of serovar *hardjo* infection of cattle is estimated to be 2 to 15%. In certain locations,

prevalence rates of > 30% have been reported.

Two types of serovar *hardjo* have been defined on the basis of genetic differences. Type hardjoprajitno is the reference stain for serovar *hardjo,* has only been isolated from cattle in Europe, and is used to formulate the *hardjo* component of leptospiral vaccines used in the U.S. Type hardjo-bovis, has been isolated from cattle worldwide and is the only type of serovar *hardjo* that has been isolated from cattle in the U.S.

The clinical signs of serovar *hardjo* infection in cattle vary. Most often the infection is persistent and clinically inapparent. Acute disease caused by *hardjo* is rare in the U.S. and is characterized by fever, anorexia, agalactia, and in some cases mastitis. Cattle which become infected during pregnancy may have late-term abortions, stillborn or weak calves, or clinically normal, infected calves. A syndrome of infertility characterized by failure to settle or delayed return to heat is also described and may occur in cows infected near the time of breeding or in those with persistent infection of the reproductive tract.

Diagnosis of leptospirosis in cattle caused by serovar *hardjo* is difficult. This is because infected animals often develop low antibody titers to the organism and the leptospires are difficult to isolate or identify in infected animals. The advantages and disadvantages of the currently available diagnostic techniques are summarized in Table 3. Numerous approaches have been investigated to improve diagnosis of leptospirosis in cattle and swine. These include development of enzyme immunoassays to detect antibodies against leptospires, improved procedures for culture, and nucleic acid probes. Most of these newer diagnostic tests are only available in a few laboratories.

Table 3. Diagnostic tests for bovine leptospirosis.

Test	Sens	Spec	Speed	Cost	Availability
Serology	+ +	+ +	+ + +	+ + +	+ + + +
FA	+ +	+ / -	+ + +	+ +	+ +
Culture	+ + +	+ + +	-	-	+ / -
DF Microscopy	+	-	+ + +	+ + +	+ +
Histopathology	+	+	+ +	+ +	+ + + +

Serology is the most commonly used technique to identify animals infected with *L. interrogans.* Serology is inexpensive, reasonably sensitive, and widely available. However, vaccination and cross-reacting antibodies can interfere with interpretation of serological results. A particular problem with serovar *hardjo* infections in cattle is that the serologic response of the animal is often poor and some infected animals have antibody titers below that detected by typical serologic tests. ELISA tests have been developed to detect antibodies against serovar *hardjo* but this test is not available in diagnostic laboratories.

Fluorescent antibody tests are available to identify leptospires in tissues or body fluids of cattle. The availability of this test is increasing and the test can be used on frozen tissues. However, the fluorescent antibody test is optimally sensitive and requires considerable expertise to interpret. In addition, the fluorescent antibody conjugate currently available is not serovar specific, and therefore serologic examination of the animal is still required to determine the infecting serovar.

Histopathology with the use of silver stains is a useful technique to identify leptospires in animal tissues. This is the only routinely used diagnostic technique which can be used on formalin-fixed tissues. However, histopathology is insensitive and the infecting serovar cannot be determined.

Isolation of serovar *hardjo* is the definitive method of diagnosis and allows identification of the infecting serovar. Unfortunately, culture of leptospires is expensive, time-consuming, and difficult. Culture of tissues to detect leptospires is rarely available at diagnostic laboratories.

Coordination between the diagnostic laboratory and the veterinarian is required to maximize the chances of making an accurate diagnosis of serovar *hardjo* infection. It is advisable to contact the 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 herds, it may be necessary to consult reference or regional diagnostic laboratories which have expertise in the diagnosis of this infection.

Vaccination with killed-whole-cell vaccines containing 5 serovars of leptospires is the major method used to control leptospirosis in cattle in the U.S. In general, vaccines are effective in preventing leptospirosis in cattle caused by serovars *icterohaemorrhagiae, grippotyphosa* and *pomona.* Vaccines to prevent infection and disease caused by host adapted leptospires appear to be less effective. Vaccines to protect cattle from infection with serovar *hardjo* have a very short (< 2 months) duration of immunity. Field data in the U.S. suggests that *hardjo* vaccines are not providing optimum protection of cattle from infection with serovar *hardjo.* Improvement in efficacy of *hardjo* vaccines is necessary. Cattle in areas that have a high incidence of *hardjo* infection should be vaccinated 3 to 4 times per year. In other areas of the country, two vaccinations annually may be sufficient.

Antibiotic treatment is another approach to control of serovar *hardjo* infection. The organism is sensitive to a variety of antibiotics and antibiotic treatment may assist in the elimination of clinical signs. However, antibiotic therapy is relatively ineffective in eliminating the carrier state in cattle infected with serovar *hardjo.* The most commonly used antibiotics are dihydrostreptomycin, penicillin, and oxytetracycline.

In herds in which *hardjo* infection is endemic, the incidence of abortions, weak calves, etc., can be reduced by assuring that heifers are exposed to the older cows and therefore, exposed to *hardjo,* prior to breeding. In some herds, however, this may lead to breeding difficulties associated with a persistent infection of the reproductive tract. Another disadvantage to this approach to management of

hardjo infection in a herd is that infected cattle may remain persistently infected and shed *hardjo* in their urine for long periods of time serving as a reservoir of infection for other animals and humans.

References

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