Guide to Leishmaniasis
Dogs with canine Visceral Leishmaniasis

The Disease, its Distribution, and Preventive Measures

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Visceral Leishmaniasis (VL) is a slow wasting disease caused by a Leishmania parasite. In Europe and the United States, the species found in dogs is Leishmania infantum (Duprey et al., 2006). In tropical areas of the world, this infection is due to bites from sand flies which suck blood from infected dogs or people and then bite a dog or person without infection leading to spread of the parasite (Figure 1). Leishmania parasites enter white blood cells underneath the skin after a bite, get into the blood stream and multiply within these cells in the spleen and liver, resulting in a chronic condition characterized by weight loss, tiredness, decreased appetite, and anemia (Petersen, 2009). The spleen, liver and lymph nodes become enlarged and blood work abnormalities appear. Bleeding disorders resulting in bloody noses or blood in the stool are not uncommon. In later stages of disease, kidney failure and other more severe problems occur. Crusty skin disease is also not uncommon in infected, symptomatic dogs. This appears as non-itchy, raised reddish bumpy areas near the eyes, or on the face, ears, axillary region (armpits or feet. Dogs also can develop abnormally long, brittle nails. Co-infection with other diseases such as intestinal worms, tick-borne diseases and additional stresses such as pregnancy, poor nutrition, overexertion, and being lower in the kennel pecking order can trigger appearance of clinical signs. Anderson et al published the first account of VL in American foxhounds in Oklahoma in 1980. It is thought that the disease was most likely introduced by acquiring hounds from hunts in Southern Europe. VL was more recently recognized in the US Foxhound in 1999, when one kennel of New York hounds was found to have significant numbers of hounds infected with canine VL. There are kennels across the US, including the Midwest, which currently harbor VL. In endemic areas all breeds of dogs are susceptible to VL. Within the US, due to the parasite’s ability to be transmitted from dam to pup, other breeds of dogs which originate from endemic areas, including Neapolitan Mastiffs, Corsicas and Italian Spinones, have also been found to be infected with the VL parasite.

**Spread of Leishmaniasis**

Spread of VL in tropical areas is primarily due to bites of sand flies transmitting the parasite between dogs and sometimes humans (Figure 1A). In the United States, while sand flies exist in many states with demonstrated cases of canine VL, there have been no Leishmania-infected sand flies trapped in areas around kennels. The primary means of transmission is from infected dams to their offspring (Boggiatto et al, 2011 in press), as well as due to blood to blood contact through biting, wounds, and possibly sexually between infected males and females (Figure 1B). The parasite cannot survive in the environment out of the fly or animal for more than a few seconds. There have not been any cases of hound to human spread of this disease in the US.

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**SIGNS OF CANINE VL**

- Weight loss/Poor condition
- Lack of energy
- Decreased appetite
- Anemia
- Crusty areas on skin
- Overgrowth or brittle nails
- Bloody nose
- Bloody stool
- Watery or goopy eyes
- Big lymph nodes/swollen glands
- “Pot-bellied” appearance

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**FIGURE 1.**

**A. Classical Leishmania infantum Lifecycle**

1. Promastigotes transform to amastigotes in the sandfly gut and migrate to proboscis
2. Sandfly takes a bloodmeal from infected mammalian host and injects infected macrophages
3. Amastigotes transform into promastigotes inside macrophages, multiply and spread to various tissues
4. Promastigotes injected into skin of naïve mammalian host taken up by macrophages

**B. Proposed Leishmania infantum Lifecycle in Foxhounds in the United States**

1. Cells infected with Leishmania infantum passed to pups during gestation and/or birth and/or nursing
2. Direct contact (such as fighting) with blood containing infected cells between infected and uninfected foxhounds
Clinical Signs

The most common treatment in the United States is oral Allopurinol used daily for 3-24 months duration. Please consult your veterinarian for infected kennel.

