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NEWSLETTER

4th International *Bartonella* Meeting

Uppsala, Sweden

Evelyn E. Zuckerman, Editor **Winter 2005** **Vol. 4, Number 1**

A Personal Note: **Dr. Hardy, an avid scuba diver and underwater photographer, is very fortunate to have missed being in the path of the recent Indian Ocean tsunami by a mere 28 days. He was scheduled to be the seminar speaker and to be diving with the Society of Aquatic Veterinary Medicine in the Andaman Islands, India beginning January 23, 2005. The Andaman Islands were the first landmass north of the earthquake and were severely damaged by the tsunami.**

In This Issue:

In the winter 2005 issue of the NVL Newsletter we will cover the remaining, non-veterinary, content of the 4th International *Bartonella* Meeting that was held August 26-28 at The Evolutionary Biology Centre, Uppsala University in Uppsala, Sweden. We will cover the human *Bartonella* clinical reports and *Bartonella* genomic and pathogenesis papers.

The remaining scientific presentations comprised 12 papers concerning human *Bartonella* and 15 papers concerning *Bartonella* pathogenesis and their genomes. We will summarize selected papers in each category. Although many of these reports are technical, the observations are very relevant to our studies of *Bartonella* in cats and dogs.

Human *Bartonella* Clinical Reports:

Clinical Manifestations of Bartonella Infection. **JE Koehler, University of California San Francisco, San Francisco, CA**. Dr. Koehler, the leading clinician studying human *Bartonella* diseases, described her work with the occurrence of *Bartonella* induced fevers in HIV infected patients and the development of a primate macaque model of *Bartonella* pathogenesis. Although the classical presentation of cat scratch disease (CSD) lymphadenopathy is usually recognized, the less obvious signs of *Bartonella* infection are often never diagnosed. Dr. Koehler studied 382 HIV infected patients with fever and found *Bartonella* etiology in many more than previously reported. Overall, 18% (68/382) of patients were infected with *Bartonella henselae* or *Bartonella quintana*. She concluded that *Bartonella* infection should be sought in patients with fever of unknown origin. She also reported that there was no adverse outcome for the pregnancy, or to the fetus, in 2 pregnant women infected with *Bartonella*.

Dr, Koehler also established a macaque model of *Bartonella* infection. She found that only *Bartonella quintana*, and not *Bartonella henselae*, was able to induce a bacteremia when inoculated into macaques. This animal model will allow the study of the natural course and pathogenesis of *Bartonella* infections in primates.

Bartonella koehlerae, A New Human Pathogen Causing Culture-Negative Endocarditis. **B. Avidor, *et al*., Kaplan Medical Center, Rehovot, Israel.** Dr. Avidor and his colleagues reported that *Bartonella koehlerae* was identified for the first time, in the aortic valve, as a human pathogen causing culture-negative endocarditis. The causative agent had been misidentified as *Bartonella henselae* (Schattner, A. et al. 2003 Lancet 361:1786). *Bartonella koehlerae* is a *Bartonella* species carried by domestic cats and has been isolated from several stray cats in Israel.

Cat Scratch Disease Without Lymphadenopathy **M. Tsukahara and H. Tsuneoka, Yamaguchi University School of Medicine, Yamaguchi Kohseiren Nagato Hospital, Nagato, Japan.** A total of 185 patients were serologically positive for *Bartonella henselae*. Of these seropositive cases, 155 (83.8%) had regional lymphadenopathy while the other 30 (16.2%) had no lymphadenopathy. Of the 30 patients without lymphadenopathy, prolonged fever occurred lasting more than 7 days 25/30 (83.3%) and 14 days 11/30 (36.7%). Ten of the 30 (33%) patients without lymphadenopathy had systemic complications including optic neuroretinitis 5/10 (50%), Parinaud's oculoglandular syndrome 2/10 (20%), hepato-spenic granulomas 2/10 (20%), and 1/10 (10%) juvenile rheumatoid arthritis. The absence of lymphadenopathy was significantly associated with both prolonged fever and the presence of severe complications.

Editor's Note: **This is one of the most important observations reported. We have similar data from numerous case studies of the owners of *Bartonella* infected cats who developed severe *Bartonella* disease symptoms without the CSD prodrome of lymphadenopathy (tip of the iceberg below). We feel than many *Bartonella* disease symptoms are misdiagnosed due to the lack of the classic CSD regional lymphadenopathy that is familiar to most physicians.**

Cat Scratch Disease: The Tip of the *Bartonella* Iceberg

High Prevalence of Antibodies to Bartonella in Patients with Infected Cat Bites. **K. Westling, *et el.* Karlinska University Hospital Huddinge, Stockholm, Sweden.** Seventy-four patients with infected cat bites, who were seen in emergency wards, were studied for serological evidence of *Bartonell*a infection. Convalescent sera were available from 35 of the 74 patients. Antibody to any *Bartonella* was found in 44/74 (60%) patients. Seroconversion was observed in 8 patients. Of interest is the fact that only 1-2% of Swedish cats are infected with *B. henselae* whereas 67% are seropositive for *B. grahami*, a species isolated from small rodents in Sweden and Europe. Twenty-six % of the people with cat bites in this study, who were seropositive for *Bartonella*, were reactive to *B. grahami*.

