Small Animal Vector-Borne Disease: Diagnostic Challenges and Interpreting Test Results

Barbara Qurollo, MS, DVM
Research Assistant Professor
Dept. of Clinical Sciences, NCSU-CVM
Vector-Borne Disease Diagnostic Laboratory
Barbara_Qurollo@ncsu.edu

Vector-Borne Disease (VBD) Diagnostics

1. When, What and How of VBD Diagnostics?

2. PCR and Serology

3. Cases and Consults
   (Ehrlichia, Rickettsia, Babesia, Bartonella, Lyme Borrelia, and Leishmania)

When to Test for VBD

1. Routine Screening
   a. Is flea/tick prevention working?
   b. annual HW testing
   c. animal has returned/brought to US from overseas

2. Clinical Indications
When to Test for VBD

**Clinical Signs**
- Fever
- Bleeding disorders (petechial, epistaxis)
- Polyarthritis / lameness
- Lymphadenopathy
- Anterior uveitis
- Vasculitis
- Splenomegaly
- Icterus
- Ticks or fleas present

**CBC/Biochem/UA**
- Anemia
- Thrombocytopenia
- Hyperglobulinemia
- Hypoalbuminemia
- Leukopenia
- Leukocytosis
- Lymphocytosis
- Proteinuria
- Hyperbilirubinemia

**Diseases**
- IMHA / ITP
- Kidney Disease
- Vasculitis
- Granulomatous Dz
- Endo/myocarditis
- Lymphoma

What VBD to Test For?

Things to consider other than clinical signs...
- Regional VBD Prevalence
- Co-infections
- Travel History
- Dog Breeds

Regional VBD Prevalence

Travel History

- Determine the travel history (overseas?)
- More dogs from shelters are being relocated
- More dogs being rescued with unknown travel history
Breeds at Risk for specific VBDs

- Babesia gibsoni, B. conradae
- B. canis
- Leishmania infantum
- “Bully” breeds
- Greyhounds
- Foxhounds

How to test for VBD?

Considering...
- shared clinical signs
- expansion of vectors
- pet relocation efforts
- unknown travel histories
- any breed can be infected

Molecular (PCR) and/or serological panels, where you screen multiple VBDs, may be a better option!

Paired PCR and Serology?

A study at NCSU-VBDDL resulted in 4-58% ↑ in recognition and detected more dogs with infection and co-infections

- In a group (I) of healthy dogs, 10% more blood donor would be excluded
- In a group (II) sick dogs (seroneg), 3% more VBD id w/ added PCR in likely low risk cases
- In a group (III) of sick dogs (seropos), 4% more VBD id w/ added PCR
  - If only PCR was used, 92% of VBD seropositive dogs missed
  - In a group (IV) of sick dogs (PCR+), 54% more VBD id w/ added serology
  - If only serology used, 13% individual infections would have been missed

Maggie et al. Comparison of antigenic and molecular panels for diagnosis of vector-borne diseases in dogs.
Parasites & Vectors 2014. 7:127
### VBD: PCR and Serology

**Polymerase Chain Reaction (PCR)**

- **IFA**
- **ELISA**

**Serology**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Serology (serum)</th>
<th>PCR</th>
<th>Best Samples for PCR</th>
<th>Tissues for PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma phagocytophilum</td>
<td>IFA*</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Spleen, BM, LN</td>
</tr>
<tr>
<td><em>Ehrlichia</em> canis, E. chaffeensis</td>
<td>IFA*</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Spleen, BM, LN</td>
</tr>
<tr>
<td>Bartonella</td>
<td>IFA</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Spleen, BM, LN</td>
</tr>
<tr>
<td><em>Rickettsia</em></td>
<td>IFA</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Various tissue</td>
</tr>
<tr>
<td>Babesia</td>
<td>IFA</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Spleen, liver, spleen, liver, kidney, LN, BM</td>
</tr>
<tr>
<td>Cytauxzoon</td>
<td>N/A</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Spleen, liver, spleen, liver, kidney, LN, BM</td>
</tr>
<tr>
<td><em>Borrelia burgdorferi</em> hemotrophic mycoplasma</td>
<td><em>IFA</em>, ELISA</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>Potential LN or skin or tick bite</td>
</tr>
<tr>
<td>Leishmania</td>
<td>IFA, ELISA</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>LN, BM, spleen, conjunctiva, skin</td>
</tr>
<tr>
<td><em>Cytauxzoon</em></td>
<td>N/A</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>?</td>
</tr>
<tr>
<td><em>Hemoproteus</em></td>
<td>N/A</td>
<td>Yes</td>
<td>Anticoag-whole blood</td>
<td>LN or BM aspirate into small volume anticoag-whole blood</td>
</tr>
</tbody>
</table>

### How to send samples?

Always check with the diagnostic lab, but in general...

