When canine leptospirosis is on the differential diagnosis list both clinicians and dog owners want quick and accurate testing. Dogs infected with leptospirosis are at serious risk for hepatic and renal disease, and death. As a zoonotic disease, the potential risk to family members and clinic staff is an additional concern.

Leptospirosis is a disease caused by infection with one of the more than 250 types of bacteria in the genus *Leptospira*. These bacteria live in warm, wet environments such as damp grass, standing water, mud, and lakes. Under ideal conditions, the bacteria can survive more than three months outside the body. Leptospires are excreted through the urine of infected animals and disease transmission most often occurs through mucous-membrane contact with the urine of infected animals or contaminated food, bedding, soil, or water. Dogs can also be infected from close contact with another infected dog, and through the bite of, or by eating, an infected animal. Raccoons, skunks, opossums, rats, cows, and pigs are all known carriers of the infection.

Leptospirosis in dogs can cause a broad range of clinical signs in any combination. The most common early indicators of *Leptospira* infection are: anorexia; polyuria, oliguria, or anuria; lethargy; vomiting; diarrhea; and abdominal pain. Even with prompt, exhaustive medical care, 10 to 15 percent of infected dogs may die. Untreated, many infected dogs die of kidney or liver failure.

Diagnostic Options

The gold standard for diagnosis of leptospirosis is a positive culture. However, culturing this bacterium is rarely attempted in diagnostic laboratories because of the difficulty and time it takes for a successful isolation.

Serology has long been the mainstay of leptospirosis diagnostics. The Bacteriology Laboratory at the MSU VDL conducts over 80,000 serologic assays for leptospirosis annually and performs the Microscopic Agglutination Test (MAT) daily, Monday through Friday. Because the test is performed daily, results are usually reported the same day samples are received—samples received later in the day will be reported the following day. Our canine leptospiral MAT (test code 95055) includes antibody titers against the following six leptospiral serovars: Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona. Titers against serovar Autumnalis are available in our expanded panel (test code 95066) or as an add-on (test code 95054) by request. The prevalence of serovars has changed over the last few decades and is influenced by geography and exposure to reservoir hosts. Currently, the most prevalent serovars are thought to be Grippotyphosa, Icterohaemorrhagiae, Pomona, and Bratislava.

An interpretation is included in each MAT report at no extra charge and additional consultation is also available. As with other serology assays, vaccination complicates the interpretation of titers. Therefore, please provide a leptospiral vaccination history if it is available. This vaccination history is used...

(continued on page 2)
in combination with the appropriate clinical history and test request on the submission form to ensure the appropriate tests are requested, and to provide context for the interpretation. For example, please indicate on the submission form if the dog is ill (test is for clinical purposes) or if the test is being requested as part of a vaccine screen (test code 95996) to determine whether the dog needs to be given a booster dose of leptovaccine.

In a clinical case, performing a **PCR assay** can detect the leptospiral DNA. The PCR assay (test code 95053) is a quick test with a two to five day turnaround time for a diagnosis. However, due to the transient bacteremic phase of the disease, leptospira will not be detected by PCR in blood after the first few days of infection. Urinary shedding of leptospires is a sequelae of the disease; however, the bacteria may not be present (detected by PCR) in the urine of infected dogs until about five to seven days after they become ill. Antibiotic treatment will also negatively affect the chances of finding leptospires in urine using any method.

Testing with one sample/assay for leptospirosis is not ideal and a combination of serology and organism detection is recommended.

To provide our companion animal (and zoo animal) clients a more complete diagnostic package, we have combined the traditional leptospira serology assay (MAT) with PCR. The MSU VDL's new leptospirosis panel (test code 95068) includes PCR (blood or urine) and MAT at a discounted price.

**Sample Types and Requirements**

**Remember that antibiotics will negatively impact the detection of leptospires. Please collect urine and blood samples for PCR PRIOR TO beginning antibiotic treatment.**

**PCR:**
- 1 mL of whole blood or 5-10 mL of urine
- Ship samples on ice
- Samples should reach the lab within 24-48 hr post collection; we recommend using the MSU VDL insulated mailer with UPS overnight return shipping

**MAT:**
- 0.5 mL of serum or plasma
- Refrigerate sample until shipment
- During hot months, we recommend shipping with an ice pack using the MSU VDL insulated mailer with UPS overnight return shipping
- Separated serum that has been stored ≤ 10° C may be tested up to 1 month post collection

**MAT & PCR:**
- 0.5 ml serum (or plasma) for MAT AND 5-10 mL urine (or 1 mL whole blood) for PCR
- Ship overnight with an ice pack; we recommend using the MSU VDL insulated mailer with UPS overnight return shipping

**For More Information**

For more detail please see the MSU VDL catalog of available tests or call 517.353.1683.

To aid clinicians in discussions with dog owners, we also offer a client education guide, “Leptospirosis: What Every Dog Owner Should Know” which is available on our website at animalhealth.msu.edu under Client Education. This guide can be printed or shared with clients electronically. Please contact us if you would like printed copies sent to your clinic.

**References:**


Involvement in Key Federal Networks Helps the Michigan State University Veterinary Diagnostic Laboratory Protect Animal Health Nationwide

The Michigan State University Veterinary Diagnostic Laboratory (MSU VDL) is a member of several federal networks charged with protecting human and animal health. Active collaboration and partnership with other member laboratories as well as the federal agencies managing these networks enables the MSU VDL to contribute to animal health protection efforts nationwide.

