



# NATIONAL VETERINARY LABORATORY

P.O. Box 239, 1Tice Road  
Franklin Lakes, NJ 07417  
877-NVL-LABS (877-685-5227)  
[www.natvetlab.com](http://www.natvetlab.com)

## NEWSLETTER

### *Bartonella* Screening and Therapy Evaluation Tests<sup>©</sup>

Evelyn E. Zuckerman, Editor

Winter 2013

Vol. 12, Number 1

#### In This Issue:

In the winter 2013 issue of the NVL Newsletter we will describe the 3 *Bartonella* tests done by this laboratory: 1) screening FeBart<sup>®</sup> Test, 2) FeBart<sup>®</sup> Kitten Retest for Incubation Test (RT-I) and 3) the therapy evaluation test- the Therapy Titration Test. These tests determine the antibody responses of cats or dogs to *Bartonella* infection.

#### *Bartonella* Tests:

Pet cats can be infected with at least 6 species of *Bartonella* and any age cat is susceptible to infection.

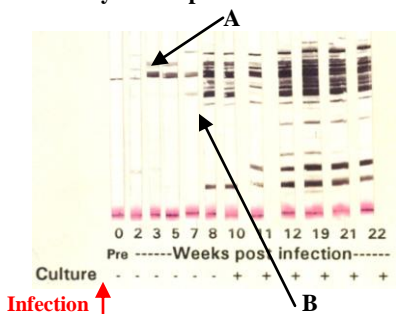


Cat and dog fleas and ticks all can carry and transmit *Bartonella* from cat to cat. Like many other chronic bacterial and viral infections such as FIV, *Borrelia* and *Ehrlichia*, antibody to *Bartonella* co-exists with infection and, in most cats, does not clear the infection for years if ever (Figures 1 & 2). Presently there are 5 blood tests available to detect *Bartonella* spp.: 1-3) serology- western blot, ELISA, and IFA tests, 4) PCR DNA detection, and 5) culture.<sup>1-7</sup> However, it must be emphasized that *Bartonella* often are only intermittently present in the peripheral blood (bacteremia) of infected animals which can make culture and PCR methods problematic.<sup>5</sup> Intermittent bacteremia does not alter the serologic detection of antibodies to *Bartonella*. Several veterinary laboratories now offer IFA and ELISA serologic, PCR and culture tests but none of them offer therapy evaluation tests.

#### Serologic Detection of Early Infection:

Figure 1

##### Antibody Development After Infection

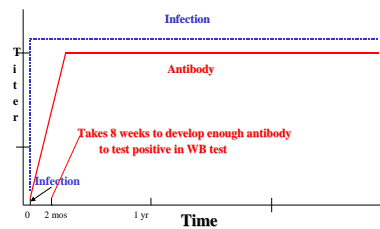


We studied the development of antibody against *Bartonella* by inoculating a small

amount of infected blood into an adult cat and following the development of antibodies against the bacteria and the ability to isolate the bacteria from the blood. As can be seen in Figures 1 & 2, it takes about 3 weeks after infection for antibody to begin to form (Figure 1 A) and 8 weeks before there is enough antibody to consider this cat serologically positive (Figure 1 B). Bacteria were first isolated from the blood at week 10. The kinetics of antibody formation is important for our understanding of the infection of kittens, under 6 months of age, and the ability to detect infection by serological tests. This is the reason we recommend the retesting, by the FeBart<sup>®</sup> Retest for Incubation Test (RT-I), of all kittens 6 months old or younger who test negative or +1 (uninfected).

Figure 2

#### *Bartonella* Infection Coexists with Antibody



#### FeBart<sup>®</sup> *Bartonella* Test:

The FeBart<sup>®</sup> *Bartonella* screening test is a western immunoblot (WB) serological test for detection of antibodies against the structural proteins of *Bartonella*.<sup>1,2</sup> The western immunoblot is the most sensitive and specific (accurate) serological test compared to immunofluorescence (IFA) and ELISA tests.<sup>8</sup> It is also able to detect cross-reactive antibodies to all 6 feline *Bartonella* species.

