

North Carolina State University

College of Veterinary Medicine

Vector Borne Disease Diagnostic Laboratory



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www.cvm.ncsu.edu/docs/ticklab.html

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Sensitivity of Bartonella PCR

Hunter Blanton, Ph.D.

We are constantly working to improve the specificity and sensitivity of our molecular diagnostics and want to share with you our latest sensitivity results on the detection of *Bartonella henselae*.

The sensitivity of PCR assays to detect microorganisms is usually determined by comparing the number of microorganisms observed by visualization or colony growth, to the number of times that particular number of organisms is detected using PCR. Unfortunately *B. henselae* cannot be easily detected visually and it is extremely difficult and costly to culture. Therefore we assayed known numbers of a segment of *B. henselae* DNA. This segment is present twice in the *B. henselae* genome so two copies of this segment equal one genome equivalent in our assay. Results of our work are presented in the following table.

Preliminary findings on the frequency of detection of *Bartonella henselae* genome equivalents using the VBDDL Bartonella genera PCR protocol.

<i>B. henselae</i> Genome Equivalents	Frequency of Detection (percentage)
50	40/40 (100)
37.5	20/20 (100)
25	18/20 (90)
12.5	17/20 (85)
5	11/20 (55)
1	0/20 (0)

In short, our assay can detect the presence of 37 organisms all the time, 12 organisms most of the time and 5 organisms half of the time in standard sample volumes.

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Expanding our horizons: the potential of *Bartonella* transmission by ticks

Your participation is needed for an epidemiology survey

Due to the ability of *Bartonella* spp. to reside within red blood cells of a diverse number of animal hosts, cats, dogs, humans, cows, rodents, and other animals there is opportunity for the potential uptake of these blood-borne bacteria by a variety of arthropod vectors. In the past decade, focus has begun to shift to ticks as potential vectors of *Bartonella* species. Ticks are important vectors in the transmission of human diseases, reportedly second only to mosquitoes⁸. Disease transmission by ticks is generally seasonal, particularly in the spring and summer, as humans and dogs encroach on tick-laden environments. Many tick species will feed on a wide range of hosts, which only complicates studies that attempt to determine if ticks can transmit *Bartonella* species.

Several studies (see website for complete article) have provided strong circumstantial evidence supporting tick transmission of *Bartonella* species. At this point, many questions have yet to be answered.

Sarah A. Billeter, a PhD graduate student funded by the VBDDL, is the poor soul responsible for answering some of these elusive questions. Sarah is hoping that interested veterinarians could collect brown dog ticks, *Rhipicephalus sanguineus*, from dogs or kennel environments. If removed from dogs, it would be greatly appreciated if 2 mls of whole blood and 1ml of serum from the dog be included with the tick specimens. Engorgement status is not important; ticks do not necessarily need to have fed.

Please contact Mrs. Billeter at 919-513-0778 or at her email address (sabillet@ncsu.edu) with any questions. She would greatly appreciate your help!!

Babesiosis: Diagnosis and Treatment

Adam Birkenheuer, DVM, Ph.D.

- There are at least 8 genetically unique *Babesia* species or subspecies that can infect dogs and two in cats including; *Babesia canis canis*, *B. c. vogeli*, *B. c. rossi*, *B. gibsoni*, *B. conradae*, *Theileria annae*, *B. felis* (Feline), *B. canis presentii* (Feline) and several unnamed yet genetically unique *Babesia* spp. This may affect test interpretation because most laboratories only test for 2 canine species and cross reactivity is not always present. Knowledge of which species are tested for is important for both antibody and DNA testing.

Risk factors

- Breed
 - American Pit Bull Terriers: *Babesia gibsoni*
 - Greyhounds: *Babesia canis vogeli*
- History of tick attachment
- Splenectomized animals develop more severe clinical disease.
- Immune suppression may cause clinical signs and increased parasitemia in chronically infected dogs.
- History of a recent dog-bite wound is a risk for *B. gibsoni* (Asia) infection.
- Recent blood transfusion from a subclinically infected donor.

Disease (one or more of the following are good reasons to test)

- Anemia (typically regenerative)
- Thrombocytopenia (more common than anemia in some studies and can occur without anemia)
- Hyperglobulinemia
- Splenomegaly
- Icterus
- Pigmenturia
- Screening of blood donors
- Screening of breeding animals
- Normal? Some recovered animals have no detectable abnormalities yet remain persistently infected

Testing

- Polymerase chain reaction (PCR): Amplification of a specific piece of DNA from the organism of interest. Since *Babesia* lives in red blood cells, EDTA anticoagulated whole blood is the sample of choice for *Babesia* PCR testing. Obtain samples BEFORE treatment, since treatment may reduce number of organisms and result in false negative test results. The PCR test used by the VBDDL can detect parasitemias of 0.00000073% or about 1300 fold fewer organisms than microscopy.
- Indirect fluorescent antibody (IFA): Detection of antibodies against the organism of interest. Serum is the typical sample tested for antibodies.
- Light microscopy: Detection of *Babesia* organisms in red blood cells. Parasitemia of infected animals can range from 0.0001% to >10% of the red blood cells. Thin stained peripheral blood smears are the typical sample tested. Smears made from capillary blood (ear or toenail) may improve organism recovery.