Combating Antimicrobial Resistance
A number of bacteria that cause disease in animals can also infect humans through regular, casual contact and others can infect humans through the food supply. State veterinary diagnostic laboratories, like the MSU VDL, routinely test samples collected from clinically ill animals for bacterial pathogens and often perform antibiotic susceptibility testing on isolated pathogens to determine which antimicrobial agents will potentially be most effective. The MSU VDL’s Bacteriology Laboratory performs approximately 24,000 cultures, susceptibilities, and isolate identifications per year. Ensuring that the antimicrobial agent selected for treatment is effective and approved for use in that species is not only essential to ensuring successful treatment, but to helping to reduce or prevent the development of antimicrobial resistance (AMR) in bacteria due to inappropriate use of these important medications.

AMR is widely considered to be one of the greatest threats to human and animal health. Two federal networks, the National Animal Health Laboratory Network (NAHLN) and the Veterinary Laboratory Investigation and Response Network (Vet-LIRN) have current projects focused on AMR. The MSU VDL is working with both projects. The MSU VDL began participating in the Vet-LIRN AMR whole genome sequencing project in January 2017 and entered the second phase of that project in July 2018. In the first phase, the laboratory collected monthly the first four isolates of *Salmonella* (from any host), *Escherichia coli* (from dogs), and *Staphylococcus pseudintermedius* (from dogs). In the second phase, the laboratory will continue to collect the phase one isolates, as well as the first 12 other isolates for which it conducts antibiotic susceptibility testing each month, excluding isolates collected for the NAHLN project.

The MSU VDL has been one of 19 veterinary diagnostic laboratories participating in the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) AMR pilot project. The NAHLN project focuses on specific bacteria in particular species: *Escherichia coli* (cattle, swine, poultry, horses, dogs, cats); *Salmonella enterica* (cattle, swine, poultry, horses, dogs, cats); *Mannheimia haemolytica* (cattle); and *Staphylococcus intermedius* group (dogs, cats). Data collection for Year 1 of the NAHLN pilot project will conclude at the end of December 2018.

The goal of both projects is to develop an antimicrobial resistance surveillance program to follow AMR trends in bacteria of veterinary importance, which includes establishing a “bank” of representative bacterial isolates and associated AMR data. Tracking AMR data from routinely isolated pathogens across the United States and collecting isolates into a centralized data bank has the potential to help protect both humans and animals. This is because a centralized data collection and reporting process means data can be monitored for trends in antimicrobial resistance to help identify new or emerging drug-resistance profiles and to help monitor the usefulness of antibiotics over time.

Protecting Animal Foods
In late May 2018 the U.S. Food and Drug Administration, Center for Veterinary Medicine (FDA CVM) recognized more than 20 FDA employees, two employees from the Oregon State Veterinary Diagnostic Laboratory (OSVDL), and three employees from the MSU VDL’s Toxicology Laboratory with an FDA Group Recognition (Agency Crosscutting) Award. The award was given because of the individuals’ contributions to a pentobarbital in pet food investigation and recall. Toxicology samples from a dog submitted for necropsy were collected by the OSVDL and submitted to the MSU VDL for testing. When the MSU VDL identified pentobarbital in both the dog’s stomach contents and in the food can residue,
Dr. John Buchweitz, toxicology section chief, notified the OSVDL and worked closely with the FDA field agent. The ensuing investigation involved collaboration among the three organizations and ultimately led to a recall of contaminated pet foods that could have posed a risk to other dogs nationwide. In addition to Dr. Buchweitz, Dr. Andreas Lehner, analytical chemist, and Margaret Johnson, toxicology laboratory manager, were also recognized as a part of the award group.

“These two recent examples of our partnerships and involvement with the federal networks of which we are a member illustrate the importance of the work that is done at the MSU VDL and across the nation to help protect animal health,” says Rachel Reams, director of the MSU VDL. “Because animal health and human health are so closely connected, our efforts often benefit people as well. The laboratory’s everyday diagnostic caseload means that veterinarians and animal owners across the country get the results they are counting on and we are prepared to step up at a moment’s notice when the need arises.”

**About the NAHLN and Vet-LIRN**

The National Animal Health Laboratory Network (NAHLN) is a cooperative effort between two USDA agencies and the American Association of Veterinary Laboratory Diagnosticians. The goal of the NAHLN is to provide a nationally coordinated network of federal, state, and university-associated laboratories that provide animal health diagnostic testing to detect biological threats to the nation’s food animals, thus protecting animal health, public health, and the nation’s food supply. In October 2017, the MSU VDL was awarded Level 1 laboratory status in the NAHLN and retained Level 1 status for 2018. Out of 51 total member laboratories in 2018, only 15 were awarded Level 1 status.

The MSU VDL is also a member of the FDA’s Veterinary Laboratory Investigation and Response Network (Vet-LIRN) whose mission is to promote human and animal health by collaborating with veterinary diagnostic laboratories in order to provide scientific information, build laboratory capacity for routine and emergency response, and train scientists; as well as helping the Center for Veterinary Medicine (CVM) investigate potential problems with CVM regulated products such as animal feeds and animal drugs. The network provides the means for rapid response to reports of animal injury and establishes protocols to facilitate veterinary diagnostic reporting to FDA.

In addition to the NAHLN and Vet-LIRN, the MSU VDL is a member of the Laboratory Response Network (Centers for Disease Control and Prevention), and the Food Emergency Response Network (USDA-FDA). Participation in these networks positions the VDL to assist other State or Federal agencies in the event of a biologic threat or to threats to food safety that may impact humans and animals.