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Relationship Between Degenerative Joint Disease, Pain, and *Bartonella* spp. Seroreactivity in Domesticated Cats

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Background: Recently, a potential association was identified between *Bartonella* exposure and arthritides in mammalian species other than cats.

Hypothesis/Objectives: We hypothesized that *Bartonella* exposure is associated with more severe degenerative joint disease (DJD) and a greater burden of DJD-associated pain in client-owned cats.

Animals: Ninety-four client-owned cats (6 months to 20 years old), ranging from clinically unaffected to severely lame because of DJD.

Methods: Using physical examination and radiography, pain and radiographic scores were assigned to each part of the bony skeleton. Sera were tested for *Bartonella henselae*, *B. koehlerae*, and *B. vinsonii* subsp. *berkhoffii* (genotypes I, II, and III) antibodies using immunofluorescence antibody assays. Variables were categorized and logistic regression used to explore associations.

Results: Seropositivity to *Bartonella* was identified in 33 (35.1%) cats. After multivariate analysis controlling for age, total DJD score (OR, 0.51; 95% CI, 0.26–0.97; $P = .042$), appendicular pain score (OR, 0.33; 95% CI, 0.17–0.65; $P = .0011$), and total pain score (OR, 0.35; 95% CI, 0.17–0.72; $P = .0045$) were significantly inversely associated with *Bartonella* seroreactivity status, indicating that cats with higher DJD and pain scores were less likely to be *Bartonella* seropositive.

Conclusions and Clinical Importance: Based upon this preliminary study, *Bartonella* spp. seropositivity was associated with decreased severity of DJD and decreased DJD-associated pain in cats. Additional studies are needed to verify these findings, and if verified, to explore potential mechanisms.

Key words: *Bartonella* spp.; Cats; Degenerative joint disease; Pain; Radiographic; Seroreactivity.

Research over the last 10 years has highlighted the high prevalence of radiographic evidence of degenerative joint disease (DJD) in cats[1–4] and has shown that a spectrum of clinical signs can be associated with DJD in cats.[5–7] Most authors agree that the prevalence of DJD in cats is strongly and positively associated with age.[2,4] Other work has shown that in association with the increase in radiographic DJD, musculoskeletal pain increases, whereas mobility and the ability to perform activities decreases.[2,8]

Despite the high prevalence, currently, little is known about the etiology of DJD in cats.[9] Recently, based upon gene microarray data, immune system dysfunction was found to be associated with DJD in cats,[10] but the relationship between is not clear.

There is increasing interest in the potential role of systemic infections in the etiology of joint disease in mammals. In dogs, synovial specimens from 43 dogs diagnosed with degenerative rupture of the cranial cruciate ligament were tested for presence of bacterial DNA.[11] Of those, 37% were found to be PCR positive with mixtures of environmental bacterial nucleic acids found. In their discussion, the authors suggested that these bacterial mixtures or their products could promote the development of synovitis. Additional work by the group suggested that bacterial load is unlikely to be a primary pro-inflammatory factor, but the authors suggested dysregulation of immune responses within synovial tissues might be dependent upon an environmental microbial trigger.[12] *Bartonella* spp. have been implicated as a cause of lameness. One study in dogs evaluated the relationship between lameness and seroprevalence of *Bartonella* spp. antibodies, and found a positive association between lameness and arthritis-related lameness and *Bartonella* spp. seroreactivity.[13] It has also been suggested that immune dysregulation occurs in dogs experimentally infected with *Bartonella vinsonii* subsp. *berkhoffii* genoptype I, which could predispose these dogs to autoimmune or immune-mediated diseases such as polyarthritis.[14] In

Abbreviations:

CrCL	cranial cruciate ligament
DJD	degenerative joint disease
FELV	feline leukemia virus
FIV	feline immunodeficiency virus
FVRCP	feline viral rhinotracheitis, calicivirus and panleukopenia
LPS	lipopolysaccharide
PCR	polymerase chain reaction
TLR-4	toll like receptor 4

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horses, 1 study found a high prevalence of *Bartonella* spp. bacteremia in lame horses as compared to controls.[15] In an uncontrolled study, 62% of 296 human patients selected by a rheumatologist for testing were found to be positive for antibodies against *Bartonella* spp.[16]

Despite these findings in other species, the high prevalence of DJD in cats, and the fact that cats are the natural host for *Bartonella*, to the authors' knowledge, no studies have evaluated if there is any relationship between DJD and associated pain and *Bartonella* exposure in cats. On the basis of the findings in other species, we hypothesized that *Bartonella* spp. seropositivity in cats is associated with more severe radiographic DJD and a greater burden of DJD-associated pain. The aim of this study was to explore a potential relationship between radiographic DJD, pain assessed on palpation during orthopedic evaluation and *Bartonella* spp. seropositivity in a population of domestic cats.

