Background

Leptospirosis, a disease caused by spirochetes belonging to the genus *Leptospira*, is the most common zoonosis worldwide. There are a host of *Leptospira* species, both pathogenic and saprophytic. Currently used genetic classifications by DNA hybridization identify at least 19 species (13 pathogenic and six saprophytic). Seven species – *L. interrogans*, *L. borpetersenii*, *L. santarosai*, *L. noguchii*, *L. welli*, *L. kirshneri* and *L. alexanderi* are the main agents of leptospirosis.

Serological classification on the basis of surface antigens identifies 24 serogroups and 250 serovars based upon the expression of surface lipopolysaccharide. Serovars with overlapping antigenic determinants are classified into a larger serogroup. Each serovar has a definitive host responsible for maintenance of the organism within the environment.

Typically definitive or maintenance hosts become infected at a young age and harbor and shed leptospires (urine) and manifest minimal to no disease. Animals generally have more severe clinical signs when infected with serovars to which they are not adapted. Almost every mammal can serve as a carrier of leptospires leading to urinary shedding. Once excreted in the urine, leptospires can survive in moist environments for months to years given optimum environmental conditions. Freezing and UV radiation inactivate leptospires. Rats are the major carriers in most human infections. Humans are considered incidental hosts for leptospirosis. Animals can serve as either maintenance hosts or incidental hosts for leptospirosis, depending on the infecting serovar. Humans and animals contract leptospirosis from exposure to spirochetes in urine or in urine contaminated water.

Vaccination of dogs against *L. icterohemorrhagiae* and *canicola* appears to have decreased the prevalence of these forms of leptospirosis but there has been a resurgence of canine leptospirosis associated with *L. pomona*, *grippotyphosa* and *bratislava* serovars. Newer canine *Leptospira* vaccines include *L. grippotyphosa* and *L. pomona* in addition to *L. icterohemorrhagiae* and *L. canicola*. Early studies showed a greater risk for large, herding breed dogs but there appears to be an increasing predilection for small, terrier breeds.

Leptospirosis can be manifest as syndromes varying from peracute to chronic. Peracute leptospiremia can cause fatal or near fatal
shock with few premonitory signs. Acute signs of leptospira infection include pyrexia, shivering, myalgia, vomiting, dehydration and icterus. Chronic manifestations include chronic renal disease with polyuria, polydipsia, anorexia, vomiting and weight loss, and chronic liver disease with development of ascites, hepatencephalopathy and weight loss.

Diagnosis

Diagnosis of leptospirosis by antibody serology is problematic. Serum antibodies reflect exposure to leptospiral antigens which could come from leptospires in the environment or from vaccines. Furthermore, leptospiral antibodies can be documented in healthy animals. There is also a lag time to antibody development post exposure and persistence of antibody in some instances for months to years past recovery. Attempts to interpret the significance of leptospiral antibodies have focused on assessment of antibody titers to various leptospiral serovars using the microscopic agglutination test (MAT). The MAT uses a panel of live leptospires in culture against which serial dilutions of patient sera are reacted in microtiter plates. The titer is last dilution in which >/= 50% of the leptospires remain agglutinated. The complexity of this assay may account for the reported discordance of results.

Some have advocated interpreting the highest MAT serovar as the infecting serovar. Emphasis on interpretation of MAT antibodies to various serovars is fraught with error. A number of studies have demonstrated inconsistencies in MAT antibody results between laboratories and presence of MAT antibodies to serovars to which animals have not been exposed. The highest MAT antibody titers are often not to infecting serovars. For example, antibodies to *L. autumnalis* are frequently reported in dogs and often represent the highest titer, yet *L. autumnalis* has never been documented in the United States and this serovar is not included in vaccines. This phenomenon is thought to represent cross reactivity to *L. pomona*. Studies of infected humans with culture proven leptospirosis have shown accurate prediction of the infecting serovar in < 50% of the cases. Acute and convalescent MAT antibody serology has been recommended to increase the accuracy of test interpretation. This requires sampling 7 - 14 days apart to assess for a 4 fold rise in titer consistent with recent infection. Antibody treatment can reduce the rise in titer. Furthermore, natural infection with leptospires can induce MAT antibodies which persist for years and post vaccinal MAT titers can persist at high levels if ongoing exposure to field strains occurs.