In 1995, the concordance of WB serology with isolation of *Bartonella* in culture was as follows: WB negative or +1= 96% culture negative, whereas WB +3 or +4= 53% culture positive.<sup>1</sup> The poor WB +3 or +4 serological/culture concordance may have been due to the use of inadequate culture techniques available at that time, the use of only one point blood draws, which did not take into consideration the intermittent bacteremia that is common in *Bartonella* infected animals, and the fact that some of the cats had been treated with antibiotics before blood was drawn for culture. However in a small study, we have recently found

100% agreement with 8 WB +4 cats by culture isolation at a commercial laboratory that now employs improved culture and PCR techniques (Hardy, WD, Zuckerman, EE and Broadhurst, J Unpublished observations).

#### *Bartonella* Proteins- Cat Serum

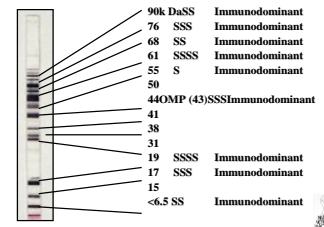


Figure 3 FeBart<sup>®</sup> screening test: *Bartonella* WB proteins recognized by the cat's specific *Bartonella* antibody "immune fingerprint."

The WB is an expensive and labor-intensive immunological technique but it is considered the "Gold Standard" technique for detection of antibodies in diagnostic samples. The WB is the most sensitive AND specific antibody assay available as it gives a "finger-print" of the animal's specific immune response to each diagnostic protein of an organism (Figure 3).<sup>8</sup> In order to be considered serologically positive, cats must have antibodies to at least 7 *Bartonella* proteins and any cross-reacting antibodies to bacteria such as *Chlamydia* can be identified and discounted (Figure 4). There are cross reacting *Chlamydia* and *Bartonella* antibodies which can be problematic for ELISA and IFA serological tests since they give an all or none color change as their end point and any of the multiple antibodies, including the non-diagnostic cross reacting antibodies, can be responsible for the positive color end point.

#### FeBart<sup>®</sup> Western Blot (WB) Test

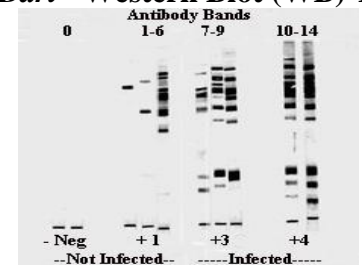


Figure 4 Revised grading system for the FeBart<sup>®</sup> Western Blot (WB) Test. - and +1= not infected, +3 & +4= infected. Antibody against at least one low molecular weight protein must be present to be seropositive.

### Kittens and Bartonella:

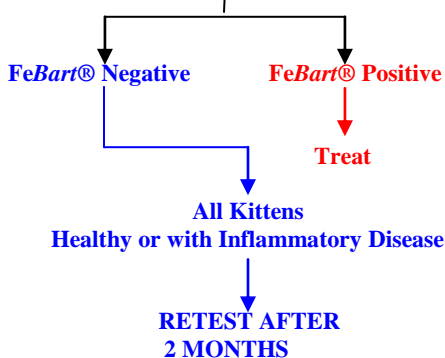
Kittens quickly become welcome members of many households each year and the special friends of children in those households. Children often allow kittens to lick their faces, to eat from their plates and to sleep in their beds. They also play more vigorously with the kittens than do the adults in the households thereby receiving playful bites and scratches more frequently than the adults. *Bartonella* organisms are found in the blood plasma, inside erythrocytes and endothelial cells and in tissues of infected cats. In order to be transmitted to people, the organism must be present on the claws (scratch), in the mouth (bites) or on the fur (contact- no abrasion) of infected cats. Infected kittens are rapidly growing and have changing dentition leading to the probability that *Bartonella* can leak into the oral cavity. The loss of kitten teeth or oral trauma due to rough play, chewing and playful fighting, can lead to *Bartonella* in the oral cavity. Cats groom themselves frequently thereby depositing *Bartonella* organisms from the oral cavity onto their fur or claws. The fact that kittens and children are both more playful toward each other presents the conditions needed for the zoonotic transmission from kittens to children.