<table>
<thead>
<tr>
<th>Sample</th>
<th>Container</th>
<th>Storage</th>
<th>Transport</th>
<th>Dealt</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Red (orange) or blue (heparin)</td>
<td>4°C or -80°C</td>
<td>Cooked O/N</td>
<td>Serology (IFA, ELISA)</td>
<td></td>
</tr>
<tr>
<td>Anticoagulated-whole blood</td>
<td>Lavender top with EDTA, light blue top with buffered citrate, yellow green top with heparin</td>
<td>4°C or -80°C</td>
<td>Cooked better, cool O/N shipping</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Synovial Fluid</td>
<td>sterile container, purple or red top</td>
<td>4°C or -80°C</td>
<td>Cooked O/N</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>sterile container, purple or red top</td>
<td>4°C or -80°C</td>
<td>Cooked O/N</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Tissue Aspirates</td>
<td>sterile saline</td>
<td>4°C or -80°C</td>
<td>Cooked O/N</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Tissue (fresh biopsy)</td>
<td>sterile saline or a little sterile saline or in moistened sterile gauze in a small vial</td>
<td>4°C or -80°C</td>
<td>Cooked O/N</td>
<td>PCR</td>
<td></td>
</tr>
<tr>
<td>Tissue (Formalin- fixed)</td>
<td>4°C or -80°C</td>
<td>4°C or -80°C</td>
<td>Cooked O/N</td>
<td>PCR</td>
<td></td>
</tr>
</tbody>
</table>

O/N = over night
RT = room temp
4°C = fridge
-20°C = freezer
PCR: Amplifies pathogen DNA

- Collect 1-2 mL
- Extract DNA
- PCR contains primers, buffer, polymerase
- Target gene on pathogen DNA
- Individual PCRs/well
- DNA amplification of Target gene
- Positive PCR: Billions of copies of Pathogen DNA that we can measure/sequence

How do we see the results and verify the pathogen?
- Conventional PCR run on a gel (size)
- Quantitative PCR (melting temp)

For both cPCR and qPCR, we can then sequence the amplicon to compare the ATCG...

DNA marker

PCR: A positive PCR = Active Infection

- Amplifies pathogen DNA (living or dead organisms)
- Broad detection of all species in a genus or very species-specific
- A positive PCR is conclusive that the pathogen is present (assuming the veterinarian and the laboratory practice good QC to not contaminate samples)
- Most PCRs are sensitive enough to detect 1-2 organisms per sample

...if it’s in the sample
...if it's in the sample

- A single sample from a single time-point
- EDTA-anticoagulated whole blood = best sample for most VBDs (exceptions include Lyme Borrelia and Leishmania)
- **Acute phase**: most VBD = higher pathogen load in blood.
- **Late or chronic phase**: usually lower pathogen load in blood... but still worth testing.
- Pathogen load may be decreased with antibiotics or antiprotozoal use before blood collection... but still worth testing.

PCR: A negative PCR can NOT rule-out infection

**False Negatives can occur when...**

1. The pathogen is not in the sample submitted (blood vs. tissue)
2. Correct sample but pathogen is below the limit of detection (low pathogen load)
   - *Rickettsia* is in the blood for short period of time before moving to endothelial cells.
   - *Bartonella* is usually present at very low numbers in canine blood
   - Therapy initiated before sample collection
3. If the PCR assay is not designed to amplify the pathogen
   - The assay primers may be very specific and not amplify a similar pathogen in the same genus

