Microbial culture of blood samples and serologic testing for bartonellosis in cats with chronic rhinosinusitis

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Objective—To assess the role of Bartonella spp in chronic rhinosinusitis (CRS) by determining detection rates for the organism by serologic testing and microbial culture of blood samples for Bartonella spp in cats with CRS and control cats (cats with other nasal diseases, cats with systemic illnesses, and healthy cats).

Design—Prospective case-control study.

Animals—19 cats with CRS, 10 cats with other nasal diseases, 15 cats with systemic illness, and 15 healthy cats.

Procedures—Serologic testing for Bartonella clarridgeiae and Bartonella henselae and microbial culture of blood samples were conducted in all cats. In cats with CRS and cats with other nasal diseases, a nasal biopsy specimen was submitted, when available, for tissue PCR assay to detect Bartonella spp.

Results—9 of 19 cats with CRS had positive results for serologic testing for 1 or both Bartonella spp; whereas, 4 of 10 cats with other nasal diseases, 2 of 15 cats with systemic diseases, and 4 of 15 healthy cats had positive results for serologic testing to detect Bartonella spp. These values did not differ significantly among groups. Microbial culture of blood samples yielded B henselae in 1 cat with a nasopharyngeal abscess. The PCR assay for Bartonella spp in nasal tissues yielded negative results for 9 of 9 cats with CRS and 5 of 5 cats with other nasal diseases.

Conclusions and Clinical Relevance—A role for Bartonella spp in the pathogenesis of CRS in cats was not supported by results of this study. (J Am Vet Med Assoc 2008;233:1084–1089)

Chronic rhinitis or rhinosinusitis is one of the most common chronic disorders in the feline population and is characterized by excessive mucus accumulation; nasal discharge; sneezing; and, in some cats, turbinate destruction. The underlying pathogenesis of CRS in cats is unclear, and the disease is likely multifactorial with viral infection (ie, FHV-1), secondary bacterial infections, and poorly regulated or improper immune responses all playing a role in the pathogenesis. Currently, treatment is aimed at the consequences of nasal dysfunction rather than at the cause of the condition and focuses on decreasing nasal inflammation and eliminating secondary bacterial infections. Other treatments that have not been extensively evaluated include immune modulation, which can have some potential benefit, and antiviral agents.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>bp</td>
<td>Base pairs</td>
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<tr>
<td>CRS</td>
<td>Chronic rhinosinusitis</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>FHV-1</td>
<td>Feline herpes virus-1</td>
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<td>SPF</td>
<td>Specific pathogen free</td>
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Bartonella spp are implicated in rhinitis and epi-staxis of dogs, although controversy surrounds the role of this organism in clinical disease of the nasal cavity in dogs. Nonetheless, many veterinarians in private clinical practice test for Bartonella spp and use azithromycin to treat cats with CRS partially on the assumption that Bartonella organisms may be contributing to disease progression in affected animals. Traditionally, Bartonella spp were not believed to be pathogenic organisms in cats; instead, they were primarily a zoonotic concern because they cause suppurrative lymphadenitis in people (ie, cat scratch disease). However, research has revealed that inoculation of these bacteria into SPF cats can cause histologic inflammatory changes in multiple organ systems. The hypothesis of the study reported here was that cats with CRS would have evidence of greater exposure to Bartonella spp as determined on the basis of serologic response and isolation of organisms from microbial culture of blood samples, compared with results for control cats with other nasal diseases, systemic diseases other than CRS, and cats.
with no signs of diseases (ie, healthy cats). In addition, we hypothesized that there would be a positive correlation between results of PCR assay of nasal tissues for *Bartonella* DNA in cats with CRS, compared with results for cats with other nasal diseases. These results would support a role for *Bartonella* spp in the pathogenesis of CRS in cats.

**Materials and Methods**

**Animals**—The study involved 4 groups of cats. The study group consisted of cats affected with CRS (group 1). Three control groups comprised cats with other nasal disease (group 2), cats with systemic illness (group 3), and healthy cats (group 4). All procedures performed in the study were approved by the University of California, Davis, Animal Care and Use Committee, and client consent was obtained for use of all cats.