Bartonelloses and Other Louse-Borne Infections in 934 Homeless of Marseilles. **P. Brouqui. Unite des Rickettsies, Universite de la Mediterranee Marseilles, France.** Homeless

people are particularly exposed to ectoparasites. Dr. Brouqui and his medical team found that 22% of the homeless were infested with lice and *Bartonella quintana* was isolated from blood culture of 50 people (5.3%). *Bartonella quintana* was found in the erythrocytes and erythroblasts as well as the dental pulp of bacteremic patients. Interestingly, these chronically bacteremic patients were non-febrile. The uncontrolled louse infestation of this population should alert health professionals to the possible re-emergence of louse-borne infections (*Rickettsia prowazekii, Bartonella quintana, and Borrelia recuuentis*).

Bartonella Infections among Homeless in Sweden. **C. Ehrenborg, *et al.* Uppsala University, Uppsala, Sweden.** This group studied 50 homeless people during a one-year period in Sweden. They found an unusually high *Bartonella* seroprevalence of 62% in the homeless compared to 14% in a matched control group. The 14% prevalence in the control group is also very high. No louse infestations were observed in the homeless people. The species of *Bartonella* seroreactivity was not determined.

A homeless man in Uppsala Sweden.

Editor's Note: *Bartonella quintana* is mainly a human *Bartonella*. It has only been found in people and recently in 1 cat. Humans are the main natural reservoir. The finding of *B. quintana* in the dental pulp is relevant to our observation of the *Bartonella* induced oral inflammatory diseases in cats. *Bartonella henselae* is the prototypic *Bartonella* in cats but has been found in dogs and humans as well.

Evidence of Bartonella sp. In Questing Adult and Nymphal Ixodes ricinus ticks from France and Co-infection with Borrelia burgdorferi sensu lato and Babesia sp. **L. Halos, *et al.* Ecole Nationale Veterinaire, Maisons-Alfort, France.** This group examined 92 questing ticks in northern France for coinfection with *Bartonella, Borrelia burgdorferi sensu lato* and *Babesia sp*. by PCR. *Bartonella* was detected in 9% of the ticks. One tick was infected with all 3 pathogens. Ticks represent a major vector for *Bartonella* transmission to humans and animals.

Bartonella Genomics:

***The Louse-borne Human Pathogen Bartonella quintana is a Genomic Derivative of the Zoonotic Agent Bartonella henselae*.** **SGE. Andersson, *et al.* Uppsala University, Uppsala, Sweden.** Dr. Andersson and her collaborators have sequenced the complete genomes of 2 human pathogens, *Bartonella quintana* (1,581,384 bp) and *Bartonella henselae* (1,931,047 bp). They conclude that *Bartonella quintana* was derived from *Bartonella henselae*, millions of years ago, through the loss of 18% of the genome and genomic islands (bacteriophage regions) and thus genome mobility. These genomic changes may be the reason that *Bartonella quintana* is mainly restricted to humans whereas *Bartonella henselae* is very capable of infecting cats, dogs, and people. In comparison to other Alpha-Proteobacteria, the elimination of a few thousand genes is characteristic of a shift to intracellular animal environments and vector-mediated transmission pathways. This team, and others around the world, is investigating the genes responsible for the pathogenic characteristics of all *Bartonella*. The information is being generated at an extremely rapid pace.

***Sequencing the Bartonella tribocorum Genome*.** **S. Schuster, *et al.* Max Planck Institute for Developmental Biology, Tubingen, Germany.** This group has sequenced the genome of *Bartonella tribocorum*, the *Bartonella species* originally isolated from Norwegian rats. This genome is very large (2.69Mb) compared to *Bartonella henselae* (1.93 Mb) and *Bartonella quintana* (1.58 Mb).[1,2] *Bartonella tribocorum* has genetic sequences derived from an insect virus which may be important in the biology of transmission by insect vectors. In this regard, *Bartonella henselae* can replicate in the flea gut.

Bartonella melophagi: a New Endosymbiont? **M. Vayssier-Taussat, *et al.* Ecole Nationale Veterinaire, Maisons-Alfort, France.** This is an observation that relates to the paper directly above regarding *Bartonella* life cycles in insects. *Bartonella* DNA has been found in the *Hippoboscidae* flies of the genera *Hippobosca*, *Lipoptena* and *Melophagus*. *Melophagus ovinus* flies are a permanent parasite of sheep. Although the *Bartonella* DNA was present in all adult (n=38) and pupae (n=14) *Melophagus ovinus*, no *Bartonella* was recovered in culture. By genome analysis, this *Bartonella* is considered a new species, *Bartonella melophagi*. None of the sheep parasitized by this fly were infected with this new species of *Bartonella*. It appears that this new species of *Bartonella* is an endosymbiont, living symbiotically only within this fly with no transmission to sheep. This is the first example of a *Bartonella* confined to an insect "vector."

Bartonella Pathogenesis:

***Role of the Type IV Secretion System VirB/D4 in Bartonella Pathogenesis*.** **C. Dehio, *et al.* University of Basel, Basel, Switzerland**. Dr. Dehio and his group have made great progress in the elucidation of bacterial virulence factors required for *Bartonella* pathogenesis using cultured human endothelial cells. They have identified the bacterial type IV secretion system (T4SS) VirB/D4 as an essential pathogenicity factor in *Bartonella*.[3] T4SS are multi-component transporters that allow bacteria to transfer protein or DNA into a wide variety of target cell types. VirB/D4 T4SS of *B. henselae* mediates most virulence attributes of this pathogen in endothelial cells.[4] These include: 1) massive rearrangements of the actin cytoskeleton, which results in formation of *Bartonella* aggregates and their uptake into the target cell, 2) NF kappa B-dependent proinflammatory activation, leading to cell adhesion molecule expression and chemokine secretion, and 3) inhibition of apoptotic cell death, resulting in enhanced endothelial cell survival. In total, these factors lead to cell invasion (erythrocyte and endothelial cells), tissue inflammation, prolonged cell survival, and proliferation of endothelial and inflammatory cells (macrophages). In people, this results in bacillary angiomatosis, a tumor like proliferation of capillaries in the skin and various organs. **Editor's Note: Similar lesions and processes occur in *Bartonella* infected cats.**

