Canine Leptospirosis: An update on diagnosis, treatment and prevention

Richard E. Goldstein  DVM DACVIM DECVIM-CA
Cornell University, Ithaca, NY rg225@cornell.edu

INTRODUCTION

Leptospirosis is an important world-wide zoonosis, caused by an infection with a pathogenic species of the genus *Leptospira*. These are highly motile obligate aerobic spirochetes that share features of both Gram-negative and Gram-positive bacteria. Dark field or phase contrast microscopy is necessary to visualize these bacteria since they stain so poorly. This genus is classified today based on genetic determinations. Most of the commonly diagnosed canine pathogenic serovars are still classified (as before) as belonging to the *L. interrogans* species although the serovars *grippotyphosa* and *ballum* are now classified as belonging to the *L. borgpetersenii* and the species *L. kirschneri* respectively. This talk will include a general overview of the current literature regarding canine leptospirosis. This will include the most recent thoughts on the epidemiology of the disease, the pathogenesis, the methods of testing for the disease, prevention and vaccination as well as clinical signs and treatment.

Serovars: Historically *L. icterohaemorrhagiae* and *L canicola* were most common and the ones found in the bivalent vaccine. In the past 10 years multiple reports of increased incidence of the disease have been published with little or no cases of the above serovars (Table 1). The most common serovars seen today in the United States are thought to be *L. bratislava*, *L. grippotyphosa* and *L. pomona*. Recently new vaccines have appeared on the market that include *L. grippotyphosa* and *L. pomona*. It is too soon to assess a potential serovar shift if there is one following the new vaccines. In recent years increasing incidence of *L. Autumnalis* has been documented as many commercial laboratories have added this serovar to their testing panel. Little is known about this serovar in the dog in terms of experimental infection but it is likely to emerge as an important cause of renal and non-renal leptospirosis. The following are some of the recent reviews assessing serovar incidence in different regions of North America (Table 1). Other serovars have been documented in different parts of the world.

Table 1. Recent reviews documenting the most common serovars in dogs in different areas of North America.

<table>
<thead>
<tr>
<th>1st Author (region)</th>
<th>Journal</th>
<th>Year</th>
<th>No of cases</th>
<th>Predominant Serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescott JF (Ontario)</td>
<td>Can Vet J</td>
<td>2002</td>
<td>31</td>
<td><em>autumnalis</em> <em>bratislava</em></td>
</tr>
<tr>
<td>Adin (California)</td>
<td>JAVMA</td>
<td>2000</td>
<td>36</td>
<td><em>pomona</em> <em>bratislava</em></td>
</tr>
<tr>
<td>Ribotta (Quebec)</td>
<td>Can Vet J</td>
<td>2000</td>
<td>19</td>
<td><em>grippotyphosa, pomona</em></td>
</tr>
<tr>
<td>Prescott (Ontario)</td>
<td>Can Vet J</td>
<td>1999</td>
<td>18</td>
<td><em>grippotyphosa, autumnalis</em></td>
</tr>
<tr>
<td>Birnbaum N (New York)</td>
<td>JSAP</td>
<td>1998</td>
<td>36</td>
<td><em>grippotyphosa, pomona</em></td>
</tr>
</tbody>
</table>

Reservoir hosts: Reservoir hosts vary from serovar to serovar and not all are definitively characterized (Table 2). Table 2. Reservoir hosts for the common serovars:

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Reservoir Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>canicola</td>
<td>Dog</td>
</tr>
<tr>
<td>icterohaemorrhagiae</td>
<td>Rat</td>
</tr>
<tr>
<td>grippotyphosa</td>
<td>Raccoon, Marsupials</td>
</tr>
<tr>
<td>pomona</td>
<td>Cow, Pig</td>
</tr>
<tr>
<td>hardjo</td>
<td>Cow</td>
</tr>
<tr>
<td>bratislava</td>
<td>Horse</td>
</tr>
<tr>
<td>autumnalis</td>
<td>Mouse</td>
</tr>
</tbody>
</table>