Cats evaluated at the University of California, Davis, Veterinary Medicine Teaching Hospital were included in the study. Cats were prospectively evaluated between February 2005 and March 2007. All cats evaluated for nasal disease were anesthetized. Computed tomography was performed by use of a helical high-speed scanner with 2- or 3-mm thick sections. Rhinoscopy of the rostral portion of the nasal cavities was performed by use of a 2.8-mm rigid telescope or 1.7-mm semiflexible endoscope. In all cats, both sides of the nasal cavity were assessed rhinoscopically and histologically.

A diagnosis of CRS was made on the basis of a history of mucopurulent nasal discharge for > 3 months’ duration, and CT and rhinoscopy with histologic examination of nasal tissues that failed to identify fungal infection, neoplasia, or an identifiable cause for signs of nasal disease. Specifically, CRS was diagnosed on the basis of CT characteristics of fluid density in the nasal cavity or sinuses, rhinoscopic evidence of mucus accumulation with friable and edematous turbinates, and an inflammatory response consistent with CRS (lymphoplasmacytic, histiocytic, neutrophilic, or eosinophilic) on histologic examination.

For the 3 control groups, cats with nasal disease other than CRS (group 2) qualified for the study when a diagnosis was made of a nasal condition attributable to fungal disease, neoplasia, or a foreign body. Rhinoscopy and CT were performed on all cats in group 2 to establish the disease process. Group 3 consisted of cats with systemic disease that lacked nasal disease. Cats with disorders associated with *Bartonella* infection, including abnormal CNS signs, fever, lymphadenopathy, uveitis, and stomatitis, were excluded from control group 3. Entry into these 2 control groups relied on the patient population of the small animal medicine section at the veterinary medical teaching hospital during the time frame established; age, breed, indoor or outdoor status, and flea exposure could not be controlled in these cats. Group 4 consisted of healthy cats matched on the basis of age with CRS cats. Healthy cats were client-owned cats of hospital employees (ie, veterinary students, residents, and technicians). Healthy cats did not have a history of nasal disease and were considered clinically normal on the basis of owner observations. Results of physical examinations and serum biochemical analyses were within expected limits for all healthy cats at the time samples were obtained. A CBC was not performed for the healthy cats because of limitations in funding.

*Bartonella* spp are blood-borne organisms most likely transmitted to cats through flea feces. Therefore, flea exposure was quantified as high or low for each cat on the basis of the history provided by the owner; additional information on flea exposure required subsequent communications with some owners. Cats were considered to be at low risk of flea exposure when they were solely indoor cats, had not been exposed to other cats or dogs, and had not been exposed to an outbreak of fleas during the 6 months preceding examination at our facility. Flea control was not considered as an important factor because monthly preventative medications were used in only a few cats, with the exception of cats that had been exposed because of an outbreak of fleas during the 6 months preceding examination at our facility.

**Procedures**—Serologic testing to detect *Bartonella henselae* and *Bartonella clarridgeiae* and microbial culture of blood samples to detect *Bartonella* spp were conducted in all cats in the study. In cats evaluated because of nasal disease, a biopsy specimen of nasal tissue was obtained for histologic evaluation, and when possible, an additional biopsy specimen of nasal tissue was obtained for PCR assay to detect *Bartonella* spp.

**Serologic testing**—Antibodies against *Bartonella* spp were detected by use of an immunofluorescent antibody test, as described elsewhere. Serial dilutions of serum samples were incubated on slides containing Vero cells infected with strains of *B henselae* or *B clarridgeiae*. Slides were washed and probed with fluorescein isothiocyanate–labeled goat anti-cat immunoglobulin. Fluorescence intensity was evaluated to determine the titer, and cats were considered seropositive when the titer was ≥ 1:64.

**Microbial culture**—Blood samples (2 mL) were collected into EDTA-containing tubes and stored at −70°C prior to processing. Culture techniques have been described elsewhere. Briefly, samples were thawed, centrifuged, and plated onto 5% fetal bovine serum added to 5% rabbit blood agar. Plates were incubated for 1 month at 35°C in an environment of 5% carbon dioxide. Positive identification of colonies was obtained by performing PCR-restriction fragment length polymorphism analysis of the citrate synthase gene and gene sequencing of 16S rRNA on DNA extracted from colonies that grew during culture. The number of colonies was counted.