***The Role of Bartonella Adhesin A (BadA) and HIF-1 in B. henselae Infections*.** **V. Kempf, *et al.* Institut fur Medizinische Mikrobiologie and Hygiene, Tubingen, Germany.** This group has defined different pathogenic factors induced by *Bartonella*. They have observed, *in vitro* and in bacillary angiomatosis tissues *in vivo*, that *Bartonella henselae* infection activates hypoxia-inducible factor-1 (HIF-1), the key transcription factor involved in angiogenesis, and the secretion of vascular endothelial growth factor (VEGF). *Bartonella henselae* have short hair-like structures in their cell wall called pili that enable the bacteria to move. Pili are similar to flagella but are much shorter. Infection with *Bartonella henselae* variants, that do not possess pili (pilus-negative variants), do not activate HIF-1 nor VEGF secretion indicating the importance of this bacterial surface protein in the angiogenic reprogramming of host cells. This surface protein is a non-fimbrial adhesin of *Bartonella henselae* designated as *Bartonella henselae* adhesin A (BadA). BadA mediates the binding of *Bartonella henselae* to extracellular matrix proteins and to endothelial cells. BadA is immunodominant in the antibody response of humans infected with *Bartonella henselae* and in rodents infected with *Bartonella* indicating it is expressed during *Bartonella* infections. BadA is the largest *Bartonella henselae* protein characterized to date with a size of 340 kD and, in fact, is one of the largest proteins found in any bacterium. The BadA gene is the largest gene in *Bartonella henselae*. Serologic detection of BadA in people may improve the serodiagnosis of *Bartonella henselae* infection.

References:

1. Alsmark, CM, *et al.* The louse-borne human pathogen *Bartonella quintana* is genomic derivative of the zoonotic agent *Bartonella henselae*. Proc Natl Acad, Sci. 101: 26, 9716-9721, 2004.
2. Boussau, B., *et al.* Computational inference of scenarios for alpha-proteobacterial genome evolution. Proc Natl Acad, Sci. 101: 26, 9722-9727, 2004.
3. Schulein, R., and Dehio, C. The VirB/VirD4 type IV secretion system of *Bartonella* is essential for establishing intraerythrocytic infection. Mol. Microbiol. 46: 1053-1067, 2002.
4. Schmid, MC. *et al.* The VirB type IV secretion system of *Bartonella henselae* mediates invasion, proinflammatory activation, and anti-apoptotic protection of endothelial cells. Mol. Mocrobiol. 52: 81-92, 2004.

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NEWSLETTER

Bartonella: Quick Reference Sheet

Evelyn E. Zuckerman, Editor **Spring 2005** **Vol. 4, Number 2**

In This Issue:

In the Spring 2005 issue of the NVL Newsletter we will give a complete summary (Quick Reference Sheet) of *Bartonella* infections in cats and dogs.

Bartonella Quick Reference Sheet

Background:

Bartonella are bacteria that cause chronic inflammatory diseases in any tissue in cats, dogs and humans. Cats and dogs act as reservoir hosts for many *Bartonella* species and the bacteria are found in the plasma, tissues, in erythrocytes, macrophages and importantly in endothelial cells. Cats can be bacteremic for years, or even for life, and thus serve as reservoirs for human infection.

Transmission:

Bartonella are transmitted mainly by arthropod vectors, fleas and ticks, in cats and dogs.

Although not proven, it seems likely that they may also **very rarely** be transmitted directly among cats by scratches and bites, as occurs in the zoonotic transmission from cats to humans. For the most part, direct transmission cat to cat does not occur and infected cats may be kept with uninfected cats while being treated.

Diseases:

Bartonella cause inflammatory diseases in any tissue because of their strong tendency to adhere to and penetrate endothelial cells, the components of capillaries. They induce inflammatory cytokines in those tissues and a chronic inflammation ensues. What makes some tissues, like the gingival and respiratory tissues and eye, more susceptible is unknown.

Most Accurate *Bartonella* Test:

After 5 years of research comparing culture isolation with serology, our data showed that the most accurate and reproducible test for detection of *Bartonella* infection in cats is the serologic detection of antibodies to the bacteria using the western blot (WB). Multiple studies have shown that the WB is the most accurate and sensitive serologic assay for many microorganisms. It is used in human medicine to confirm ELISA positive HIV screening tests, Lyme positive serology and several others. In veterinary medicine it is also used to confirm FIV ELISA positive serology.

Figure 1. *B. henselae* isolation (gray rounded colonies) from the blood of a 6-month-old kitten. >1,000 *Bartonella*/ml was isolated after 35 days in blood agar culture. Isolation is not a very sensitive assay for detection of *Bartonella* infections.

Comparative tests of serology and PCR for *Bartonella* detection in cats have not been performed but in humans with cat scratch disease, only 64% are PCR positive whereas 84% are serologically (antibody) positive. Detection of antibodies is an amplification system when antibodies coexist with etiological agents as they do in FIV and *Bartonella* infections in cats.

Fe*Bart*® Western Blot (WB) Test

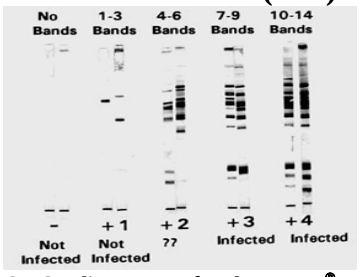

Figure 2. Grading system for the Fe*Bart*® Western Blot (WB) Test. – and +1 not infected, +2 30% of cats infected, +3 & +4 infected.