General Considerations for VBD PCR

1. A positive PCR is conclusive that the pathogen is present (good QC)
2. Good for diagnosing acute and late stage infections (depending on the pathogen)
3. Sensitivity may be decreased if the patient was on antibiotics or antiprotozoals before blood collection.
4. **Collect samples PRE-Treatment and store for potential additional dx (4°C storage OK)**
5. *the VBDDL will hold samples (blood and serum) for NO additional fee.*
6. A negative PCR can never completely rule-out infection
**VBD Diagnostic Serology**

Identifies antibodies in response to pathogen (antigen)

- **Indirect Fluorescent Antibody Test (IFA-T)**
- **Enzyme-linked immunosorbent Assay (ELISA)**
- **Western Blot**

**Indirect Fluorescent Antibody Assay (IFA)**

- **3rd layer** is the conjugate = a fluorescent dye labeled anti-dog or anti-cat IgG and IgM Ab
- **2nd layer** is the serum from a host animal prepared with dilutions: 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048, 1:4096, 1:8192
- **1st layer** is the “known” or antigen layer fixed onto slide wells.

**IFA Interpretation**

- Organisms visualized at higher host serum dilutions = greater # of antibodies in the serum of the patient
- There can be a 2-fold dilution difference in interpretation between different human readers.

**Pathogen** (can be extracellular or intracellular)

Either non-specific staining or aggregates of bacteria
This dog was identified as Rickettsia seropositive at 1:2048.

There can be a 2-fold dilution difference in interpretation between different human readers.

General Considerations for Dx Serology

1. Detects antibodies, not the pathogen so you may only be identifying a previous exposure or a cross-reaction with a similar organism.
2. Not optimal for detecting acute infections (~2 weeks to mount and antibody response)
3. A 4-fold or greater increase in titers measured ~4 weeks apart (acute and convalescent samples) → recent active infection (optimal to test samples in pair test)
4. For many VBDs, high titers (≥ 1:1024) likely correlate with infection
5. Not useful for discrimination of species or subspecies for most VBD
6. Duration of antibodies present after a pathogen has been cleared varies - good option for animals that have been on antibiotic/antiprotozoals in some diseases

SNAP 4DX Plus

Multi-analyte capability

Serum, plasma or whole blood

D. immitis (HW)
A. phagocytophilum
A. platys
E. canis
E. ewingii
B. burgdorferi (Lyme)
Ec/Eew
Bb (Lyme)
### General Considerations for SNAP 4DX Plus

1. Indicates exposure (Ab detection) or HW infection (Antigen).

2. Qualitative, not quantitative: The intensity of the color reaction does not correlate with the level of Abs.

3. Does not detect antibodies induced by Lyme vaccinations.

4. In general, non-clinical dogs seropositive for *Anaplasma*, *Ehrlichia*, or *Borrelia burgdorferi* should have a CBC performed and be checked for proteinuria (Lyme). Dogs with abnormalities should be treated. Consider PCRs or quantitative serology tests.

5. Dogs can remain positive for years (even though organism has cleared)

6. Good tool to see if preventative working?

   *Monitor those blue dots...are new blue dots showing up?*

### Interesting Cases and Consults

**unfortunate situations, flawed coping mechanisms, mayhem, and other things that happened.**

### Consult Question – Rickettsia IFA

**Q:** Results from a Canine Comprehensive Panel (Serology and PCR) showed a *positive Rickettsia rickettsii* IFA (titer of 1:128) and negative for all other VBD dx. Does the dog have Rocky Mountain Spotted Fever?

**SHORT ANSWER:** If the dog is doing ok, with only mild clinical signs, probably not.

An overarching problem with Rickettsia serological assays remains that they cannot distinguish rickettsial infections to the species level.

Abs to other spotted fever group rickettsia (SFGR) cross-react
## Other Rickettsia spp. that infect dogs

### RMSF symptoms
- R. conorii (Mediterranean Spotted Fever in humans)
- Pathogenic significance in dogs is unclear

### Other SFGR that could cross-react with R. IFA
- R. massiliae
- R. amblyommii
- R. montanensis
- R. canadensis
- R. parkeri
- R. felis *
- R. prowazekii *

* Typhus group

### Long Answer: IFA Rickettsia Interpretation

- Cross reaction occurs with other SFGR. It remains inconclusive if the cases reported with RMSF based on serology are truly caused by R. rickettsii or other SFGR.

- Serum IgG and end point titers of ≥1:256 are considered evidence of recent or current infection with SFGR.