Epidemiology: The disease is maintained in the renal tubules of the reservoir host and excreted in the urine. These hosts are typically non-symptomatic and may be able to shed bacteria for their entire life. This is also likely true for dogs secreting *L. canicola*. It is unknown whether such a carrier state exists in dogs infected with other serovars. Transmission can occur via direct or indirect contact with an infected host, urine or other body fluids.
excretions. The ideal conditions for the bacteria to survive outside the host are a warm and moist environment. Hence there is seasonality to canine leptospirosis cases with the typical highest number of cases being in the late summer and fall. The number of cases in a specific year has been correlated to the amount of rainfall the previous spring.(1)

Pathogenesis: Understanding the molecular basis for Leptospiral virulence is crucial in the effort to produce more effective vaccines. Identifying surface antigens that are expressed during active infection in vivo may also facilitate distinction between active infection and vaccination or exposure. For example, Leptospira immunoglobulin-like protein A (LigA) contains domains homologous to proteins with attachment and invasion functions and is expressed in vivo but not in vitro.(1) Leptospira organisms penetrate abraded skin or mucus membranes and replicate rapidly in the bloodstream. The sequence of events after infection likely depends on:

1. **Virulence.** Important questions include: Is there a difference between serovars? New data that will be presented would suggest that there is.
2. **Immune response.** Questions: Has the dog been vaccinated or previously exposed? How well does the vaccine protect from natural infection and is there acquired immunity after being infected with a specific serovar? Many of the commercially available vaccines have been shown to provide good short term immunity but the length of that immunity is unknown. A recent study comparing different commercially available vaccines should only a mild serological response to a series of 2 vaccinations but good immunity when challenged 1 month after the second vaccine.(3)
3. **Gender.** Some studies show males as being more likely to be clinically affected than females.

**After infection the following organs may be affected:**

1. **Kidneys:** Renal colonization. Organisms persist and multiply in the renal tubular epithelial cells causing acute nephritis. If not fatal and not treated appropriately - this MAY lead (info is mostly experimental from *L. canicola*) to chronic interstitial nephritis and a persistent carrier state.
2. **Liver:** Liver damage. Centrilobular necrosis and sub cellular damage, bile canaliculi and duct occlusion may cause icterus. This is not seen as commonly today as with *ictero*.
3. **Blood vessels:** Vasculitis and DIC due to endothelial damage – likely more common that we think.
4. **Lungs:** Pulmonary hemorrhage. This is common in severe cases in people. The incidence of canine cases is unknown.
5. **Uterus:** Abortion? Unknown significance in canine patients.
6. **Eyes:** Uveitis? Common in horses but can occur in dogs as well.
7. **Brain:** Meningitis and encephalitis have been documented in severe cases in humans. The incidence of canine cases is unknown.
8. **Immune system:** Secondary immune mediated disease (poly-arthritis, hemolytic anemia etc...). The incidence of canine cases is unknown.

Diagnosis: The diagnosis of canine leptospirosis is not definitive in most cases in veterinary practice today. It is based on a combination of appropriate clinical signs, clinico-pathological and imaging data as well as serology. The definitive diagnosis would require identifying the organism in urine or tissue which is very uncommon because of the technical difficulties of culture and direct visualization of the organism and the high sensitivity of the organism to antibiotics (a dose or two of antibiotics can cause a negative culture, urine FA and even a negative urine PCR!).

Clinical signs: The clinical signs may depend on the serovar, the virulence, the immune status of the patient and the organ targeted by the bacteria. Although most cases diagnosed today in dogs are associated with acute renal failure other syndromes exist as well. Yet unpublished data will be presented describing the frequency of clinical signs and clinico-pathological features documented in a large study recently completed by the author at Cornell University. Non-renal cases of leptospirosis will also be presented with the main clinical sequela being hepatic disease or vasculitis.

