BOVINE LEPTOSPIROSIS. A REVIEW

L. E. HANSON
Department of Veterinary Pathology and Hygiene,
University of Illinois, Urbana

SUMMARY

Bovine leptospirosis is a disease of cattle caused by infection with motile filamentous bacteria belonging to the genus Leptospira. Leptospira pomona is the most prevalent species involving both cattle and swine in the United States.

Leptospira are readily destroyed by heat, desiccation, and most chemicals, but can survive for extended periods in stream and pond water. Moving streams provide a ready mode for dissemination, since skin contacts with urine-contaminated water apparently result in the most frequent route of infection.

Fever, anemia, mastitis, and abortion are the most characteristic signs of leptospirosis in infected cattle. The kidney is affected most consistently, with the development of an interstitial nephritis.

Laboratory diagnoses of leptospirosis may be accomplished by isolation of the organism in artificial media and laboratory animals, by demonstration of organisms in kidney and liver tissues with silver stains, or by demonstration of antibodies with various serological tests. Most diagnostic laboratories routinely perform serological tests.

Although some antibiotics aid in the control of leptospirosis, treatment to be effective, must be started early in the course of the disease. Vaccination is widely practiced in all areas of the United States. Experimental studies with the various vaccines available demonstrate that a definite but not complete or durable protection is provided by these vaccines.

Leptospirosis was first recognized in Russia as a disease of cattle in 1935 (46). Nine years later, Jungherr (39) described the disease and demonstrated the organism in cattle tissues in the United States. Baker and Little (5) isolated the first organisms from American cattle 4 yr. later.

Bovine leptospirosis is now recognized as one of the major cattle diseases of the United States. The disease was estimated to be responsible for an annual loss to the cattle industry of over $100,000,000 in 1954 by the U. S. Department of Agriculture (68). Obvious losses result from deaths, abortions, and decreased milk production. Less obvious losses occur in weak calves which gain weight very slowly.

CAUSATIVE AGENTS

The spirochetes responsible for leptospirosis are highly motile, filamentous organisms which appear to be beaded, due to their tightly twisted spiral shape. An axial filament runs lengthwise through the organism, providing some rigidity. Both ends of the filament are usually bent. A characteristic type of motility is produced by the spinning of the filament along its long axis. The numerous species of Leptospira can not be distinguished on the basis of morphology. The metabolic reactions frequently used to classify most bacteria are of no value, since leptospires fail to utilize the common nutrients of test media (32, 61).

Classification is, therefore, based on variations in antigenic structures as determined by the agglutination-lysis test. When placed in contact with the
homologous serum, leptospires are either agglutinated or lysed. The presence of 10% or more of the serum titer following adsorption with the heterologous serum strain indicates that the strain belongs to a different species (78).

More than 50 serotypes or species have been recognized at the present time (3). In the United States, five species either have been isolated from cattle tissue or their antibodies detected in cattle serum. *Leptospira pomona* is the most widespread species in the United States and is responsible for the major losses due to leptospirosis. *Leptospira canicola* was isolated from a two-day-old calf in Alabama (67) and was incriminated in another case (75, 77). Three other species, *Leptospira sejroe*, *Leptospira grippotyphosa*, and *Leptospira icterohaemorrhagiae* have been detected serologically, but their isolation has not been reported from cattle.

*L. pomona* was first isolated in Australia from a person with seven-day fever in 1937 (16), and later from swine in 1939 (38) and from cattle in 1949 (65). In addition, this organism can cause disease in sheep (6), goats (48), and horses (60). Although swine appear to be the primary carriers (72), transmission may occur directly between cattle and also apparently from cattle to sheep (6). Recently, McKeever *et al.* (45) isolated *L. pomona* from raccoons, skunks, and wildcats in Georgia, and Borg-Petersen (7) from field rodents in Denmark. The presence of this organism in these animals suggests that they may be a reservoir which could play an important role in initiating outbreaks in domestic animals. Antibodies have been detected in the sera of an appreciable number of deer in various areas of the United States (24, 62, 76), but the possible role of deer as disseminators has not yet been determined.

