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NEWSLETTER

Veterinarian's Questions about *Bartonella*!©

Evelyn E. Zuckerman, Editor **Spring 2014** **Vol. 13, Number 2**

In This Issue:

In the spring 2014 issue of the NVL Newsletter we will address questions that veterinarians have asked us about *Bartonella* including: what test is best, how do I manage multi cat households, what is the best antibiotic to use for therapy, and do I test cats who show no clinical signs?

Basic Concepts:

Bartonella are ubiquitous bacteria that infect many animals including humans, cats, dogs, farm animals and most wildlife. There are more that 30 species of *Bartonella*, but *Bartonella henselae* is the most common species found in cats, dogs and humans. Pet cats are infected by fleas and are the natural reservoir for the most, six, species of *Bartonella*.[1] Since cats live in close proximity to humans, they pose the greatest zoonotic threat. In the past 10 years the concepts and knowledge regarding *Bartonella* have changed from an "unimportant group of bacteria" where there was no diagnostic test and no effective therapy to the realization that "these are pathogenic and dangerous zoonotic bacteria where there are now several diagnostic tests and effective therapy."

Question 1: What is the best test to detect *Bartonella* infection in pet cats and dogs?

There are 2 basic methods, direct or indirect, of detecting any pathogen in animals or humans. Direct methods detect the whole or parts of a pathogen directly in the blood or tissues, whereas indirect methods detect the reaction of the animal or human to a pathogen.

Direct Detection Methods:

1. Isolation in culture.
2. Detection of antigens of the pathogen.
3. PCR- detection of the nucleic acid (DNA).
4. Visualization in tissues by special stains.

Indirect Detection Methods:

1. Serology- detection of specific antibodies against the pathogen.

Presently there are 5 tests available to detect *Bartonella spp.*: Serology- 1-3) western blot, ELISA, and IFA tests, 4) PCR DNA detection, and 5) culture.[1-6] **However, it must be emphasized that *Bartonella* often are only intermittently present in the peripheral blood (bacteremia) of infected animals which can make PCR and culture problematic.[7]**

Culture from Blood:

Bartonella are very slow growing and fastidious, requiring special media for growth.[4-6] Isolation is proof of infection (bacteremia), whereas a negative culture may simply have been taken at a time when the organism was not circulating. Positive cultures must be confirmed to be *Bartonella* by PCR which increases the cost significantly.

PCR from Blood:

PCR is a very sensitive DNA amplification test for the presence of *Bartonella* DNA. A negative result does not rule out the presence of *Bartonella* DNA due to the possibility of intermittent bacteremia.[3]

Serology:

Serologic methods for detection of antibodies directed against *Bartonella* have been employed more than any other technique in the literature for detection of *Bartonella* infections in cats, dogs and people. ELISA and IFA antibody tests result in an all or none color development (Figures 1 & 2) whereas WB tests result in a specific multiple antibody profile (Figures 3 & 4).[2] Multiple studies have shown that the WB is the most specific and sensitive serologic assay. Large, well designed, comparative studies of serology, culture, and PCR for *Bartonella* detection in cats have not been performed.

IFA *Bartonella* Test

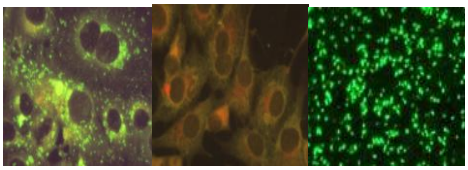

Figure 1. The IFA *Bartonella* test developed in our laboratory was not as accurate or as reproducible as our western blot test for detecting *Bartonella* infected cats. Left panel: antibody-positive test showing apple green fluorescence of *Bartonella* in infected cells. Middle panel: antibody-negative test- no fluorescing bacteria. Right panel: positive fluorescing bacterial preparation.

ELISA *Bartonella* Test

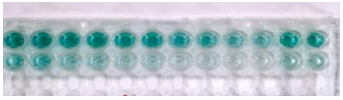

Figure 2. Our *Bartonella* ELISA test was the least accurate and reproducible serological assay.

Western Blot *Bartonella* Test:

WB detection of a profile of at least 6 antibodies against *Bartonella* proteins (Figure 3) insures the specificity of the technique.[2] We are able to detect infection with all 6 known species infecting cats and dogs since the sera of infected cats and dogs are cross-reactive for all infecting *Bartonella* species (Figures 4 & 5). In addition, cross-reacting antibodies to other bacteria are discounted by WB. As with other chronic pathogens such as FIV, HIV, and *Borrelia* (Lyme), antibodies to *Bartonella* co-exist with the organism and rarely are sterilizing of the infection. However, about 2-3% of *Bartonella* infected cats do not produce antibodies and are thus negative by WB. We also utilize the WB technique for dogs (Figure 5). WB tests are accurate, reproducible, require only 24 hours, and are cost effective. WB also eliminates the problem of cross reactive *Chlamydia* antibodies that may occur in ELISA and IFA tests.

Fe*Bart*® Western Blot (WB) Test

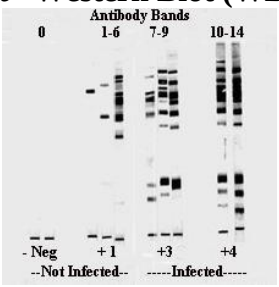

Figure 3. Grading system for the Fe*Bart*® Western Blot Test. – and +1: not infected, and +3 & +4: infected.