Treatment

There are multiple diagnostic tests for canine Visceral Leishmaniasis available. These include parasite culture, Immunofluorescent Antibody (IFA) testing, kinetic Enzyme Linked Immunosorbent assay (kELISA)-based serologic testing, Real Time Polymerase Chain Reaction (RT-PCR) analysis and Necropsy (Figure 2). Culture of parasites from bone marrow, lymph node, or splenic biopsy is a direct definitive method of diagnosis. This method is limited as it is highly invasive to get these samples and may be negative even when clinical disease is present. IFA testing involves looking for antibodies to the parasite in the dog’s blood at different dilutions, e.g. “titers”. This method is more sensitive than biopsy with culture and may detect approximately 92% of clinically positive dogs, but does cross-react with antibodies to Trypanosoma cruzi, found in the Southeastern US. This test is much less sensitive in asymptomatic dogs. ELISA-based serologic testing is another means of measuring antibodies made by the dog’s immune system to fight against the parasite. kELISA is a kinetic test detecting anti-Leishmania antibodies in canine serum. This test has a sensitivity of approximately 95% of symptomatic dogs and due to its kinetic nature, will not be positive against T. cruzi. Similar to the IFA test, the kELISA has a more limited effectiveness in asymptomatic dogs. RT-PCR conducted in an experienced, well-equipped laboratory may be the most sensitive diagnostic test available (Figure 2). This test detects Leishmania infantum-specific kinetoplast (unique parasite organelle) DNA, therefore does not cross-react with T. cruzi (Chagas’ disease agent) or other species of Leishmania. It should be noted however, that RT-PCR is a direct method of detecting parasites in the blood and is highly sensitive. Therefore, RT-PCR may detect parasites early in infection months or years before the development of clinical disease. In addition, some animals testing positive on RT-PCR at a low level may control the parasite and never develop clinical infection. The ability of these dogs that do not have positive titers, but are RT-PCR positive, to spread disease is not fully understood. It should also be noted that in infected dogs, a positive RT-PCR is dependent on presence of parasites within the diagnostic sample(s), which can lead to false negative results. Necropsy is the animal equivalent of an autopsy. This process, also known as a post-mortem examination, is performed on a dog after death and looks at the changes in all parts of the body both by the naked eye, or grossly, as well as on a microscopic level. There are characteristic changes in the spleen, liver, lymph nodes, kidneys and other organs which are consistent with chronic infection with L. infantum. In addition, sometimes microscopic parasites can be observed in dog white blood cells. It is not possible to be 100% certain that these are Leishmania parasites just based on microscopy, and additional tests mentioned above are used to confirm the diagnosis.

Treatment for positive dogs in the United States is oral Allopurinol used daily for 3-24 months duration. Please consult your veterinarian for further details.

Diagnosis of Canine VL

The effectiveness of diagnostic testing depends upon the state of disease in the animal, the source (blood, tissue biopsy, autopsy) and handling of sample(s) used and the type of test being utilized. The two most sensitive tests for infection are RT-PCR and kELISA, which can detect infection months to years prior to the development of clinical signs. The CDC IFA is an accurate indicator of soon-to-be clinical disease. All of these methods are useful tools in the arsenal against this disease, but all have significant gaps during the course of disease during which infected animals without any clinical signs may go undetected (Figure 2). Due to these gaps in testing, it is advisable to use at least two different tests to provide an accurate clinical assessment of any dog’s Leishmania infection status.

Diagnostic Tests and the Stage of Infection

- Biopsy and Culture
  - Definitive if positive
  - Invasive
  - Often negative even when disease present
- CDC “titers”-IFA testing
  - Good detection immediately prior to and during clinical disease
  - Limited detection in early Infection
  - Cross-reacts with other parasite diseases (T. cruzi)
- kELISA
  - Good detection in clinical disease
  - Limited in early detection, but picks up 1+ yrs before IFA titers
- ISU RT-PCR
  - Detects parasites
  - Indicates infection, not clinical disease
  - No cross reaction with other parasites

FIGURE 2.

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DIAGNOSTIC STRENGTHS AND WEAKNESSES

- Biopsy and Culture
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  - Invasive
  - Often negative even when disease present
- CDC “titers”-IFA testing
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TESTING: WHO, WHAT, WHERE?

At this point, there is no perfect test, so it is best to use information from more than one test to make informed decisions. Both dogs and bitches to be bred should be tested prior to breeding. To be certain that VL is not in your kennel, you should test all hounds in the kennel.

To run CDC titers, kELISA and ISU or other PCR test, you should:

- Have a skilled blood-drawer take at least 2ml or cc of blood into both a red top and green or purple to tube PER DOG.
- Label each tube with the dog’s name or a number which matches to a key with all dogs ordered numerically. Also provide date of birth and any other important history on each hound sample enclosed-e.g. losing weight lately, has been getting bloody noses, dam, sire and/or other close relatives had a titer back in 2000, etc.
- Call Dr. Peteresen’s lab (319) 335-4148 to notify them samples are coming. For information on packaging, please see https://ahdc.vet.cornell.edu/docs/Shipping_Patient_Specimens_to_the_AHDC.pdf
- Send samples by an overnight courier to:

  **Cornell (kELISA):** ($20/sample, must arrive by Tuesday to run that week)
  Animal Health Diagnostic Center
  Cornell University
  Ithaca, NY 14851

  **University of Iowa (RT-qPCR):**
  Petersen Laboratory
  2501 Crosspark Road
  Center for Emerging Infectious Diseases
  MTF-B109
  University of Iowa
  Iowa City, IA 52242
  319) 335-4148

  **Michigan State (Serology):** ($25.50 sample)
  DCPAH
  4125 Beaumont Road
  Lansing, MI 48910
  (517) 353-1863

  **NC State (Serology/PCR):** (Serology $20 sample, PCR $50/sample, send with Test Request Form):
  NCUS/CVM, VBDDL, RM462A
  1060 William Moore Drive
  Raleigh, NC 27607
  (919) 513-8279

Prevention and Management

**Visceral Leishmaniasis** is a chronic disease which can remain in undetected animals for months or years before clinical disease is detected. There are limitations in the efficacy of all current diagnostic tests. Preventing introduction of canine VL into uninfected hunts and reducing the spread of canine VL within infected hunts must be the goal of all individuals within the Foxhound community. The following recommendations are not exclusive and additional recommendations of disease management may further increase the effectiveness of management.