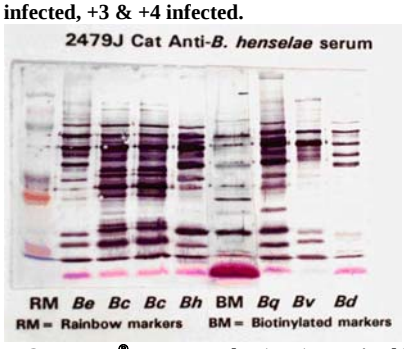

Figure 3. Fe*Bart*® Western Blot (WB) Test is able to detect all 6 feline *Bartonella* and is able to detect the cross-reacting proteins from other *Bartonella*. This figure shows the detection of proteins from *Be B. elizabethae*, *Bc B. clarridgeiae*, *Bh B. henselae*, *Bq B. quintana*, *Bv B. vinsonii*, and *Bd B. weissii* by an infected cat's serum. Thus, our WB test can detect any *Bartonella* that infects cats or dogs which is not always the case for the IFA or ELISA *Bartonella* tests (see below).

IFA *Bartonella* Test

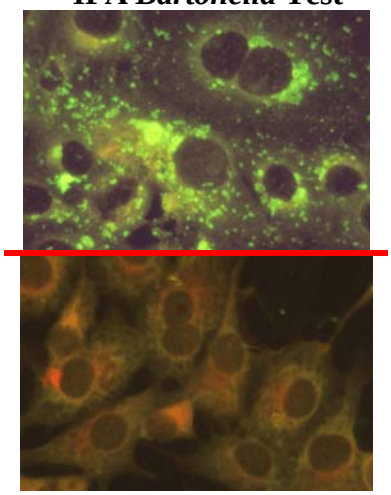

Figure 4. The IFA *Bartonella* test developed in our laboratory was not as accurate or as reproducible as our western blot test for detecting *Bartonella* infected cats. *Bartonella* were grown in feline cells in cell culture and used as targets for the detection of antibody in cat sera. Top panel: antibody-positive test showing apple green fluorescence of *Bartonella* in infected cells. Bottom panel: antibody-negative test.

PCR *Bartonella* Test

Figure 5. PCR test developed with our collaborators to compare our different *Bartonella* isolates. Only 64% of people with cat scratch disease are PCR positive whereas 84% are antibody positive.

ELISA *Bartonella* Test

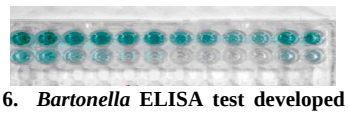

Figure 6. *Bartonella* ELISA test developed in our laboratory was the least accurate and reproducible of all the serological assays.

Public Health:

Bartonella originating from cats can infect people and cause at least 24 chronic inflammatory diseases and may even rarely cause fatalities. Contrary to some publications, **MORE IMMUNOCOMPETENT PEOPLE ARE INFECTED BY CAT *BARTONELLA* THAN IMMUNOSUPPRESSED PEOPLE.** However, immunosuppressed people are more likely to have severe consequences and more likely to die from their *Bartonella* infection.

Treatment:

Bartonella are susceptible to several antibiotics. Azithromycin has been shown to be the most effective antibiotic for *Bartonella* infections in humans and cats. It should be noted that high dose long-term therapy is required since many, though not all, *Bartonella* live intracellularly in erythrocytes, macrophages and endothelial cells.

Antibiotics of choice are:

Azithromycin: 10 mg/kg SID for 21 or more days
Rifampin*: 10 mg/kg SID for 21 or more days
Doxycycline: 10 mg/kg BID for 6 weeks (careful of esophageal strictures)
Only infected cats, WB +3 or +4, should be treated.

*** Rifampin can be used as a single drug. However, we have had numerous reports of allergic reactions in cats treated with rifampin. Reactions consist of reddened and itchy ears, face, nose, and paws, swelling of the face and paws, abdominal pain and general unease.**

DO NOT TREAT UNTESTED CATS OR CATS THAT ARE WB +1 OR -NEGATIVE WITH AZITHROMYCIN. Azithromycin is an excellent human antibiotic and we must use it specifically and judiciously in veterinary medicine.

Therapy Evaluation:

The Comparative Titration Test is the **ONLY** way to determine if therapy has eliminated *Bartonella* infection. The regular screening WB will remain positive 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 1:2,048,000. The titration test compares the titer of antibody in the original sample (saved in our freezer) with the post-therapy sample taken:

6 MONTHS OR LONGER AFTER THE END OF THERAPY.

The titration test is more expensive because, unlike the screening single WB Fe*Bart*® test, we must use 8 WB strips, 4 for the original sample and 4 for the 6-month post therapy sample, to determine the comparative endpoints (Figures 7, 8 & 9). If the titer decreases 4 fold or greater the therapy has been successful in eliminating *Bartonella*. There is no reason to re-titer a cat that has already had a 4 fold or greater decrease titration test result. We cannot accurately test to determine if a cat has been re-infected.

Therapy Titration Tests

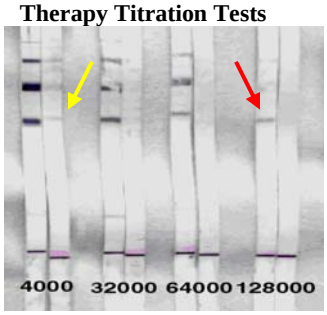

Figure 7. A 32 fold antibody titer decrease after azithromycin therapy. Pre-therapy titer 1:128,000 (red arrow) and post therapy titer 1:4,000 (yellow arrow). This indicates successful removal of the *Bartonella* infection.