Several workers (26, 58) have detected significant levels of *L. grippotyphosa* and *L. sejroe* antibodies in the serum of American cattle. These species have not been reported from cattle. Although signs of leptospirosis have usually not been associated with the presence of *L. sejroe* serum antibodies (26), abortion, reduced milk flow, and loss in weight were associated with the development of *L. sejroe* and *L. grippotyphosa* antibodies in cattle in Illinois (4). *L. grippotyphosa*, however, has been isolated from the raccoon in Georgia (45).

The members of the genus *Leptospira* can be cultivated in liquid, semisolid, and solid media. Cultivation requires a medium enriched with 10 to 15% mammalian serum. Rabbit serum is most commonly used. Various liquid media have been developed for the propagation of *Leptospira* (13, 64). Semisolid media are used in some laboratories for maintenance of cultures, since the organisms multiply more slowly than in the liquid cultures, making fewer transfers necessary. The organisms can be cultivated to a limited degree on solid media (19). The colonies grown on solid media show some variation in morphology. Temperatures of 28 to 29°C appear to be optimum for growth, while temperatures above 30°C cause inactivation and death (14). Pasteurization temperatures, therefore, render milk safe for human consumption.

Although leptospires are easily destroyed by heat, sunlight, desiccation, chemical disinfectants, and strong acids and bases, they can survive in relatively wide ranges of environmental conditions. A moist environment with moderate
temperatures is best. Considerable variation in survival time has been reported in many types of environment. Surface waters in streams and ponds apparently provide the most common media of dissemination and, therefore, have been studied extensively. Van Thiel (73, 74) detected virulent organisms for as long as 22 days in surface water. Leptospires were detected for seven days in nonsterile river water and for 94 days in Seitz-filtered river water by mouse inoculation (50). Okazaki and Ringen (50) detected live organisms for as long as 183 days in water-saturated soil. The pH of the water is important as demonstrated by Chang et al. (15). They found that *L. icterohaemorrhagiae* would survive in fresh water for only 28 hr. at pH 5, but would survive for as long as 30 days at pH 7. Its survival time was reduced to 18 to 20 hr. in salt water (15).

Kirschner and McGuire (41) observed that *Leptospira hyos*, *L. icterohaemorrhagiae*, and *L. pomona* were lysed by milk from cows, goats, and man. The antileptospiral effect of the milk was not reduced by storage, pasteurization, or heating for 5 min. at 80° C.

The survival time of leptospires in the urine of animals and man has been studied by various workers. Davidson and Smith (21) found that *L. icterohaemorrhagiae* could survive as long as 6 hr. in the urine from persons not exposed to Leptospira. Van der Hoeden (69) found specific agglutinins and lysins in the urine of infected men, dogs, and rats.

**PATHOGENESIS**

Leptospires may enter the body of an animal by several routes. Penetration of the abraded skin of feet and legs in wading of streams, field ponds, and marshy areas is probably the most frequent portal of entry in cattle. The organisms can also enter the mucous membranes of the eyes, nose, and the mouth.

The organisms multiply quite rapidly in the blood stream after penetration of the skin. The incubation period is three to seven days. Elevation of body temperature varying from 103 to 107° C. is usually the first detectable sign of leptospirosis. Organisms may be isolated from the blood of cattle during the period of demonstrable fever and until antibodies are detectable in the blood. Reinhard and Hadlow (53), using the agglutination-lysis test, detected antibodies in cattle as early as four days, but in some animals antibodies were not detected until 18 days following artificial exposure.