**Histologic evaluation**—Biopsy specimens of nasal tissues were immersion-fixed for 12 hours in neutral-buffered 10% formalin and embedded in paraffin. 4-μm-thick sections were cut and stained with H&E. All biopsy specimens were evaluated by a veterinary pathology resident and the board-certified veterinary pathologist on duty at the time of submission. Special stains were applied as needed to define the disease process.

**PCR-restriction fragment length polymorphism**—Colonies were scraped off the agar in the culture plates and suspended in 100 μL of sterile water. The bacte-
Material suspension was heated at 100°C for 15 minutes and then centrifuged at 15,000 × g for 10 minutes at 4°C. Supernatant was diluted 1:10 and then used as a template for amplification of the gltA and 16S RNA genes. Primers and methods described elsewhere were used to amplify approximately 1,500 bp of the gltA gene and 1,65 sps were also compared between CRS cats (group 1) and other cats of group 2; all 5 had negative results, including 2 cats that were seronegative for 1 or both Bartonella spp. A biopsy specimen of nasal tissue was obtained for PCR assay from 5 of 10 cats of group 1, and all had negative results. Six of these 9 cats were seropositive for 1 or both Bartonella spp. A biopsy specimen of nasal tissue was obtained for PCR assay from 5 of 10 cats of group 2; all 5 had negative results, including 2 cats that were seropositive for 1 or both Bartonella spp.

Power of the study was calculated at 0.43 for B henselae and 0.42 for B clarridgeiae. To achieve sufficient power

**Results**

The study comprised 19 cats in group 1, 10 cats in group 2, 15 cats in group 3, and 15 cats in group 4. In group 2 cats, 3 had nasal neoplasia (2 with carcinoma and 1 with lymphoma), 2 had aspergillosis, 2 had a foreign body, 2 had nasal discharge secondary to primary lung disease (on the basis of a normal appearance during nasal imaging and histologic examination of nasal biopsy specimens as well as a diagnosis of bronchial disease), and 1 had a nasopharyngeal abscess. In group 3 cats, 6 had primary bronchial disease, 3 had diabetes mellitus, 2 had inflammatory bowel disease, 1 had renal disease, 1 had a megacolon, 1 had FeLV with myelodysplasia, and 1 had severe orthopedic disease.

Median age for cats of group 1 was 7 years (range, 2 to 16 years), which did not differ significantly from that of cats of group 2 (median, 12.5 years; range, 3 to 16 years), 3 (median, 9 years; range, 3 to 19 years), or 4 (median, 6 years; range, 2 to 15 years). However, the age of cats of group 4 was significantly (P = 0.03) less than that of cats of group 2.

Overall, 19 of 59 (32%) cats had positive results when serologic testing was used to detect 1 or both Bartonella spp (Figure 1). Positive results for serologic testing were evident in 9 of 19 cats of group 1, 4 of 10 cats of group 2, 2 of 15 cats of group 3, and 4 of 15 cats of group 4 (Table 1). No significant difference was evident among groups with regard to seropositivity for B henselae, B clarridgeiae, or both. When controlling for flea exposure (high vs low) by use of exact logistic regression analysis, no significant difference was detected among seropositivity rates among all groups.

Microbial culture of blood samples to detect Bartonella organisms yielded negative results in all cats of groups 1, 3, and 4. One cat of group 2 had a positive result (1 colony-forming unit/mL) for B henselae type II. The final diagnosis in that cat was a nasopharyngeal abscess. There was no significant difference in culture results among groups.

A biopsy specimen of nasal tissue was obtained for PCR assay from 9 cats of group 1, and all had negative results. Six of these 9 cats were seropositive for 1 or both Bartonella spp. A biopsy specimen of nasal tissue was obtained for PCR assay from 5 of 10 cats of group 2; all 5 had negative results, including 2 cats that were seropositive for 1 or both Bartonella spp.