### Bartonella Tests for Kittens:

All newly introduced kittens, at any age, should be screened for *Bartonella* infection at their first examination. Since the FeBart® test is a test for antibody against various *Bartonella* proteins, a positive test in a kitten may represent maternal antibody or kitten antibody. The western blot technique is so sensitive that it can detect maternal antibody up to 7-8 months in many kittens. Irrespective of the source of antibody, all FeBart® test positive kittens should be considered infected and treated for their infection. Some kittens with maternal antibody will be treated needlessly; however, truly infected kittens are too dangerous not to treat.

FeBart® test negative kittens, 6 months old or younger, present a different problem for the practitioner. The negative test is most likely (83% or greater) to represent a truly uninfected kitten. However, we have retested FeBart® negative kittens, younger than 6 months of age, 8 weeks later, and found 17% of them converted to FeBart® test positive.

### Updated Bartonella Test Algorithm for Kittens 6 Months Old or Younger



The first negative test was apparently taken, during the 8-week period, between infection and the kitten's production of antibody. *Bartonella* appear to be able to infect young kittens and

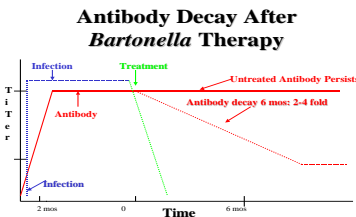
induce an inflammatory disease before the development of detectable antibody.

### Bartonella Therapy Evaluation

#### Therapy Titration Test:

The most practical method to determine if the therapy for *Bartonella* infection has been successful is to monitor the antibody levels. A decrease in the antibody titer indicates successful bacterial therapy. As with any antigen or infectious agent, initial stimulation leads to rising antibody titers whereas, removal of the antigen or infectious agent, leads to eventual decrease in antibody titer. Therapeutic responses of many human disease agents are monitored by serological assays for antibody titer decreases: *Helicobacter pylori*- Gastric ulcers, *Treponema pallidum*- Syphilis, *Borrelia burgdorferi*- Lyme disease, *Brucella abortus*-Brucellosis, *Porphyromonas gingivalis*- Periodontal disease, *Burkholderia pseudomallei*- Melioidosis, *Coxiella burnetii*- Q Fever, and *Bartonella henselae*- CSD.

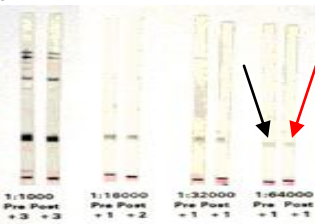
Figure 5



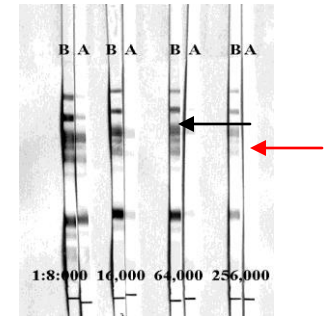
As seen in Figure 5, *Bartonella* infection leads to the production of detectable antibody, at high titers, by 8 weeks- rising titer. We found no decrease titers in 19 of 19 untreated *Bartonella*-infected cats (Figure 6). Conversely, removal of *Bartonella* by antibiotic therapy leads to the SLOW decrease in antibody titer as shown in Figures 5 & 7. It takes 6 MONTHS for the titer to decrease (antibody catabolism) 2 to 4 fold after clearance of *Bartonella*. When antibiotic therapy fails there is no decrease in antibody titer and re-treatment is required (Figure 8).