Leptospires may be released in various excretions of the body, but the most important avenue is the urine. Leptospira are most consistently present in kidney tissue; they enter the kidney early in the infection and later localize in the renal tubules. They are released into the tubules and carried through the urinary bladder and out of the body with voided urine. Most workers have found relatively few leptospires in cattle urine and have had difficulty isolating the organisms. However, Gillespie et al. (31) observed large numbers of *L. pomona* in the urine of cattle. In the febrile stages of the disease, organisms may be isolated from the blood and occasionally have been detected in milk (5). Several isolations have been made from aborted fetuses (20, 52), however, most isolation attempts have failed.
The onset of fever, which is often accompanied by anorexia and inappetence, may be followed within a day by anemia, albuminuria, hemoglobinemia, and a sudden decrease in milk flow. The milk secreted is usually viscous and yellowish, but no marked swelling of the udder is detectable. In some animals, extensive hemolysis occurs, causing the milk to appear pink and the urine dark red. Icterus of most body tissues is present in severe cases. Deaths may occasionally occur. Although cattle of all ages are susceptible, the disease is usually most severe in calves and feeder cattle. Abortions may occur in pregnant animals, usually taking place ten to 16 days following acute signs (23, 43, 47). Ferguson et al. (23) suggested that toxemia rather than direct infection of the fetuses may be responsible for abortions, since many workers have failed to isolate organisms from the aborted fetuses. On the other hand, Fennestad et al. (22) demonstrated leptospires in a number of fetuses and believed that invasion of the fetus by leptospires is the factor responsible for abortion in cattle. Bridges (8) suggested that failure to isolate leptospires is due to death and disintegration of organisms after the fetus dies. TePunga and Bishop (66) suggested that interruptions of the fetal maternal cotyledon junction may be caused by localized lesions. Morter et al. (49) stated that they found lesions in the cotyledon which supported this theory. Also, Morse (47) reported that retention of fetal membranes following abortion occurred in 20% of the outbreaks in Wisconsin.

The lesions in bovine leptospirosis are primarily confined to the kidneys. In the acute stage, petechiae are often present on the surface of the kidneys and may be accompanied by hemosiderin deposits. Small white foci are frequently observed on the surface of kidneys following the acute stages of leptospirosis (17, 33, 53). Microscopic examination of affected kidney tissue reveals an interstitial nephritis. Leptospires can often be demonstrated in affected tubules when silver stains are used (8).

The liver tissue may be markedly yellow and congested in severe cases, and petechiae and icterus may be observed in various mucous membranes.

The clinical signs of bovine leptospirosis may be highly suggestive, but are not pathognomonic of the disease. Due to the varied signs produced, and the frequent absence of some of them in many outbreaks, serological and isolation techniques are required for a definite diagnosis.

Serological diagnosis is based on the detection of antibodies primarily in the blood, although other body fluids are occasionally used. The agglutination-lysis test is apparently the most accurate test, although it is somewhat cumbersome, since live organisms must be used for the antigen and the test has to be read under a darkfield microscope. Several plate agglutination tests which have been developed are widely used by laboratories and practicing veterinarians in screen-
ing sera for positive reactions. However, the plate agglutination tests are less sensitive than the agglutination-lysis test and do not measure the antibody concentration. A capillary tube agglutination test using a formalin-killed antigen is also used and has the advantage over the plate agglutination-lysis test of measurement of antibody concentration. Van der Hoeden (71) has developed a milk agglutination test. At present, some laboratories use the rapid plate test for screening samples, and either an agglutination-lysis test or a capillary test to determine the antibody concentration (63). All of these tests are species specific, which necessitates repetition of each of the antigens. Galton et al. (27) recently developed combination antigens, each of which contains a group of several species to be used for screening purposes. Once a group has been identified with these combined antigens, single species antigens can be used for further identification.

A complement-fixation test is also used in some cases, but the complement-fixing antibodies do not appear as early and they do not persist as long as the agglutination-lysis antibodies (81). Cox (18) has developed a hemolytic test which appears to be genus specific rather than species specific.

Detection of antibodies alone does not constitute a positive diagnosis. Vaccination of cattle with *L. pomona* bacterin can also cause formation of antibodies which are detectable by serological tests. Positive reactions at titers of 1:100 or more by the agglutination-lysis or capillary tube test are usually considered significant. Since titers persist for years, the present disease may or may not be associated with *L. pomona*. Therefore, detectable antibodies are important only if their titer rises within a period of several weeks following clinical evidence of the disease. Correlation of an increase in antibody level during a short period of time with the presence of clinical signs provided a reliable method of leptospirosis diagnosis.