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Assessment of leptospiral IgM antibodies carries some of the downfalls of MAT antibody serology but offers some advantages over the MAT. IgM antibodies rise more rapidly post exposure but also dissipate more rapidly. Leptospiral IgM antibodies appear 3 – 10 days post exposure. Vaccination associated IgM antibody waned in the majority of dogs by 12 weeks post vaccination and in all dogs by 22 weeks. In contrast, MAT antibodies appeared later and persisted later post exposure and vaccine. Given the rapid rise of IgM antibodies, repeat testing 3 days following a negative IgM antibody result will demonstrate seroconversion avoiding the need for 7 – 14 day delay for convalescent MAT serology.

Culture and polymerase chain reaction (PCR) assays can directly detect leptospires in blood or urine. Because of the difficulty and prolonged turnaround time, culture is an impractical test for patient management. Although PCR offers promise in detecting leptospires in blood in early infection and in urine later in the disease process (> 10 days post infection), studies assessing PCR results in infected animals have yielded mixed results. Antibiotic therapy can reduce sensitivity of both culture and PCR although multiple doses of antimicrobials may be required to cause false negative PCR as this assay detects both viable and nonviable organisms.

Given the MAT insensitivity in early infection as well as the hazardous and subjective nature of the MAT assay, the ACVIM 2010 Small Animal Consensus Statement on Leptospirosis advocated use of Leptospira IgM serology for early diagnosis of canine leptospirosis.

**Leptospira IgM Serology Interpretive Guidance**

A positive Leptospira IgM test result indicates exposure to *L. canicola*, *icterohemorrhagiae*, *grippotyphosa* or *pomona/autumnalis* and should be considered supportive of the diagnosis of leptospirosis if the dog has not been recently vaccinated and the clinical signs, history, physical examination findings and other laboratory results support the diagnosis.

Ancillary testing (serum chemistry, hematology, urinalysis) and molecular testing (Leptospira PCR in urine or blood) can aid in making a definitive diagnosis of leptospirosis.

A negative IgM test result indicates no exposure to *L. canicola*, *icterohemorrhagiae*, *grippotyphosa* or *pomona/autumnalis* has occurred in the time window of 3 – 10 days up to 6 months prior to testing.

Repeat testing for IgM antibodies 3 days following a negative test can confirm exposure status.

Negative IgM test results support ruling out leptospirosis provided there is an appropriate time course since exposure.

Ancillary testing (serum chemistry, hematology, urinalysis) and molecular testing (Leptospira PCR in urine or blood) can aid in ruling out leptospirosis.

Studies show *Leptospira* vaccination IgM antibody wanes in the majority of dogs by 12 weeks and becomes undetectable in all dogs by 26 weeks post vaccination.

**Ordering Information**

To order *Leptospira* testing, use the following test codes:

- Leptospira IgM: VLEPT
- Leptospira PCR: VLEPTSO
References


DON’T LIMIT YOUR LIVER BIOPSY: HOW TO COLLECT DIAGNOSTIC LIVER BIOPSYS

Anne Kincaid, DVM, Diplomate ACVP, Marshfield Labs Veterinary Services

The goal of performing a liver biopsy is to obtain an accurate diagnosis to guide patient therapy. At Marshfield, pathologists want to obtain the most information possible when examining these samples. Follow these steps to ensure maximum benefit from your biopsy specimens.

The first step to getting the most out of your liver biopsies is to provide a full history and clinical findings. Congenital vascular disorders are a good example since the histologic findings are stereotypical and the final diagnosis can only be made with the clinical information indicating the presence or absence of a shunt vessel. Ideally, the following information should be provided: species, breed, age, gender, brief history, overview of clinical signs/findings and levels of biochemical alterations. Specific numbers for biochemical alterations should be provided rather than just a history of liver enzyme elevation. Why was the biopsy taken? What did the liver look like? If a mass or masses are present, what were the sizes? Hepatic masses greater than 3 cm in diameter are more likely to be neoplastic. Providing the appropriate history will not influence the pathologist’s diagnosis but will help the pathologist in making the appropriate clinical/pathology correlations.
Diffuse conditions such as lipidosis/steatosis or lymphoma are more likely to be diagnosed in smaller biopsies. Laparoscopic or wedge biopsies taken via laparotomy are better for multifocal processes. Necrosis from toxic liver injury may vary considerably in the degree of parenchymal involvement and a single needle biopsy can lead to the wrong conclusion regarding the overall status of the liver (Figure 1).