Treatment

- Imidocarb dipropionate (FDA approved; 6.6 mg/kg SC or IM every 1–2 weeks) and diminazine aceturate (not FDA approved; 3.5–7 mg/kg SC or IM every 1–2 weeks) decrease morbidity and mortality in affected animals. This may completely clear *B. canis* infections but not *B. gibsoni*. Combination therapy of azithromycin (10 mg/kg PO q24h for 10 days) and atovaquone (13.5 mg/kg PO t.i.d for 10 days) is the treatment of choice and the only treatment that can potentially clear *B. gibsoni* (Asia) infections in dogs. In a controlled study 85% of dogs cleared the infection after treatment. Metronidazole (25–50 mg/kg PO q24h for 7 days), clindamycin (12.5–25 mg/kg PO b.i.d. for 7–10 days), and doxycycline (10 mg/kg PO b.i.d. for 7–10 days) have been reported to decrease clinical signs but not to clear infections.
- Primaquine phosphate (1 mg/kg IM, single injection) is the treatment of choice for *B. felis*.

QUESTIONS RELATED TO INTERPRETATION OF THE IDEXX SNAP 4DX

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Veterinarians play a central role in the diagnosis, treatment and prevention of tick-transmitted infectious diseases of companion animals. Veterinarians also play an increasingly important role in advising the public as to the zoonotic potential of organisms that are transmitted from ticks to pets or to their owners.

The SNAP 4DX test detects *Dirofilaria immitis* antigen, antibodies directed against *Borrelia burgdorferi* C-6 peptide, antibodies to two synthetic *Ehrlichia canis* immunodominant proteins and antibodies to *Anaplasma phagocytophilum* immunodominant peptides.

Interpretation of a Positive Ehrlichia canis Reaction:

Following introduction of the SNAP test, the VBDDL participated in a collaborative study with IDEXX laboratories to determine the extent to which *E. canis* SNAP positive, healthy dogs are IFA seroreactive and actively infected based upon PCR testing. In 86 *E. canis* SNAP+ samples submitted by regional veterinary hospitals from untreated dogs, 58% of the dogs were thrombocytopenic, 99% IFA seroreactive and 14% PCR +. It does appear that some dogs, naturally infected with *E. canis*, are capable of mounting an effective immunological response that may eliminate the organism. These dogs would mount an antibody response detectable by SNAP or IFA, but have normal hematological findings and would be PCR negative. (**Caution:** A negative PCR result can support immunological or therapeutic elimination of an

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infection, but can never completely confirm that an individual animal is not infected with a given organism.) When veterinarians tested sick dogs, the presence of antibody appeared to correlate with active infection, regardless of the level of the antibody titer. This may not be true in the healthy dog population. If a positive *E. canis* SNAP result is obtained on a healthy dog, I would recommend examination of a complete blood count prior to treatment. If the dog is anemic, neutropenic, thrombocytopenic or hyperglobulinemic, then treatment with doxycycline 5 mg/kg every 12 hours for 4 weeks (See ACVIM Consensus Statement on Canine Ehrlichiosis) would be recommended. If the complete blood count values are within normal reference ranges, treatment may not be indicated. Alternatively, the SNAP test result can be confirmed by IFA testing and the current infection status of the dog can be determined by PCR testing.

Although our experience to date is limited, some dogs may remain *E. canis* SNAP positive one year after an initial positive SNAP test result and following appropriate treatment with doxycycline. When tested by both IFA and PCR (one year post-treatment) neither antibodies nor DNA was detected suggesting that the infection was therapeutically or immunologically eliminated. This preliminary observation should be considered when repeating an annual test on a dog that was previously *E. canis* positive by SNAP.

Anaplasma phagocytophilum

Anaplasmosis, caused by *A. phagocytophilum*, is characterized by an acute, febrile illness in cats, dogs, horses and human beings. *Anaplasma phagocytophilum* can also infect numerous other wild animal species that serve as reservoir hosts for subsequent transmission by *Ixodes scapularis*, *Ixodes pacificus* and perhaps other tick species.

