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Canine Leptospirosis
Diagnostic Technique

Major T. C. Jones*

LEPTOSPIROSIS is an important disease problem, both in animals and in man. Since the causative organism Leptospira icterohemorrhagiae and Leptospira canicola may be transferred from a dog to its owner, or vice versa, early diagnosis is essential. The clinical picture, although suggestive, is commonly non-specific, but final diagnosis must be made by laboratory methods.

Leptospirosis should be suspected in a dog presenting symptoms of gastro-enteritis with acute collapse and fever, with or without jaundice or nephritis. The disease has the characteristics of septicemia in its early stages and if the animal survives, the organism tends to localize in the kidneys. In the later stages, therefore, symptoms of kidney involvement are to be expected. A rather large number of dogs recover clinically from leptospirosis but many carry the organisms in the kidney and eliminate them through the urine. These potential carriers are a problem in themselves. Wild rats become carriers of Leptospira icterohemorrhagiae in much the same way.

Serological Tests

The agglutination test is a most valuable tool in diagnosis of leptospirosis. As soon as the disease is suspected, a blood sample should be taken, the serum separated by clotting, and the clear serum submitted to the agglutination test. Two tests are available. The microscopic test consists of observation through the microscope of agglutination of Leptospira in the presence of serum. Dissolution of the organisms also is seen, hence the test is usually called the “agglutination-lysis test.” (1) The macroscopic, or rapid plate, test is the choice under most conditions because of its simplicity. In our hands it has proved highly satisfactory. (2) However, it is not as sensitive as the microscopic test but this is no disadvantage. Stained antigen, consisting of a heavy suspension of Leptospira plus gentian violet is mixed with serial dilutions of serum on a glass plate and warmed slightly. The test may be read within 5 minutes, gross clumping of the organisms indicating a positive reaction. As a control, known positive serum is usually tested with the unknown.

Interpretation

Interpretation of reactions to this agglutination test must be cautious. In the early stages of the disease no agglutinins may be present in the serum. For this reason a second sample should be tested 3 to 5 days after the first. A rise in agglutination titre or the appearance of agglutinating antibodies following an initial negative reaction are of diagnostic significance. A ten fold increase in titre under these circumstances is an indication of leptospiral infection. Therefore, an initial titre of 1:10 followed by agglutination in dilutions of 1:100 or higher in the second test should be considered positive. The titre usually reaches 1:1000 or higher later in the course of the disease.

Cultural Methods

If at all possible, cultural methods should be used as an aid to the diagnosis

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of leptospirosis. The most satisfactory medium in our experience has been Fletcher's Broth (3) which is prepared as follows:

1. Make a 12 per cent suspension of freshly drawn rabbit serum in distilled water. Inactivate for 40 minutes at 56° C.

2. Add 6 cc. of nutrient or meat extract agar (2.5 percent agar, pH 7.4) to each 100 cc. of the 12 per cent rabbit serum suspension. Adjust reaction to pH 7.4.

3. Pipette into Wassermann or Loeffler tubes, 6 cc. to each tube. Sterilize at 56° C for 1 hour on each of 2 successive days.

Blood cultures are of diagnostic value when taken early in the disease. The best method is to collect about 5 cc. of blood with aseptic precautions using a Luer syringe. The blood is then placed in 0.5 cc. amounts in each of 10 tubes of Fletcher's Broth prepared as described above. These cultures are then incubated at 32° C for 10 days. Growth is indicated by a cloudy zone in the upper part of the media. This supernatant fluid is examined with the darkfield microscope for Leptospira. The cultures are re-examined at frequent intervals during a 30-day period before being considered negative.