- Single serum end point titers <1:256 could be:
  1. An infection at an undetermined time (past infection, early response to recent)
  2. Weak cross-reactivity with another SFGR other than RMSF.

- A 4-fold or greater increase in IgG titer between 2 serum samples drawn 2-4 weeks apart and tested in parallel is considered presumptive evidence of a recent or current infection.

- Antibody is variably absent for 1-2 weeks after onset of symptoms, so an animal with a negative IFA may still have an early Rickettsia infection.

### Acute v. Chronic: Rickettsia rickettsii detection

<table>
<thead>
<tr>
<th>Time</th>
<th>PCR (+) at 5 DPI</th>
<th>Seropositive 1:64 at 10-13 DPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPI</td>
<td>Body Temp (°C)</td>
<td>R. Rickettsii IgG</td>
</tr>
<tr>
<td>5 DPI</td>
<td>thrombocytopenia, anemia; 6-11 DPI: petechial, neurological signs</td>
<td></td>
</tr>
</tbody>
</table>
Rickettsia rickettsii (RMSF)

From a public health perspective, it is important that veterinarians recognize and accurately diagnose RMSF to educate the client as to the potential risk to the family.

- Principle target host cell = endothelial cells = VASCULITIS
- Fever, lethargy, inappetence, petechiae, muscle pain, CNS signs, ocular and nasal discharge, scrotal edema
- CBC: anemia and severe thrombocytopenia followed by secondary thrombocytosis; marked monocytosis; leukocytosis

From a public health perspective, it is important that veterinarians recognize and accurately diagnose RMSF to educate the client as to the potential risk to the family.

- Dogs can be sentinels for RMSF → always explain risk to owners if dog's (+)

Rickettsia Dx Summary

- Dogs that present with a high fever, signs of vasculitis → start treatment (Doxy 5-10 mg/kg BID)
- PCR is best at detecting early infections, serology best later in infection
- Other SFGR can cross react with Rickettsia rickettsii IFA, often are <1:256
- Dogs can be sentinels for RMSF → always explain risk to owners if dog’s (+)
- Dogs infected with RMSF that recover should have life-long immunity

Consult Question – E. canis

Q: A dog was SNAP®4DXPlus® Ehrl positive 6 months ago with thrombocytopenia. Treated for 30 days with doxycycline and clinical signs resolved. The dog is doing well clinically but the owner requested a follow up test.

VBD results: All PCRs (-), Ec IFA 1:64 (+), Rr 1:128 (+), Snap®4DXPlus® Ehrl (+), Do I treat him again?

A: Probably not, but maybe.

Titers may be left over from original infection and SNAPs can remain positive for years or maybe new infection because E. canis antibodies are not protective.

Find out if dog’s been on tick prevention. Recommend BIC if no abnormalities probably don’t need to treat.
Consult Question – Ehrlichia

Q: 2 year old MC Boxer Mix - presented for intermittent fever (106 F) and non-regenerative (mild-moderate) anemia of just over 1 week duration. Serology was sent to NCSU (did not run PCR b/c the dog was started on Doxy a few days prior to presenting).

E. canis IFA 1:512 (+) and Rickettsia IFA 1:256 (+); Snap®4DX Plus was negative.

How can the Ec IFA be positive and the Snap®4DX Plus be negative?

A: Snap®4DX Plus and E. canis IFAs are usually concordant, but not always

- Ec IFA uses whole cell antigen and may have more Ehrlichia spp. cross-reactivity
  (Panola Mountain Ehrlichia, E. chaffeensis, E. multi)

- Snap®4DXPlus uses synthetic peptides designed from a few OMP and usually doesn’t detect antibodies below 1:256

11-year-old, castrated male Scottish Terrier from NC

CBC / Biochemistry
mild thrombocytopenia
↑ atypical lymphocytes
↑ ALP, ↑ ALT

Serology
R. rickettsii (1:64), E. canis IFA (1:1024), Snap®4DX Plus, Ehrlichia (-)

Ehrlichia PCR
Panola Mountain Ehrlichia (PME) sp. in a Dog
16S rRNA PME (100%), gltA PME (100%), map1 PME (100%), negative for other Ehrlichia spp.