Clinical Application: The SNAP 4DX test detects antibodies to a synthetic *A. phagocytophilum* immunodominant protein. Due to serological cross reactivity, the test will also be positive in dogs previously exposed to *Anaplasma platys*, the cause of cyclic canine thrombocytopenia. *A. platys* appears to be transmitted by the brown dog tick, *Rhipicephalus sanguineus*. Based upon PCR testing, canine anaplasmosis is frequently encountered in sick dogs and people in the northeastern, north central and north western United States and southern Canada. Due to transmission by the same tick vector (*I. scapularis* or *I. pacificus*) in the United States, co-infections with *B. burgdorferi* and *A. phagocytophilum* are common in "Lyme-endemic" regions. Dogs infected with *A. phagocytophilum* can develop only mild illness or perhaps no clinically apparent illness at all. Dogs co-infected with *B. burgdorferi* and *A. phagocytophilum* are more likely to develop severe disease signs. Experimentally, dogs can develop chronic *A. phagocytophilum* infection in the absence of clinical signs of disease. The extent to which natural infection following tick transmission results in chronic infection

or induces chronic disease manifestations is unknown. In contrast, infection with *A. platys* can induce chronic infection, accompanied by a moderate to severe cyclic thrombocytopenia, but generally without accompanying severe clinical manifestations. An *Anaplasma* SNAP+ dog that has resided in the southeastern United States is more likely to have been exposed to *A. platys*, which is a relatively common tick borne infection, as compared to *A. phagocytophilum*, which is infrequently transmitted to cats, dogs, horses or human beings in the southeastern US (similar to data relative to patterns of *B. burgdorferi* transmission).

Treatment: Treatment of healthy *Anaplasma* SNAP+ dogs in Lyme-endemic regions is not currently recommended as there is limited evidence that detection of antibodies correlates with chronic infection due to *A. phagocytophilum*. PCR could be used to establish chronic or recurrent infection due to *A. phagocytophilum*. *Anaplasma* SNAP+ dogs from the southeastern US, Central or South America may be infected with *A. platys*, which would warrant treatment if the dog was thrombocytopenic (remember the decrease in platelet numbers is cyclic) or PCR+. Both infections are thought to respond to doxycycline. The duration of treatment has not been clearly established for either infection so dogs are generally treated for 4 weeks as proposed for *E. canis* infections. Co-infection with *E. canis* and *A. platys* causes more severe thrombocytopenia and may be more difficult to elicit a cure with doxycycline. The influence of co-infection on treatment outcome is unknown.

In healthy dogs, *B. burgdorferi*, *E. canis* and *A. phagocytophilum* test results should be recorded in the patient record. This information would be useful to the clinician if the dog develops compatible disease manifestations at some future time. For questions related to our testing capabilities, contact the VBDDL at North Carolina State University at 919-513-8279 or by e-mail (Julie_Bradley@ncsu.edu). You can also visit our website at: (www.cvm.ncsu.edu/docs/ticklab.html) for diagnostic request forms and other information.

This information is provided to veterinarians as a service of the College of Veterinary Medicine, North Carolina State University.

----- **How Do I Send Samples?**

All packages should be sent overnight on ice packs to:
NCSU-CVM
RM 462A
4700 Hillsborough Street
Raleigh, NC 27606

Samples sent through the mail take an average of 7 days to reach our laboratory. Please send samples overnight via FedEx, DHL, or UPS. This provides a paper trail, should a sample get mishandled. The VBDDL is closed on the weekends. Should a sample be ready for shipment on Friday, refrigerate and ship on Monday.

Cytauxzoon Test Update and Clinical Trial

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Cytauxzoon felis is a tick-transmitted protozoal disease of domestic cats that is typically characterized by acute onset of fever, bi-cytopenia or pancytopenia, and icterus. This disease is endemic to the south and southcentral United States and has been most recently recognized in North Carolina, Virginia and South Carolina.¹

Cytauxzoonosis is typically identified between April and September which correlated with the time we most frequently identify ticks on cats. Historically this disease has been considered to be fatal in nearly 100% of infected cats. Some studies have demonstrated that some cats survive infection. The VBDDL has recently developed a PCR test for *C. felis* that can be run on a STAT basis (24-72 hour turnaround) to aid in rapid and accurate diagnosis of this infection.² In addition, the VBDDL is collaborating with Dr. Leah Cohn at the University of Missouri in performing a clinical trial evaluating two treatments for cytauxzoonosis. Preliminary data suggests that one of these treatments is associated with survival rates of over 60%. If your practice is in an endemic region that sees multiple cases per year and you are interested in participating in our trial please contact Dr. Adam Birkenheuer (ajbirken@ncsu.edu).

Visit our website for more information:

- Updated Diagnostic Request Form
- Updated PCR Testing information
- Articles in this news letter in full and with references.

www.cvm.ncsu.edu/docs/ticklab.html



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