The screening test for infection, the FeBart® test, is too sensitive to use for detection of decreased titers since it is performed at a single dilution of 1:100. Many infected cats have titers of 1:256,000 or greater. Therefore, the Therapy Titration Test is required for determination of successful *Bartonella* therapy. This test consists of a total of 8 western blots, 4 blots for the pre-therapy sample (which we store in our freezers) and 4 blots for the post-therapy sample. Serial dilutions are tested for each sample to obtain end point titers for before and after therapy. A 4 fold or greater titer decrease indicates successful elimination of the bacteria. **PLEASE DO NOT REQUEST THE SCREENING FEBART® TEST FOR THERAPY EVALUATION.**

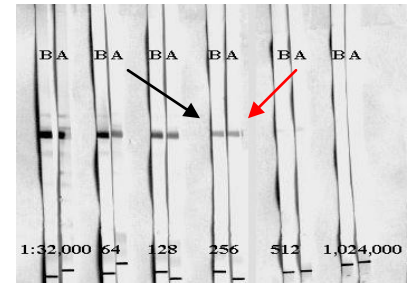
Figure 6



**No Therapy:** There is no decrease in titer after 3 years in this untreated *Bartonella*-infected cat.



**Figure 7 Successful Therapy:** *Bartonella*-infected cat treated with azithromycin- 10 mg/kg once daily for 21 days. There is a 16-fold decrease in titer which indicates effective *Bartonella* therapy. Pre-therapy titer (B) is >1:256,000 (black arrow) whereas the post-therapy titer (A) is 1:64,000 (red arrow).



**Figure 8 Therapy Failure:** Azithromycin failed to decrease the titer, Before-therapy titer (B) is 1:256,000 (black arrow) and the after-therapy titer (A) is also 1:256,000 (red arrow).

### References:

- Hardy, WD, Jr, Zuckerman, EE, Gold, JWM, Baron, P, Kiehn, TE, Polsky, B, and Armstrong, D. Immunogenic proteins of *Bartonella henselae* defined by western immunoblots with naturally infected cat sera. 95th General Meeting, American Society for Microbiology, Washington, D.C., May 21-25, 1995.
- Litwin CM, Martins TB, Hill HR. Immunologic response to *Bartonella henselae* as determined by enzyme immunoassay and Western blot analysis. Am J Clin Pathol 108:202-209, 1997.
- Hardy, WD, Jr., Zuckerman, E, Corbishley, J. Seroprevalence of *Bartonella*-infection in healthy and diseased cats in the United States and Caribbean: Evidence for *Bartonella*-induced diseases in cats. International Conference of the American Society for Rickettsiology, Big Sky, Montana, Aug. 17-22, 2001.
- Hardy, WD, Jr., Zuckerman, EE, Corbishley, J, Gold, JWM, Baron, P, Polsky, B, Gilhuley, K, Kiehn, TE, and Armstrong, DA. Efficacy of high dose, long duration Doxycycline or Azithromycin treatment for *Bartonella* infections in pet cats. International Conference of the American Society for Rickettsiology, Big Sky, Montana, August 17-22, 2001.
- Kordick, DL, Papich, MG, and Breitschwerdt, EB. Clinical and pathologic evaluation of chronic *Bartonella henselae* or *Bartonella clarridgeae* infection in cats. J Clin Microbiol 37: 1536-1547, 1999.
- Vermeulen, MJ, et al. Low sensitivity of *Bartonella henselae* PCR in serum samples of patients with cat-scratch disease lymphadenitis. J. Med. Microbiol. 57:1049, 2008.
- Maggi, RG., Duncan, AW. Novel chemically modified liquid medium that will support the growth of seven *Bartonella* species. J Clin Microbiol 2005. 43(6): 2651-2655.
- Harlow, E., and Lane, D. "Antibodies: A Laboratory Manual." Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1988.

**Bartonella references can be obtained at:**  
[www.nlm.nih.gov/](http://www.nlm.nih.gov/)  
 ©2013 National Veterinary Laboratory, Inc.