Ehrlichia Dx Summary

- Snap®4DXPlus + (+) w/ clinical signs → tx (Doxy 5 mg/kg BID or 10 mg/kg SID 30 days)
- Snap®4DXPlus + (+), no clinical signs → PCR (+/- panel) and/or blood work
  (PCR and/or abnormal BW then treat)
  (PCR and/or normal BW may not need to treat)

- Dogs can remain Ehrlichia Snap®4DXPlus + (+) for years
- Snap®4DXPlus and E. canis IFAs are usually concordant, but not always
- Ehrlichia infections can cause clinical signs similar to lymphoma such as: monoclonal gamopathies, atypical lymphocytes, clonal expansion of T-cells.
Consult Question-Babesia

**Q:** Acute and convalescent *B. canis* IFA titers dropped from 1:128 (acute) to 1:64 (convalescent). Is there any significance or value to paired IFA titers in babesiosis?

**A:** In acute Babesia infections, we can see an increase in convalescent samples; however, we see a lot more “newly diagnosed, chronic Babesia” so it is less common for us to actually detect a four-fold increase.

Monitor treatment response with PCR at 60 and 90 days post-tx. No good data on Babesia titers after treatment.

A dog with clinical signs of babesiosis that is also seropositive:
1. Run a PCR to identify the species and determine best treatment method
2. If PCR is negative or you can’t run PCR, chose a treatment and assess response (signs of disease, not the titer)

---

**Babesia: Treatment Recommendations**

- **B. gibsoni, B. conradae, B. microti**
  - Atovaquone (13.5 mg/kg q8h PO) and Azithromycin (10 mg/kg q24 PO) for 10 days

- **B. canis (vogeli) and Babeisa spp. coco**
  - Imidocarb diproionate (6.6 mg/kg IM or SC), repeat in 14 days
  - Pretreatment with atropine or glycopyrrolate minimizes cholinergic adverse effects

* There are not enough data to determine if parasitemia is cleared consistently

---

**Consult Question:**

**April 2015:** 5 yr MC schnauzer, that recently moved from TX to Canada. He has a waxing-waning low-grade fever, decline in appetite, hyperglobulinemia, mild anemia. His clinical signs resolve temporarily after 30 days of doxycycline.

**Serum Tick Panel**
- Bgib IFA - neg
- Bc IFA - neg
- Ec IFA - neg
- Bartonella IFA - neg
- SFG Rickettsia IFA - neg
- Snap4DXplus® (Anx/Ee/Few/Lyme/HW) – neg

**Q:** What other vector-borne disease should I look for?

**Answer:** Add PCR panel
Added PCR Panel

Babesia 18S PCR – neg
Bartonella PCR – neg
Anaplasma PCR – neg
Ehrlichia PCR – neg
Rickettsia PCR – neg
Bartonella PCR – neg
Mycoplasma PCR – Mycoplasma hematoparvum

Is M. hematoparvum REALLY is the cause (or only cause) of his waxing-waning low-grade fever, decline in appetite, hyperglobulinemia, mild anemia?

Follow up: Clinical signs resolved on doxycycline...yay!
But clinical signs returned several months later and the dog was treated with doxy.
Dog got better...yay!
But clinical signs returned several months later...

February 2016: Waxing-waning low-grade fever, decline in appetite, splenomegaly, thrombocytopenia (65K).

Does this dog need to be treated for Mycoplasma infection off and on forever?

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesia LSU PCR (new PCR)</td>
<td>B. microti-like</td>
</tr>
<tr>
<td>B. microti-like</td>
<td></td>
</tr>
<tr>
<td>Bartonella PCR</td>
<td>neg</td>
</tr>
<tr>
<td>Anaplasma PCR</td>
<td>neg</td>
</tr>
<tr>
<td>Ehrlichia PCR</td>
<td>neg</td>
</tr>
<tr>
<td>Rickettsia PCR</td>
<td>neg</td>
</tr>
<tr>
<td>Bartonella PCR</td>
<td>neg</td>
</tr>
<tr>
<td>Mycoplasma PCR</td>
<td>neg</td>
</tr>
</tbody>
</table>

1. confirmed
2. April 2015 sample
- Bab LSU PCR (+) B. microti-like
- He was treated with 10 days of Atovoquone / Azythromycin
- Monitored for clearance with PCR, post treatment
  - 60 days –PCR negative
  - 90 days –PCR negative
- All clinical signs of disease have remained absent!