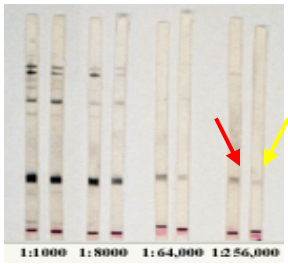

Figure 8. No antibody titer decrease after azithromycin therapy. Pre-therapy titer 1:256,000 (red arrow) and post therapy titer 1:256,000 (yellow arrow). This indicates unsuccessful removal of the *Bartonella* infection and this cat should be retreated with rifampin.

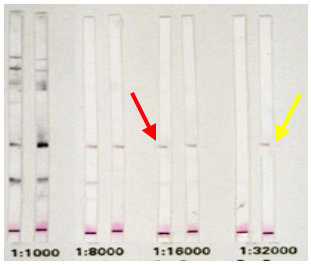

Figure 9. A 2 fold antibody titer INCREASE after azithromycin therapy. Pre-therapy titer 1:16,000 (red arrow) and post therapy titer 1:32,000 (yellow arrow). This indicates unsuccessful removal of the *Bartonella* infection and this cat should be retreated with rifampin.

Therapy Results:

We have evaluated 1,996 therapy titration tests as of August 2004 (Figures 10). Compared to the 20 cats who were not treated and had no titer decrease, 84% of treated cats had titer decreases of 2 fold or greater (Figures 10 & 11). These data show that appropriate antibiotic therapy can eliminate *Bartonella* infections in cats.

Figure 10

Therapy Titer Evaluation: *Bartonella*-Infected Cats

Titers:	#	Increase 2x or > WB Titer	No Decrease WB Titer	Decrease WB Titer: 2 Fold	4 Fold >
No Therapy	20	6	14	0	0
%	100%	30%	70%	0%	
Therapy	1,996	44	271	379	1,302
%	100%	2%	14%	19%	65%
			16%	84%	

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Antibiotic Comparison: 1,996 Therapy Titer Evaluations

Antibiotic	↑	No ↓	2 F	4 F	8 F	16 F	32 F	64 F	128 F
Azithromycin n 1,849	39 16%	258	356	790	314	67	18	4	3
Rifampin n 126	4 13%	12	20	46	39	4	1	0	0
Doxycycline n 21	1	1	3	8	5	3	0	0	0
Totals: 1,996	44	271	379	844	358	74	19	4	3
%	2 16%	14	19	42	18	3.7	1	0.2	0.1

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Figure 11. Titer reduction comparisons of azithromycin, rifampin and doxycycline therapy for *Bartonella* infection. All 3 antibiotics are effective, but azithromycin appears to be the most practical and has the least adverse side effects.

Laboratory Test Submission Forms: Data Requested

We have always requested that ALL clinical information be filled in on our laboratory Test Submission Forms and will ask that any missing information be faxed back to us. Why are we so OBSESSED with obtaining all the required information on our Test Submission Forms? The reasons are given below:

Age & Diagnosis: Both MUST be indicated in order to permit us to give you a proper interpretation of our test result. This is important both **MEDICALLY** and **LEGALLY**. For example, we recommend a re-test of any kitten less than 6 months old with an inflammatory disease who tests +1 or negative. Why? Because 17% of such kittens are infected and *Bartonella* is inflaming the tissues before enough antibody has been produced for us to detect. Yes, 17% retest positive (infected) 8 weeks later and thus are a **ZOONOTIC RISK TO THEIR OWNERS. These infected kittens may be missed if the age and diagnosis information is not indicated on the test submission form. If the cat's age is unknown, please estimate relative to younger or older than 6 months.**

Therapy Data: We **INSIST** that all therapy outcome data be recorded on the bottom of our Test Submission Form (Did the cat improve with therapy? What percent improvement occurred?). We need this information to interpret our titration data and give you the PROPER recommendation for re-treatment when it is indicated.

LEGAL LIABILITY: There have been, and will be, legal instances concerning a veterinarian's responsibility in *Bartonella* testing and recommendations to cat owners, especially owners of kittens in households with children. With complete information given by you on the Test Submission Form, and with correct recommendations based on our test results, we can give appropriate public health recommendations and be legally without fault. We insist on this for your and our protection.

***BARTONELLA* ARE SIGNIFICANT PUBLIC HEALTH RISKS TO VETERINARIANS, THEIR STAFF, AND CAT OWNERS**.

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NEWSLETTER

The Controversy Regarding Feline *Bartonella* Pathogenicity in Cats

Evelyn E. Zuckerman, Editor **Summer 2005** **Vol. 4, Number 3**

In This Issue:

The Summer 2005 issue of the NVL Newsletter will discuss the continuing controversy regarding the pathogenicity of feline *Bartonella* in cats. Several prominent academic feline clinicians on the VIN Message Boards and in their scientific presentations continue to question the ability of feline *Bartonella* to cause disease in pet cats and to fulfill Koch's Postulates. We will explore the scientific facts and misconceptions that exist regarding this controversy.

What are the Criticisms?

1. Feline *Bartonella* do not fulfill Koch's Postulates as the cause of disease in cats, their natural reservoir host.

2. So many healthy cats are infected with *Bartonella* that you cannot determine the disease association in infected cats with inflammatory diseases.

What can we agree on?

1. Most *Bartonella* researchers and academic veterinary clinicians agree that *Bartonella,* derived from cats by zoonotic transmission, cause at least 22 human inflammatory diseases.