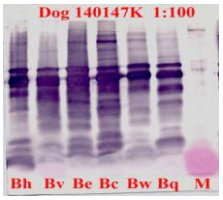

Figure 4. Left. Fe*Bart*® Western Blot test is able to detect all 6 feline *Bartonella* and is able to detect the cross-reacting proteins from other *Bartonella*. This figure shows the detection of proteins from *Be B. elizabethae*, *Bc B. clarridgeiae*, *Bh B. henselae*, *Bq B. quintana*, *Bv B. vinsonii*, and *Bd B. weissi* by a *Bh* infected cat's serum.

Figure 5. Right. Seropositive dog showing cross-reactivity to 6 *Bartonella* species: *B. henselae*, B. *vinsonii*, *B. elizabethae*, *B. clarridgeiae*, *B. weissi (bovis)*, and *B. quintana* (M= mol. wt. markers).

Therapy Evaluation Test:

The comparative WB titration test is the **ONLY** serological method to determine if therapy has eliminated *Bartonella* infection. The regular screening WB will remain positive, in about 90% of cats, for years even after elimination of infection because it is performed at a 1:100 serum dilution and infected cats can have very high antibody titers, some ≥1:1,024,000. The comparative titration test compares the titer of antibodies in the original pre-therapy sample with the post-therapy sample taken **6 MONTHS OR LONGER AFTER THE END OF THERAPY.** Therapy has been successful in eliminating *Bartonella* if there is a 4 fold or greater titer decrease.[8,9]

Summary:

1. Detection of *Bartonella* antibodies (serology) has been used most often in the world's literature as the test for *Bartonella* infection.
2. WB is the most accurate serological test- it accounts for other bacterial cross-reacting antibodies.
3. Contrary to culture and PCR of blood, WB serology does not rely on *Bartonella* being present in the blood.
4. WB is the most sensitive and specific serologic assay.
5. WB can be used to evaluate therapy- elimination of infection.
6. WB is rapid and economical.

Question 2: How do I manage a multi cat household when the first cat I tested is *Bartonella* infected?

Do I treat the remaining cats without testing them?
Do I test the remaining cats and treat only those infected?

The Answer:

Scientific based veterinary practice dictates testing the remaining cats and treating ONLY the test positive cats.

Scientific Rationale:

1. On average, 58% of healthy cats living in multi cat households are **NOT** infected with *Bartonella*.

Table 1
Healthy Cat Risk Factors for *Bartonella* Infection

Risk Factor	No Tested	% Infected	Risk Factor (X)
None	840	20%	X
Multi Cat Hh	26,973	41%	2.1X
Exposed to *Bartonella* + Cat	7,229	54%	2.7X
Totals:	74,531	42%	2.1X

Hh= household Many cats had multiple risk factors. 12 years of tests to 11-4-2011

2. Treating untested healthy cats results in treating 58% uninfected cats.
3. Such treatment may induce antibiotic resistant bacteria.
4. Therapy may be more costly than testing.
5 Therapy evaluation tests are not possible for a previously untested infected housemate since no pre-therapy sample was submitted.
6. Therapy evaluation tests are important as about 12% of treated cats are not cured of their infections and must be retreated.

Our Recommendations:

1. Discuss the public health aspects of *Bartonella* infected cats with your clients.
2. Recommend testing all cats in the household.
3. Treat only those cats that are test positive.
4. Recommend therapy evaluation tests 6 months after the end of therapy.
5. If the owner refuses to test all cats in the household, note that refusal and date in your records.
6. Implement stringent flea control for the household.

Question 3: I have heard that azithromycin induces drug resistance in *Bartonella* quickly and that doxycycline should be used instead. What do you recommend?

A recent in vitro study of several antibiotics against *Bartonella* demonstrated induction of azithromycin drug resistance after 2 passages whereas doxycycline, pradofloxacin and enrofloxacin induced resistance after 5 passages.[10] We have recommended azithromycin therapy for *Bartonella* infected cats for the past 15 years and have had very good results in eliminating the infection, >88%, and have not had any reports of drug resistant *Bartonella* being isolated from treated cats. Thus, we still recommend azithromycin for therapy since it is given once daily and has very few side effects in cats. Others recommend doxycycline which is also effective, but must be given twice daily for 6 weeks. This creates potential owner compliance problems and increases zoonotic risks for owners who treat their cats. In addition, doxycycline capsules may cause esophageal strictures.

Question 4: Three months ago I treated a cat with azithromycin for severe gingivitis with no clinical improvement. Now I tested the cat and it is +4 strongly positive, what do I do?

Unfortunately we cannot accurately perform the comparative therapy titration test to evaluate elimination of *Bartonella* since you treated before testing and there is no pre-therapy blood sample in our freezer for comparison.[8] This creates a problem for you, especially if there is an immunosuppressed person in the household and is a good reason not to treat a cat for a suspected *Bartonella* infection without first testing.

Question 5: Why would I test a cat for *Bartonella* who has no clinical sign?

Since *Bartonella* infections are chronic, possibly life long infections in cats, identifying infected healthy cats is important for: 1) preventing the development of chronic inflammatory diseases in the cat, 2) removing the bacteria's reservoir for the flea vector, 3) and finally, and maybe the most important reason, to remove the chance that the infected healthy cat will transmit the infection to people. In our studies, almost half of the cases of human *Bartonella* infections came from healthy cats.[11]

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***Bartonella* references can be obtained at:**
www.nlm.nih.gov/ or natvetlab.com