Isolation from Urine

After the disease has passed the septicemic stage, Leptospira are not found in the blood but may be present in the kidneys and liver. Isolation of organisms from the urine may be accomplished in the following manner:

The dog is first given a dose of 2.5 to 5.0 gm. of sodium bicarbonate or sodium citrate per orum to alkalinate the urine. Twenty-four hours later, the dog is catheterized and a urine specimen collected with aseptic precautions. The urine is then centrifuged at high speed and the supernatant fluid decanted. The sediment, suspended in saline solution, is then placed in 0.5 cc. amounts in each of 10 tubes of Fletcher’s Broth or injected into laboratory animals.

One technique for presumptive differentiation of *L. icterohemorrhagiae* and *L. canicola* is accomplished by injecting about 5 cc. of an active culture of the organism intraperitoneally into a rabbit. Fourteen days later the agglutination test is performed with this rabbit’s serum using antigen from both strains. Agglutination with the homologous antigen is in much higher titre.

Animal Inoculation

The laboratory animal of choice is the hamster (4) in which fatal disease follows injection of either *L. icterohemorrhagiae* or *L. canicola*. Young animals weighing 25 to 30 gm. are most suitable. When the hamster dies or becomes moribund (6 to 10 days following inoculation) *Leptospira* can be demonstrated in the kidneys, liver or blood by culture or darkfield examination.

Young guinea pigs less than 100 gm. in weight) are susceptible to infection with *L. icterohemorrhagiae* but not to *L. canicola*. Since hamsters may be infected with either strain, inoculation of both guinea pigs and hamsters may be used in presumptive differentiation of the strains.

In the acute cases, the gross lesions resemble those of other acute septicemias. Petechial hemorrhages are seen on serous surfaces, particularly on the pleura. Hemorrhagic gastro-enteritis may be in-
icated by the presence of dark, "tarry" blood in the intestine with hemorrhages in various parts of the intestinal wall. Icteric discoloration of all the tissues is usually very striking, although some cases are encountered in which this lesion is not prominent. In the kidney, vascular engorgement with petechial hemorrhages in the medulla are usually seen.

The microscopic lesions in this acute stage have been well described by Bloom. (5) Hemorrhages may be seen in the gastrointestinal tract, liver, spleen, lungs and kidneys. Focal necroses are often encountered in the liver and stomach.

The kidneys usually show degenerative changes in the tubules with acute inflammation in the interstitial tissues. The diagnosis is based upon identification of Leptospira in sections stained by silver impregnation. The Kerr modification of the Warthin-Starry method is highly satisfactory. (6)

In clinically recovered (asymptomatic) or long-standing cases, the lesions in the kidney are usually striking. Grossly, gray or yellowish masses are seen in the cortex along with scattered hemorrhages. The kidney may be atrophied and very tough to cut. Streaks of fibrosis may be seen radiating from the renal pelvis. The microscopic picture here is one of interstitial nephritis with secondary involvement of the nephron. The interstitial tissues contain large numbers of lymphocytes, monocytes and plasma cells and occasionally neutrophils and erythrocytes. The glomeruli may be fibrosed in some areas or even obliterated. Here again the diagnosis is dependent upon the demonstration of Leptospira by silver impregnation. The organisms are found in the tubular epithelium or occasionally free in the lumen. In some cases these organisms are readily found, but in others prolonged search is necessary.

**Summary**

In the diagnosis of canine leptospirosis, clinical symptoms are suggestive but laboratory confirmation is essential. A most useful tool is the rapid plate agglutination test. Typical symptoms with significant agglutination titre or atypical symptoms with an increasing titre are usually sufficient to establish the diagnosis. Cultures of the blood and urine in Fletcher's Broth are desirable. Inoculation of hamsters with subsequent demonstration of Leptospira by culture or darkfield microscopy should be utilized whenever possible. Direct darkfield examination of the blood, urine or tissues of affected dogs is not reliable as a diagnostic measure. Autopsy findings which are suggestive can be confirmed by finding Leptospira in silver-stained sections.

**REFERENCES**


There are 139,309 physicians in active practice in the United States during normal times; 45,154 of these are specialists, with 12,152 of this number being surgeons.