Indications for liver biopsy:
1. Abnormal serum enzymes and function tests - 30 days or more.
2. Hepatomegaly of undetermined cause.
3. Hepatic involvement in systemic disease.
4. Staging of neoplastic disease.
5. Evaluation of response to therapy or progression of disease.

Clinician concerns:
1. Hemostasis - little agreement on ideal indicators.
2. Laparoscopic biopsies - 14g for large dogs and 16g for small dogs or cats.
3. Always collect several lobes - regional variation is an issue. Sample from at least two lobes.
4. Save samples for culture or copper evaluation.
5. Provide a thorough history.

More than 10 portal tracts are considered essential for a reliable biopsy and some veterinary needle biopsies may contain 3 or fewer portal tracts (see Figure 2). There may be considerable variation in the degree of liver lobe involvement. For instance, chronic hepatitis and fibrosis are the least likely diagnoses to be uniform throughout the liver. The caudate liver lobe is frequently the most discordant and single samples from this lobe are not advised.

ALT usually peaks about 48 hours after acute injury. The half-life of ALT in the dog is about 59 hours and in the cat is 3-4 hours. By the time the ill patient gets to the veterinarian, hepatic enzyme elevation is noted in biochemistry results and the biopsy is scheduled, the hepatic repair process can clear necrotic hepatocytes and replace lost cells, leading to a relatively normal looking liver biopsy even though the ALT is still significantly elevated. Proper handling of the biopsy is important. For routine histopathology, samples should be...
placed into formalin immediately. Frozen samples are required for some immunohistochemical markers (i.e. many markers for histiocytic sarcoma). Samples for electron microscopy should be placed in the appropriate fixative at the time of collection.

Avoid excessive use of forceps to prevent crush artifact during collection. Wrap needle biopsies in tissue paper and place in plastic cassettes. Some plastic sponges have rough or pointed cut surfaces leaving artifactual perforations in the liver and should be avoided. Compression of biopsies by sponges prior to fixation can also cause significant distortion of the hepatic architecture (see Figure 2a).

Reference


INTERESTING CASE

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Biopsies from 8-year-old spayed female Beagle mixed breed canine. Biopsies were from left fourth mammary gland and described as multilobulated masses extending down into body wall musculature. Masses ranged from 1.5 cm to pinpoint in size.

The biopsies contained multiple siderotic granulomas in the fat associated with the mammary tissue admixed multifocally with hematopoietic cells. Nodules of splenosis were identified in the deep fat adjacent to the body wall musculature. The foci of splenosis had well-defined smooth muscle capsules and contained hematopoietic cells representing myeloid and erythroid precursors as well as megakaryocytes admixed with siderophages. Figure 3 represents two nodules of splenosis in the fat adjacent to

Figure 2a: Wedge biopsy with plastic sponge artefactual holes (arrows) and distorted architecture due to compression by the sponges prior to fixation.

Figure 3: Two nodules of splenosis in fat. Skeletal muscle of body wall is at the top of the image.

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Further consultation with the submitting veterinarian revealed a previous history of a dog fight resulting in severe bruising along the left flank. Evaluation of the patient at a different veterinary hospital did not demonstrate body wall injury. However, the presence of linear nodules of splenosis indicated likely penetration of the body wall, with splenic injury resulting in seeding of splenic tissue into the area of body wall injury. The siderotic granulomas and the extramedullary hematopoiesis within the subcutaneous fat were likely caused by the trauma.

**HELPFUL HINTS: CYTOLOGY**

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**Formalin Vapor and Cytology Smears**

Formalin vapors cause problems with fresh cytology smears, frequently rendering smears nondiagnostic. To prevent this artifact, prepare cytology smears away from open formalin containers and package cytology smears separate from formalin containers. If cytology smears must be shipped in the same package with formalin jars, double bag cytology smears in ziplock bags.

**Urine Sediment Smears for Cytology**

Collect urine by cystocentesis 2 hours after the morning void. Desired volume is at least 5 mL. Prepare urine sediment by centrifugation at 450g (approximately 1500 – 2000 rpm for most centrifuges) for 5 minutes. Make smears of urine sediment within 5 to 30 minutes post collection for optimal morphology.