2. Canine *Bartonella* cause several similar inflammatory diseases in dogs, their natural reservoir host.

3. The 2 human *Bartonella* (*B. quintana* and *B. bacilliformis*) cause several severe and even fatal diseases in humans, their natural reservoir host.

Let's Examine the Facts!

1. Feline *Bartonella* do not fulfill Koch's Postulates as the cause of disease in cats, their natural reservoir host.

A consideration of Koch's Postulates is necessary to address this criticism. The opening paragraph of an excellent review by Fredericks and Relman in 1996 is most relevant[1]. The co-author David A. Relman was the first to identify *Bartonella* (*Rochalimaea*) in the tissues of a patient with bacillary angiomatosis using DNA methodology.[2]

"Life has changed since the 1880s when Robert Koch elucidated his guidelines, later to be called Koch's postulates, for determining whether a microorganism is the cause of a disease. The horse-drawn buggy bumping over dirt roads has been replaced by the computer-assisted automobile speeding along paved highways. It would be absurd to expect modern cars to abide by traffic rules and standards designed for horse-drawn carriages. Yet, many continue to hold Koch's postulates as the unchanging standard for determining causation in medicine, despite a revolution in biotechnology and leaps in medical knowledge. Recent findings based on the application of new technologies, especially in the fields of microbiology and infectious disease, demand a renewed dialogue on proof of causation and revised guidelines for defining a causal relationship between a microbe and a disease."[1]

The authors continue their analysis of the modifications to Koch's postulates over the last 125 years. "The critical elements of Koch's postulates include a specific association of the microbe with the disease state; scientific concordance of microbiological, pathological, and clinical evidence; isolation of the microbe by culture on lifeless media; and reproduction of disease by inoculation of the cultured organism into a host." These stringent criteria worked well for some bacteria such as *Mycobacterium tuberculosis* but not for others such as *Vibrio cholerae* that caused cholera in some people but can also often be isolated from healthy carriers.

"Furthermore, how does one meet criteria for causation when a pathogenic microbe is also capable of a carrier state (e.g., *Neisseria meningitidis*), causing disease in one individual and not in another? In contrast to the beliefs of Koch and those of his era, we are well aware today that microbial pathogens often cause subclinical infection… Unfortunately, Koch's postulates have frequently been applied to issues of causation with a mathematical zeal that is not warranted in the biological world…. As Alfred Evans noted, failure to fulfill the Henle-Koch postulates does not eliminate a putative microbe from playing a causative role in a disease…. Serological assays offer an independent, but indirect approach to the clinician for diagnosing disease in individual patients and for studying the epidemiology of microbes in host populations."

It is apparent that Koch and his contemporaries were unaware of microbes that had long latent periods and only induced disease in a small number of people or animals that carried the infections. They were unaware of subclinical infections that occur with many viruses and bacteria (e.g., FIV, FeLV, *H. pylori* and *Bartonella* species).

Bartonella have adapted to their reservoir hosts in unique ways. They cause chronic intraerythrocytic infections with as many as 70% of the reservoir hosts, in certain geographical areas, being bacteremic at any one time. The bacteremia is the source of the vector infection (*e.g.*, fleas, ticks and lice). Some authors state that "the *Bartonella* bacteremias, result in few (and if present, very subtle) clinical signs in their specific hosts which contradicts Koch's observation that the blood of healthy humans or animals is free of bacteria."[3] This observation is certainly not true for humans infected with *B. bacilliformis* in Peru where as many as 40-85% of untreated infected people will die, one of the highest mortality rates of all infectious diseases.[4] Nor is it true of dogs infected with *Bartonella* who develop polyarthritis, lymphadenopathy, endocarditis and fever.[5,6] It is also not true for cats experimentally inoculated with *Bartonella* who develop various inflammatory diseases (see Tables below).[7,8,9]

Abnormalities (Diseases) in Cats Experimentally Infected with *Bartonella*

Abnormalities:	Diagnosis/Cats
Lymphadenopathy	13/13
Splenic follicular hyperplasia	9/13
Cholangiohepatitis	9/13
Myocarditis	8/13
Interstitial nephritis	4/13

Kordick, et al. J. Clin. Microbiol. 37:1536, 1999

Abnormalities (Diseases) in Cats Experimentally Infected with *Bartonella*

Abnormalities:	Diagnosis/Cats
Papule at injection site	5/9
Fever	9/9
Lymphadenopathy	9/9
Myositis	3/9
Lethargy	9/9
Neurological signs- aggression	7/9
Anorexia	6/9

Mikolajczyk & O'Reilly Am. J.Vet. Res. 61:375, 2000

In addition, Guptill *et al*. described reproductive disorders in experimentally infected cats.[10] Certainly these experimental studies fulfill the portion of Koch's postulates that requires induction of disease by the inoculation of the suspected pathogenic microorganism. Yet despite these published reports, the critics choose not to accept the data as proof of pathogenicity.

2. High Prevalence of *Bartonella* in Healthy Cats: Frontal and Stealth Attack Strategies in Microbial Pathogenesis.

Merrell and Falkow in a recent review in Nature, one of the 2 most prestigious international scientific journals, discuss pathogenic microbes in military terms of frontal and stealth.[11] One of their 2 examples of stealth agents is *Bartonella*. The authors describe how *Bartonella* and *Helicobacter pylori* evade the adaptive immune responses to establish chronic, if not life-long infection. They discuss the large percentage of animals or humans that are chronically infected with these agents whereas only some develop disease. The Table below summarizes the attack strategies of each class of microorganism and is relevant to our discussion of Koch's postulates and the pathogenesis of *Bartonella* in cats.