- First case of a *B. microti*-like infected dog with clinical signs of Babesiosis in North America
- Since then, we have detected 9 additional dogs with *B. microti*-like

**Things to learn from this Schnauzer...**

- Combine serology and PCR and consider repeat testing in sick dogs not responding treatment -no test is perfect!
- Just because an animal appears to respond to doxycycline, doesn’t mean it is infected with a pathogen that will be cleared by it.
- Any breed can be infected with Babesia.
- **Babesia microti-like** may be more prevalent in the North America than previously thought (detected in 8 dogs by VRDDL since 2016).

**Consult Question -Babesia**

An anemic dog with splenomegaly. Sample submitted for a Tick panel (IFA serology), results: B. gibsoni sero (+) 1:64 and B. canis sero (+) 1:512

How do I treat the *B. canis* infection?

**Answer:** There is some degree of sero cross-reactivity between *Babesia* spp. so it’s best not to use serology to speciate (tx is different for different spp.)

PCR is the best way to speciate a pathogen to determine the appropriate treatment:
- If initial PCR is *Babesia* (+), then retest at 60 and 90 days post treatment
Q. My patient was treated with Atovaquone and Azythromycin (AA) for a B. gibsoni infection and clinical signs resolved. Retest PCR 60 days later = B. gibsoni (+) and his RBC and HCT started to decrease again so we restarted the AA regimen. He’s been on the 2nd AA treatment for 5 days, dog is worsening.

Answer:
If AA fail to clear B. gibsoni, do not repeat the same treatment (~15% won’t clear with AA treatment). It is likely a strain resistant to AA. Use the following rescue:

Treatment protocol for resistant Babesiosis:
- clindamycin (25 mg/kg q12h PO)
- doxycycline (5 mg/kg q12h PO)
- metronidazole (15 mg/kg q12h PO)

given daily for 3 months.
Bartonella: Treatment Recommendations

**Dogs (treatment 28-42 days)**

- Doxycycline (or minocycline) PO (10 mg/kg) BID and Enrofloxacin PO (5-20 mg/kg) SID

  (+) endocarditis/myocarditis

  Amoxicillin IV or IM (15-30 mg/kg) SID for 7-10 days while monitoring UA for casts and periodic BUN/Cr then start anticoagulants.

  Doxycycline (or minocycline) PO (10 mg/kg) BID and Enrofloxacin PO (5-20 mg/kg) SID

  (+) CNS involvement

  Minocycline PO (5 mg/kg) SID and Doxycycline PO (10 mg/kg) BD for 4 weeks

**Cats (treat 28-42 days)**

- Pradofloxacin (5 – 10 mg/kg) PO BID (or SID)
- Doxycycline PO (10 mg/kg) BD or Minocycline 8.8 mg/kg PO SID

  (give cats water or food to wash pills down to avoid esophageal strictures)

Bartonella Dx Summary

Because cats are reservoirs for Bartonella, it is not clear to what extent it is responsible for clinical disease. More virulent strains and immune status of the host likely play a role in clinical disease.

- For cats, in general
  - 1. Treat sick, PCR (+) cats (inform owners of zoonotic potential)
  - 2. Consider treatment of sick, seropositive cats (+/- treatment)

- If you suspect or confirm Bartonellosis in dogs (PCR or seropositive), an echocardiogram is recommended (treatment is different if endocarditis or myocarditis is diagnosed).

- Monitor response to treatment in dogs with PCR (+) or IFA (decline in titers is typically seen in weeks to a couple of months with effective therapy)

- Due to lower levels of Bartonella bacteremia in canine bartonellosis, PCR amplification can be difficult (false PCR (-)). Serial blood collections or enrichment PCR (Galaxy Diagnostics) can increase the sensitivity for detection.

Consult Question-Lyme

**Q.** I have a dog that is Lyme (+) on Snap®4DX® test but does not have any clinical signs. Do I still need to treat with a course of doxycycline?

**A.** Potentially. Currently there’s no consensus on whether to treat.

Regardless of whether the dog is treated, you should:

1. Monitor for proteinuria Q 2-4 months for ~1 year
2. Discuss tick prevention
3. Discuss co-infection potential
**Borrelia burgdorferi: Treatment Recommendations**

Lyme + with signs: easy, treat it (30 days)!