Power of the study was calculated at 0.43 for B henselae and 0.42 for B clarridgeiae. To achieve sufficient power

**Statistical analysis**—Statistical analysis was performed by use of a commercial software program. Age was compared among groups by use of an ANOVA. Positive results for serologic testing and microbial culture to detect Bartonella spp were compared among groups by use of the Fisher exact test. To control for flea exposure, positive results for serologic testing to detect Bartonella spp were also compared between CRS cats (group 1) and the 3 control groups by use of exact logistic regression analysis. Analyses of power and sample size were performed by use of commercially available statistical software. Significance was defined as values of P < 0.05.

![Figure 1](image-url)
to detect differences among groups (> 0.80), a sample size of 118 cats would have been required.

Discussion

The study reported here did not reveal significant differences in seropositivity or culture results for *Bartonella* spp in cats with CRS, compared with results for cats in 3 control groups. This finding, in combination with negative results of PCR assay of nasal tissue specimens, suggests that *Bartonella* organisms did not play an important role in the pathogenesis of CRS in this group of cats. However, this study was not designed to determine the role of *Bartonella* spp in specific cats with CRS, and because of the insufficient power of the study to detect differences, specific statements regarding the role of *Bartonella* spp in CRS cannot be made. Twice as many cats would have had to be enrolled in the study to achieve sufficient power to allow for definitive recommendations.

*Bartonella* spp have been associated with respiratory disease in dogs, although their relevance and prevalence have not been widely established. *Bartonella vinsonii* has been linked with granulomatous rhinitis in dogs, and *Bartonella* spp may be associated with nasal discharge and epistaxis in dogs. However, *Bartonella* DNA was not found in nasal tissues of dogs with idiopathic lymphoplasmaclastic rhinitis. In the study reported here on nasal disease in cats, we also found no evidence of *Bartonella* DNA in any nasal biopsy specimens. Negative results for PCR assay could suggest that *Bartonella* organisms initiate the disease process but do not develop a tissue phase of infection or that the organisms are present in undetectable quantities. If nasal inflammation in CRS is localized rather than generalized, a small tissue sample may not contain infective organisms. However, molecular techniques have been used successfully to detect *B henselae* in a dog with inflammatory liver disease, a dog with peliosis hepatitis, and dogs with infective endocarditis. Another study was successful in identifying *B henselae* in a healthy population of cats by use of culture and PCR techniques similar to those used in our study. Polymerase chain reaction assay has been used successfully in identifying *Bartonella* DNA in other tissues obtained from cats as well. Tissues submitted for PCR analysis from experimentally infected SPF cats with evidence of inflammation yielded positive results in more than half of the tissues sampled, and DNA has been amplified from oral swab specimens and samples of CSF.

*Bartonella* spp can cause disease in the feline population. In 1 study, investigators reported that 100% of kittens developed fever, lethargy, lymphadenopathy, and swelling at the site of inoculation with *Bartonella* spp, which indicated that disease can be experimentally induced in cats exposed to these bacteria. Experimental inoculation of these bacteria into SPF cats can cause histologic changes in multiple organ systems and associated inflammation in the lymph nodes, CNS, liver, spleen, kidneys, and heart, although nasal tissue was not examined in that study. *Bartonella* spp have also been implicated as a cause of endocarditis and uveitis in cats. However, there is mixed support for the role of these bacteria as a pathogen in cats. Seropositivity to *B henselae* was not correlated with neurologic signs in 1 study, and investigators failed to detect an association between exposure to *Bartonella* spp and gingivitis in a preliminary study. Finally, inoculation of *B henselae* in conjunction with FHV-1 failed to reactivate ocular toxoplasmosis in chronically infected cats, which suggests that *B henselae* is not synergistic with these known pathogens.