Serology: The microscopic agglutination test (MAT) is the most commonly used test in veterinary medicine. The highest dilution of serum that agglutinates 50% of the leptospira organism is the titer. This test does have a high specificity and sensitivity, especially if it is repeated 2-3 weeks after a negative result when leptospirosis is suspected. How specific is this test for serovars? There is a large degree of cross reactivity, we assume that the serovar with the highest titer is the one causing the infection. Disappointingly, this was found to be true only in about 50% of the cases in a human study comparing MAT titers to cultures.(4) In veterinary medicine a titer to a non-vaccinational serovar of 1:800 or greater or a fourfold rise in titer is commonly thought to be suggestive of active disease. Vaccinational titers are typically low but have been documented up to 1:3200. Antibiotic therapy may cause a decrease in the MAT titer or prevent a rising titer when convalescent titers are performed. An ELISA test is the most commonly used test for screening in humans. It is not serovar specific and if used in dogs it should probably be confirmed by MAT. ELISA testing can be performed for IgM and IgG. The IgM ELISA may be positive prior to the MAT titer in an acute infection. Direct fluorescent antibodies (FA) can be useful on tissue
and occasionally urine. The organisms though tend to disappear within a day or two of therapy causing a high number of false negative results.

Newer testing that is not yet widely commercially available in veterinary medicine includes Western blot analysis and PCR. Western blots may be used in the future to differentiate titers originating from vaccinations from natural exposure. This is extremely important now that common pathological serovars have been included in the canine vaccine. We have shown that although there are many common bands in Western blots of dogs that were infected and dogs that were vaccinated the pattern is different. Natural infection causes many more bands in a more complex pattern. This technique will likely be used in the future. PCR identification of Leptospiral DNA in urine, blood, CSF aqueous humor and tissue has been used in human medicine and veterinary research. (5,6) Unfortunately we have found that even one or two days of antibiotics may cause a negative urine PCR. Thus this will likely be an excellent screening test in the future but may not replace serology or western blots completely.

**Treatment:** In addition to fluid therapy and supportive, symptomatic care for the renal, hepatic or other manifestations of leptospirosis, antibiotic therapy is indicated as early as possible whenever this disease is suspected. In addition to minimizing organ damage, early antibiotic therapy will quickly clear the leptospiremic phase and sterilize the urine thus preventing spread of the bacteria to other animals as well as to humans. The risk to people is minimal within 24 hours of antibiotic therapy but caution (gloves, avoiding contact with the urine etc.) should still be taken for the duration of the hospitalization and continued at home. Penicillin and its derivatives as well as doxycycline can be used with good results to quickly clear the leptospiremic phase and to prevent the excretion of infective bacteria in the urine. It is very reasonable then to use a penicillin derivative in a suspected case prior to receiving a definitive diagnosis. The clearing of leptospira bacteria from the tissues, eliminating a carrier stage - if one exists in dogs with serovars other than *canicola*, may be harder. Unfortunately controlled studies are lacking as to the best antibiotic protocol to use for this stage in dogs. This makes the choice of an antibiotic once a definitive diagnosis has been made more problematic. Different protocols appear to be at use in different centers, all based on the use of different antibiotics in rodent models with leptospirosis. Enrofloxacin has shown to be effective in some rodent studies and is used in combination with ampicillin in some centers. (2) Doxycycline has also shown to be effective, superior to ofloxacin in one study with infected hamsters when PCR was used to assess the efficacy of various antibiotics. At Cornell University doxycycline is typically used once the diagnosis of leptospirosis has been established. Ampicillin or doxycycline are commonly used in suspected cases while waiting for the results of serology.

**Outcome:** Survival rates for dogs with acute renal failure from leptospirosis have been shown to be approximately 80% in past studies (2,8) and in the study recently performed by the author. *L. pomona* infection was associated with a worse prognosis than other serovars in that study. Hemodialysis was shown to be beneficial in cases refractory to conservative medical management. (2)

**References:**