1. **Maintain General Hound Health** – Adequate nutrition, routine vaccinations, and strategic anti-helminthic treatment improve the overall health of hounds and so improve the hounds’ ability to avoid Canine VL. Spread of VL by fleas and ticks is unlikely, but as they carry other diseases which hinder a dog’s immune response and make dogs susceptible to clinical VL, use of topical insecticide treatments for fleas and ticks is recommended.

2. **Minimize Hound Movement and Exposure to Blood or Wounds within the Kennel** – It is likely that parasite and other disease spread can occur through blood to blood contact, bites and wounds. For untested or disease-positive hunts, minimizing mixing of animals between groups and pens and allowing adequate feeding and watering space reduces resource arguments between dogs and VL transmission. Selection of dogs that are less likely to guard resources within the kennel is also recommended. If a hound appears to be low in the pecking order, consider moving it to a different kennel group.

3. **Diagnostic Surveillance** – Routine diagnostic testing of all adult hounds is recommended annually for hunts with no test-positive hounds or semi-annually if there are test positive hounds in the kennel. The use of multiple tests (serology and RT-PCR) allows both removal of hounds prior to development of clinical illness and decision-making tools prior to breeding selection(s).

4. **Biosecurity** – All hounds being acquired, shipped, or bred should be tested prior to exchanging dogs or breeding. It is recommended that both serologic testing and PCR be conducted on all animals prior to movement into a negative kennel. For best efficacy a minimum of two consecutive negative tests is recommended. Standard Operation Protocols (SOPs) should be created for receiving new animals and handling ill animals. This includes separate areas for reception and isolation. Further information can be obtained from http://www.cfsph.iastate.edu/Infection_Control/general-prevention-for-producers.php

5. **Management of Breeding Hounds** – The most common means of VL spread in US Foxhounds is thought to be through breeding both sexual transmission and from bitch to pups. To eradicate VL from a kennel requires not breeding any RT-PCR or serology test-positive hounds. This includes males and females and should be accomplished either through spaying and neutering of positive animals or exceptionally stringent management practices. While this measure may in the short-term result in a loss of genetic potential, the long-term effects of breeding positive animals results in large numbers of positive pups and the eventual loss of greater genetic potential due to clinical VL. Lack of this means of prevention has led to one-time (or all-in-all out) culling of the vast majority of hounds in several hunts to successfully eliminate disease.

6. **Sanitation** – Although canine VL is thought to spread primarily from breeding, keeping a clean environment prevents spread of Leishmania by reducing the likelihood that hounds spread any infectious disease and become ill from Leishmania due to co-infections, such as Giardia and Cryptosporidia. Regular washing and sanitizing of kennels, food bowls, water bowls, and other materials with detergents and hot water prior to disinfection is the most important step in disinfection. Leishmania are inactivated by 0.25% bleach (6 oz. bleach to 1 gallon of water), 2% glutaraldehyde, or formaldehyde, and are susceptible to heat of 50-60 degrees C. Disinfect and allow 10 minutes of drying time prior to rinsing.

See figure 2, page 3 for more information on Leishmania diagnosis.
Protocol for Foxhound blood draw for Dr. Petersen Leish studies and diagnostic testing

1. Aseptically and with no contamination between samples, draw blood 1-2cc in green top (Heparin) or purple top (EDTA) tubes. A short history of each dog, including clinical signs of wt loss, enlarged lymph nodes, spleen or liver, epistaxis or nose bleeds, etc is very helpful. So is indication of relationship to other dogs within kennel and AKC registration #s.

2. Send these samples overnight via a currier that tracks these samples. In order to have accurate PCR assay results it is important that these samples get here quickly so that the cells are still viable on arrival, therefore it is best to overnight ship the samples to us. Please do not send on a Friday or weekend.

3. Wrap the samples well with bubble wrap so that they do not jostle. If asked, indicate that package contains scientific samples.

4. Include several cold packs to insure the samples stay cool during shipping.

5. Please contact us when you ship the sample so that we can know when to expect your sample.

If there are any questions, please email or call Dr. Petersen, 319-384-1579, christine-petersen@uiowa.edu.

Send samples overnight to:
Petersen Laboratory
University of Iowa
2501 Crosspark Rd
MTF B109
Coralville, IA 52241

Please make a check out to University of Iowa, with Petersen Laboratory on the memo line, for $20 per sample/dog to be tested.

Thank you!

The Petersen Laboratory team