One of the difficulties in the study reported here was determining the definition for a diagnosis of bartonellosis. Amplification of *Bartonella* spp DNA from blood or tissue samples is extremely sensitive and indicates that the organism may be present but does not indicate that it is necessarily viable or capable of causing a particular disease in all instances. A positive result for serologic testing is also problematic because it may indicate exposure but does not indicate infection with the organism. One cross-sectional study performed on healthy government-owned working dogs revealed that 8.7% of dogs were seropositive to *B vinsonii* subsp *berkhoffii*, which indicated that exposure to the organism does not always result in clinical disease. Microbial culture of blood samples can be difficult to perform and may intermittently yield positive results when infected animals are intermittently bacteremic. Microbial culture of blood samples is considered the criterion-referenced standard for proving infection but could underestimate the number of animals infected. In the sample of cats in our study, only 1 had positive results for *Bartonella* spp on microbial culture, and this was a cat in group 2 that was examined because of acute nasal obstruction. Rhinoscopy and CT revealed a nasopharyngeal abscess that resolved completely with antimicrobial treatment and drainage. This
was an indoor-outdoor cat with flea exposure that had no evidence of inflammation consistent with CRS on histologic examination of nasal tissues and no clinical signs consistent with bartonellosis. In our study, we performed all commonly used techniques to maximize the chances of finding an association between Bartonella spp and CRS but were unable to establish a clear relationship. We also were able to compare our group of cats with CRS with 3 separate control groups, which improved the chances of finding an association between Bartonella spp and CRS.

One limitation of the study was the relatively small number of cats enrolled. Results for CRS cats were compared with results for 3 control groups to increase the likelihood of detecting a difference in exposure to and isolation of the organism in affected cats, but the power of the study was insufficient to detect differences. Seropositivity can vary geographically and may be as high as 93% in certain areas. Ideally, cats that were from the same geographic area would be chosen as control animals for each cat with CRS; however, this was not logistically feasible. Other factors, such as age and flea exposure, can also impact results. The study design controlled for age in some but not all groups of control cats, and flea exposure was specifically evaluated; however, no significant differences were detected in the sample of cats evaluated in our study. A potential confounding factor was variability in the recording of flea exposure in the medical record (eg, indoor-outdoor status, flea preventatives or controls). A potential confounding factor was variability in the recording of flea exposure in the medical record (eg, indoor-outdoor status, flea preventatives or controls). A potential confounding factor was variability in the recording of flea exposure in the medical record (eg, indoor-outdoor status, flea preventatives or controls). A potential confounding factor was variability in the recording of flea exposure in the medical record (eg, indoor-outdoor status, flea preventatives or controls).

In the study reported here, we failed to detect an association between Bartonella spp and CRS in cats. Additional studies are required to determine factors that contribute to chronic nasal inflammation. In addition, studies are needed to determine the role of Bartonella spp in cats with chronic inflammatory diseases.

References

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Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Evaluation of perinuclear anti-neutrophilic cytoplasmic autoantibodies as an early marker of protein-losing enteropathy and protein-losing nephropathy in Soft Coated Wheaten Terriers

Karlin Allenspach et al

**Objective**—To evaluate perinuclear anti-neutrophilic cytoplasmic autoantibody (pANCA) status in Soft Coated Wheaten Terriers (SCWTs) and SCWT-Beagle crossbred dogs and to correlate pANCA status of dogs with clinicopathologic variables of protein-losing enteropathy (PLE), protein-losing nephropathy (PLN), or both.

**Animals**—13 SCWTs and 8 SCWT-Beagle crossbred dogs in a research colony and a control group comprising 7 dogs with X-linked hereditary nephropathy and 12 healthy SCWTs > 9 years old.

**Procedures**—Samples were obtained from dogs in the research colony every 6 months. At each sample-collection time point, serum concentrations of albumin, globulin, creatinine, and urea nitrogen; fecal concentration of α-proteinase inhibitor; and urinary protein-to-creatinine ratios were determined and correlated with pANCA status.

**Results**—20 of 21 dogs in the research colony had positive results for pANCA at a minimum of 2 time points, and 18 of 21 dogs had definitive evidence of disease. None of the control dogs had positive results for pANCA. A positive result for pANCA was significantly associated with hypoalbuminemia, and pANCA preceded the onset of hypoalbuminemia on an average of 2.4 years. Sensitivity and specificity for use of pANCA to predict development of PLE or PLN were 0.95 (95% confidence interval, 0.72 to 1.00) and 0.8 (95% confidence interval, 0.51 to 0.95), respectively.

**Conclusions and Clinical Relevance**—Most dogs in this study affected with PLE, PLN, or both had positive results for pANCA before clinicopathologic evidence of disease was detected. Thus, pANCA may be useful as an early noninvasive test of disease in SCWTs. (Am J Vet Res 2008;69:1301–1304)

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