- **Doxycycline 10mg/kg PO SID**

Lyme nephritis suspects (1 mo vs 3-6 mo)

Lyme + with NO clinical signs

NO consensus right now on what to do with these dogs except…check for proteinuria!

If present then treat.


Updates from ACVIM 2016

**Lepto titers**

Recheck proteinuria q2-4 months for 1st year then periodically

**Lyme nephritis suspects (1 mo vs 3-6 mo)**

- **Borrelia burgdorferi Dx**

  - **PCR:** not optimal for detecting *B. burgdorferi*
  - **Snap®4Dxplus®:** Measures antibodies to C6 antigen (no quantification)
  - **Quant-C6:** Measures antibodies to C6 antigen but can quantify response
  - No cross reaction with vaccination

Cornell Multiplex and Antech Accuplex detect antibodies to three Osp antigens

- **OspA:** vaccination typically
- **OspC:** recent infection (14-21d)
- **OspF:** chronic infection

**Borrelia burgdorferi: Additional Information**

**Cannot measure clearance with Snap®4Dxplus®**

1. dogs can remain positive (up to 2 years)
2. additional Abx treatments are not necessary if dog remains Snap®4Dxplus® positive after clinical signs resolve

**Measure decline in C6-Abs with quantitative C6 ELISA (IDEXX, Inc)**

1. decline was seen in dogs with initially higher C6-Abs, less change in dogs with low initial C6-Ab levels
2. maybe a good indicator of dogs more likely to develop Lyme nephritis? (correlation in high C6-Ab and circulating immune complexes?)

Goldstein RE, Atwater DZ. Serology and circulating immune complexes in dogs naturally infected with *Borrelia burgdorferi* before and after doxycycline therapy [abstract #13]. Proc 24th ACVIM Forum 2006;733.


- Measure decline in C6-Abs with quantitative C6 ELISA (IDEXX, Inc)
  1. decline was seen in dogs with initially higher C6-Abs, less change in dogs with low initial C6-Ab levels
  2. maybe a good indicator of dogs more likely to develop Lyme nephritis? (correlation in high C6-Ab and circulating immune complexes?)

- Goldstein RE, Atwater DZ. Serology and circulating immune complexes in dogs naturally infected with *Borrelia burgdorferi* before and after doxycycline therapy [abstract #13]. Proc 24th ACVIM Forum 2006;733.
9. 7 yo MM Chihuahua in New Orleans. The dog just got back from a month in Spain. Now, he developed a draining tract on his left cheek and bilateral blepharitis. Little improvement with systemic antibiotics. Cytology of exudate revealed pyogranulomatous inflammation (reviewed by a pathologist). Would you recommend Serology or PCR to test for Leishmania?

**Answer:**

Recommend starting with just serology (IFA) for leishmaniasis but send in a hold blood/LN aspirate in case the IFA is negative and we want to run PCR.

---

**Leishmania Dx Summary**

1. A positive Leishmania IFA in symptomatic dogs = active infection.
2. Some asymptomatic, exposed dogs may be weakly IFA positive.
3. PCR will detect both symptomatic and asymptomatic animals within limits of test.
4. For PCR detection, bone marrow or LN are best; blood is next best sample. (we recommend LN aspirate injected into small volume ≤0.5mL EDTA whole blood)
5. Serology (IFA) may cross react with *T. cruzi* (southern states)

---

**Final Thoughts...**

- Comprehensive Screening - animals are relocated more - tick ranges expanding - VBD clinical signs vague/similar
- Combine serology and PCR and consider repeat testing in sick animals not responding to treatment - no test is perfect!
- Don’t forget to check for co-infections - if you suspect one VBD, you should be looking for others - if treatment fails, look for co-infections
- Annual Screening (SNAPs for example)... is preventative working? Monitor the blue dots... are new blue dots showing up?
- Administration of antibiotics/antiprotozoals before PCR testing may result in false [-]. BUT still may be worth testing. SAVE BLOOD (fridge or freezer)
If you have a problem, you can always call us (NCSU-VBDDL)
- results don’t make sense (suspect a false (-) or false (+))
- you need a consult on a case:

NCSU-VBDDL 919-513-8279

Questions?