Frontal vs. Stealth Pathogenic Bacteria

Frontal- (Aggressive):	Stealth- (Slow-Mild):
Short incubation period	Long/ indeterminate incubation period
Acute clinical sign	Chronic clinical signs
Engages innate immune system	Engages innate immune system
Rapid multiplication	Slow multiplication
Carrier state uncommon	Carrier state common- shedding
Induce sterilizing immunity	Rarely induces sterilizing immunity
Adaptive immune system (IS)	Avoids or manipulates the adaptive IS
Yersinia & Vibrio	*Bartonella & Helicobacter*

Merrell, D.S. & Falkow, S. Nature, 430: 250-256, 2004

When Koch's postulates do not clearly establish the disease etiology of a micoorganism other methods can be used. These include seroepidemiological evidence, pathological evidence, an appropriate animal model, molecular and immunological techniques and therapeutic intervention or vaccine prevention.

Evidence Linking Microorganisms to Infectious Diseases

1. Seroepidemiological
2. Pathological
3. Animal Model (Human Model)
4. Molecular biology and immunology
5. Antibiotic therapy- intervention

1. Seroepidemiological: The combination of the experimental studies previously cited and our seroepidemiological evidence seems overwhelming in support of the association of feline *Bartonella* with inflammatory disease in pet cats.[12] The following Table summarizes our association of *Bartonella* infection with inflammatory diseases in cats.

***Bartonella*-Infection in Cats* with Inflammatory Diseases**

Diseases	No. Tested	No. Infected	% Infected	Difference / X
Healthy: No Risk Factors	840	170	20%	X
Oral Disease	19,823	9,932	50%	2.5X
Resp. Diseases	4,933	2,471	50%	2.5X
Ocular Diseases	3,767	1,820	48%	2.4X
GI Diseases	1,522	747	49%	2.5X
Skin Diseases	399	211	53%	2.7X
Other *Bart.* Diseases	2,534	1,216	48%	2.4X
Total	32,978	16,397	50%	2.5X

* Many cats had multiple inflammatory diseases, thus totals in Table exceed the total number of cats tested.

2. Pathological: The pathology induced by *Bartonella* in all species, human, dog and cats is identical. There is chronic inflammation, granuloma formation and blood vessel proliferation in any tissue due to the tendency of *Bartonella* to adhere to and infect endothelial cells that are present in all tissues.

3. Animal Model: The animal model for feline *Bartonella* pathogenicity is the human. All of the *Bartonella* diseases found in experimentally inoculated kittens and in naturally infected cats were first described in humans. These include inflammatory diseases in all systems: ocular, oral, respiratory, gastrointestinal, musculoskeletal, neurological, skin, and major viscera.

4. Molecular Biology & Immunology: Molecular studies of the mechanism of *Bartonella* disease induction are progressing rapidly and have been reviewed in a previous Newsletter (Vol. 4, No.1, 2005). One of the more interesting observations is the derivation of the human *Bartonella quintana* from the feline *Bartonella henselae* by the loss of gene sequences. *Bartonella quintana* now seems to be restricted to its human reservoir and human louse vector.

5. Therapeutic Intervention: As we have reported at international *Bartonella* meetings and in a previous Newsletter (Vol. 3, No. 4, 2004), we have been successful in eradicating *Bartonella* infection in 84% of treated cats.[13] In addition, the inflammatory diseases in *Bartonella* infected cats were markedly improved or totally resolved in 83% of the treated cats.

Summary: Do feline *Bartonella* Fulfill Koch's Postulates?

A summary of the application of Koch's postulates to feline *Bartonella* and the comparison with those pertaining to *Helicobacter pylori* is listed in the Table below. Most, but not all, are applicable to feline *Bartonella*. The experimental transmission studies and seroepidemiological findings are definitive evidence that feline *Bartonella* induce diseases in their natural reservoir host, the pet cat.

Koch's Postulates for:	*H. Pylori* Peptic Ulcers	*Bartonella* Cat Disease
Microorganism always present in the diseased tissue.	Not always	Not always
Viable microorganisms can be cultivated from the diseased tissue.	Yes, Not always	Yes, Not always
Inoculation of microorganism into susceptible animal reproduces the disease.	Yes	Yes
Microorganism can be detected in the pathological tissue from the diseased animal.	Yes	Yes, Not always

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NEWSLETTER

Interpreting the Fe*Bart*® *Bartonella* Test Results

Evelyn E. Zuckerman, Editor **Fall 2005** **Vol. 4, Number 4**

In This Issue:

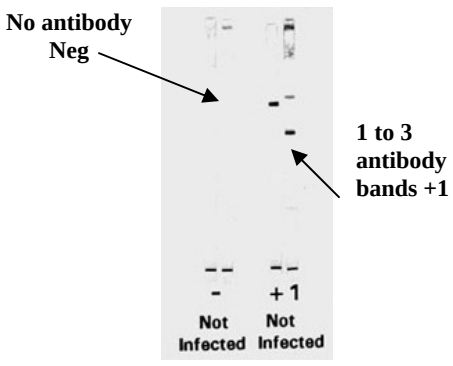
The fall 2005 issue of the NVL Newsletter will discuss the interpretations of the results of the Fe*Bart*® screening test for determination of *Bartonella* infection. What does a positive result (+3 or +4) mean? What does a negative (-Neg or +1) mean? We will discuss the various test results and what action is required for each result.