Cultures can be made from infected tissues, urine, milk, and surface water, either directly in artificial media or indirectly by inoculation of laboratory animals, followed by isolation in artificial media. Guinea pigs, hamsters, and young chickens have been used extensively in *L. pomona* studies (25, 37, 57). Some workers consider chinchillas (59) and gerbiles (70) to be more satisfactory laboratory hosts than guinea pigs.

**TREATMENT**

Antibiotics have been used extensively in the treatment of leptospirosis in cattle, man, swine, and dogs. Early studies indicate that penicillin is inhibitory but apparently primarily bacteriostatic in action (2, 12). Other antibiotics which have shown to have an inhibitory effect upon *Leptospira* are oxytetracycline, streptomycin, chlorotetracycline, chloramphenicol, erythromycin, and tetracycline hydrochloride (10, 34, 42, 55, 56, 79). Early medication is of primary importance, since the drugs are more effective early in the development of infection. Medication must be started before extensive kidney damage occurs. Administration of antibiotics in feed has been reported to decrease signs in infected herds (23, 36). Various sulfonamides have been used with very little success.
Other drugs have proved to be ineffective. Antiserum has been used in man with some success, but is not economically practical in animals.

The levels of antibiotics used in semen extenders appear to be sufficient to eliminate the semen used in artificial insemination as a mode of spread in bovine leptospirosis (11, 54).

**Vaccination**

Vaccination of cattle with bacterin is widely practiced at the present time in the United States. Although indifferent results were obtained in early studies (51), recent work has been more encouraging (28, 29, 40). The vaccines most extensively used are cultures inactivated with formalin, antibiotics, drying, and thimerosal and are standardized to a specific concentration (9). These vaccines produce varied resistance to *L. pomona* for six or more months (28, 30, 40). Calves under three months of age from dams not carrying antibodies, as well as from serologically positive dams, apparently do not respond to bacterins; therefore, most manufacturers advise against vaccinating calves until they are at least 3 months old (29).

York and Baker (80) reported that a chicken embryo bacterin inactivated by repeated freezing and thawing produced good protection. Reinhard and Hadlow (53) found that an egg-attenuated vaccine protected experimental cattle for 17 mo. Hoag and Bell (35) used an acid-heat-extracted culture of *L. pomona*, which provided protection for 1 and 2 months with little stimulation of agglutination-lysis antibodies. McDonald and Ridge (44) reported that vaccination of cows in late pregnancy provided protection of calves for the first few months of life. Alexander et al. (1) reported that significant antigenic variations exist between the types or strains of *L. pomona* which may be great enough to affect the degree of resistance produced by a vaccine prepared from a single strain.

At present, the commercially available bacterins for cattle are prepared from *L. pomona* and will not stimulate adequate resistance to the other species of *Leptospira* which have been detected in cattle in the United States. The duration of immunity following vaccination has not been adequately investigated. Only a large-scale, controlled vaccination study can demonstrate the actual value of vaccination as a control measure against bovine leptospirosis. There appears to be little point in vaccinating cattle having positive serological tests.

Since both treatment and vaccination have limited value, every effort should be made to prevent entrance of leptospirosis in a herd of cattle. Since swine are apparently the primary carriers of *L. pomona* in the United States, it is advisable to keep cattle and swine separated as much as possible.

Leptospirosis serological tests should be conducted on all animals before purchase.

Exposure should be avoided by proper drainage or fencing off of streams open to outside contamination. Feeder cattle of unknown leptospirosis status should not be pastured next to permanent beef or dairy herds.

Cattle with signs of leptospirosis should be isolated immediately, and serological tests should be made immediately and again a week or more later to diagnose the condition.
REFERENCES


(22) Fennestad, K. L., and Borg-Petersen, C. Fetal Leptospirosis and Abortion in Cattle. J. Infectious Diseases, 102: 227. 1958.


BOVINE LEPTOSPIROSIS. A REVIEW