The Fe*Bart*® *Bartonella* Test:

The Fe*Bart*® *Bartonella* test is a western immunoblot serological test for antibodies against the structural proteins of *Bartonella*.[1, 2, 3] Most, though not all, infected cats produce antibodies against 7 to 14 proteins of the infecting *Bartonella* species by 8 weeks after infection. Yes, about 4-6% of infected cats do not produce anti-*Bartonella* antibodies at any time after their infection. This is also true for approximately 16% of *Bartonella* infected people. The western immunoblot is the most sensitive and specific (accurate) serological test compared to immunofluorescence (IFA) and ELISA tests and is discussed in detail in our Vol. 4, Number 2, and Spring 2005 Newsletter. It is also able to detect cross-reactive antibodies to all 6 feline *Bartonella* species (pet cats can be infected with 6 different *Bartonella* species). In order to be considered serologically positive, cats must produce antibodies to at least 7 *Bartonella* proteins.

Fe*Bart*® Negative Tests:

Serologically negative cats are those that produce no antibody or antibody against just 1-3 *Bartonella* proteins. Antibodies against only 3 proteins probably represent antibodies cross-reactive to protein epitopes shared with other bacteria such as *Chlamydia*.

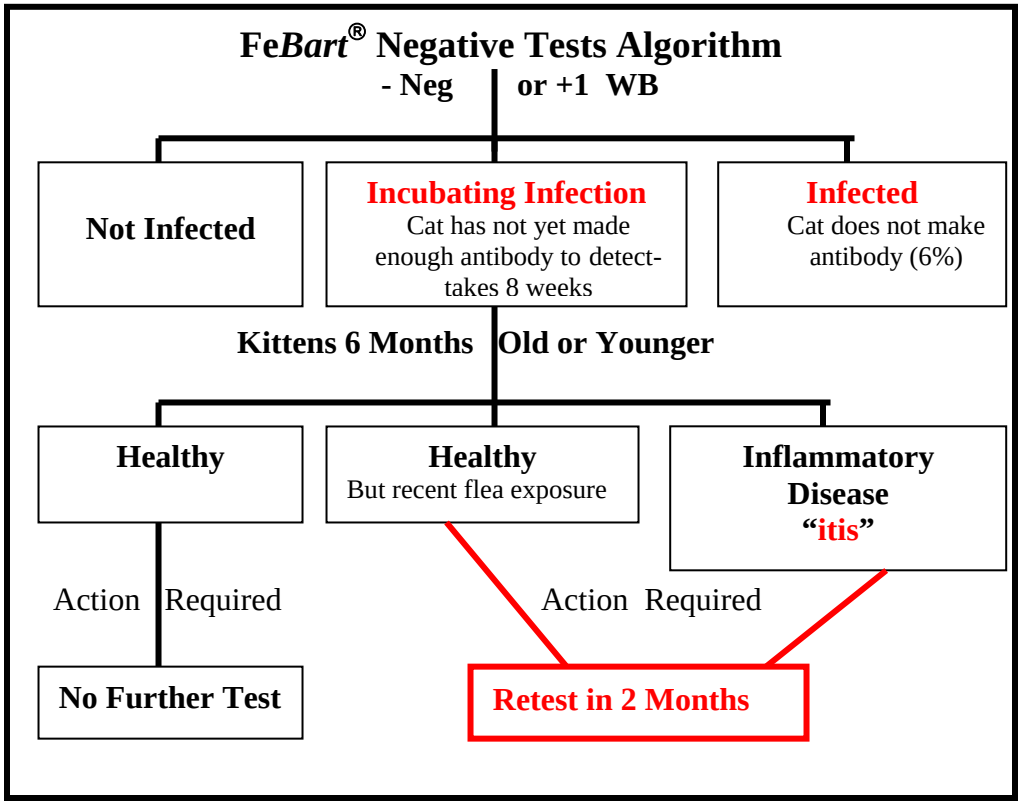


Fe*Bart*® Positive Tests:

Serologically positive cats are those that produce antibodies against 7 to 14 *Bartonella* proteins. The cross-reactive antibodies against other bacteria are discounted because of the specific *Bartonella* immunologic antibody profile.[1]

Fe*Bart*® Negative Tests Algorithm:

As shown in the box below, negative Fe*Bart*® screening tests must be interpreted based on the age and diagnosis of the cat. Kittens less than 6 months old only have 6 months to become infected and 2 months to produce antibody. *Bartonella* can cause inflammation quickly, before the production of antibody, through their ability to attach to a toll-like cellular receptor of the innate immune system which recognizes invading bacteria within hours of infection and sets off a cascade of inflammatory components to combat the infection. Thus kittens 6 months or younger with inflammatory diseases, who are Fe*Bart*® test negative, should be retested after 2 months to determine if they were incubating the infection at the time of the first test. Approximately 17% of such kittens were found to test positive on retest and were incubating the infection. **We strongly recommend against the treatment of untested cats living with test positive cats. Only serologically positive cats should be treated.**[4, 5,6]



Fe*Bart*® Positive Tests Algorithm:

As shown in the box below, positive Fe*Bart*® screening tests must be interpreted based on the diagnosis of the cat. Both healthy cats and cats with inflammatory diseases that test positive should be treated. However, the interpretation of the test result in cats with inflammatory diseases should consider the role of *Bartonella* as the cause of the disease. The algorithm below summarizes the possible etiological role of *Bartonella* as related to the test positive result.[1, 2, 3] In short, *Bartonella* may be: 1) the sole cause of the inflammatory disease; 2) a co-etiological agent along with other microorganisms; 3) not causing any of the inflammatory disease, *Bartonella* is "in the background;" and 4) *Bartonella* has been cleared by the cat's immune response and the remaining antibody is a "history of the infection."

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More than 1400 *Bartonella* references are available at: www.nlm.gov