Materials and Methods

Samples were collected for this study under clinical research protocols approved by the Institutional Animal Care and Use Committee at North Carolina State University College of Veterinary Medicine (NCSU-CVM) (IACUC numbers 05-020-O and 06-056-O). Informed owner consent was granted in each case.

Animals

This observational study used samples taken from cats (n = 100) recruited for an earlier study evaluating the prevalence of DJD, as previously described,[2] and samples taken from cats (n = 12) recruited to a study evaluating the efficacy of a diet for the alleviation of DJD-associated pain.[17] Cats from the latter study were included if they had been screened in exactly the same manner as for the former study.

Demographic and Clinical Data Collected

Data collected included: age, weight, sex, body condition score, vaccination status [rabies, feline leukemia virus (FeLV), feline viral rhinotracheitis, calicivirus and panleukopenia (FVRCP)], current tick and flea prevention status, appetite, and whether or not there were other cats in the household. In addition, the owners were asked if they thought their cat had arthritis (yes/no response).

Orthopedic examination of the appendicular and axial skeleton was performed for all cats by a single investigator (BDXL). The degree of musculoskeletal pain in response to palpation of each appendicular joint and each segment of the axial skeleton was graded as previously described[8] using a numerical rating scale: 0 = no resentment; 1 = mild withdrawal, mildly resist; 2 = moderate withdrawal, body tenses, might orient to site, might vocalize, hiss or bite; 3 = orients to site, forcible withdrawal from manipulation, might vocalize, hiss or bite; 4 = tries to escape or prevent manipulation, hisses or bites, marked guarding of the area. Scores were summed across all appendicular joints (appendicular pain score; maximum score, 64), each part of the axial skeleton (axial pain score; maximum score, 16) and a total pain score calculated from the sum of these 2 (total pain score; maximum score, 80).

In addition, a temperament score was given to each cat as previously described[8] with 0 = neutral attitude, purring, kneading; 1 = resistance to restraint; 2 = resistance to restraint, growling and hissing; 3 = resistance with biting and scratching, vocalizing and hissing and 4 = resistance with biting, scratching, vocalizing, hissing, urinating or defecating. The temperament score was assigned by a single investigator (BDXL) in all cases, and was assigned after completion of the examination.

Radiographic examination was performed as previously described.[2] Scores for severity of defined radiographic features were allocated to each appendicular joint and an overall score on a 0–10 scale assigned to each joint. These overall scores for each joint were summed to create an appendicular DJD score (appendicular DJD score; maximum score, 160). Scores likewise were allocated to each segment of the axial skeleton and summed to create an axial skeleton DJD score (axial DJD score; maximum score, 40). Addition of the appendicular DJD and axial DJD scores created a total DJD score (total DJD score; maximum score, 200). Features that were evaluated have been previously described in detail.[2,8]

Evaluation of Bartonella spp. Seroreactivity

Bartonella henselae, *B. koehlerae*, and *B. vinsonii* subsp. *berkhoffii* (genotypes I, II, and III) antigens were used with traditional immunofluorescence antibody assay (IFA) methods using fluorescein-conjugated goat anti-cat IgG (Pierce Antibody[a]) to determine the antibody titer to each *Bartonella* sp. or subspecies.[18] Briefly, isolates of *B. henselae* (strain Houston-1, ATCC #49882), *B. koehlerae* (NCSU FO-1-09) and *B. vinsonii* subsp. *berkhoffii* genotype I (NCSU isolate 93-CO-1, ATCC #51672), II (NCSU isolate 95-CO-2) and genotype III (NCSU isolate 06-CO1) were passed from agar-grown cultures into *Bartonella*-permissive tissue culture cell lines (AAE12 [an embryonic *Amblyomma americanum* tick cell line] for *B. henselae*, Vero (a mammalian fibroblast cell line) for the *B. vinsonii* genotypes, and DH82 (a canine monocytoid cell line) for *B. koehlerae*) to obtain intracellular whole bacterial antigens for IFA testing. Heavily infected cell cultures were spotted onto 30-well Teflon-coated slides (Cel-Line[a]) air-dried, acetone fixed, and stored frozen. Serum samples were diluted in phosphate-buffered saline (PBS) solution containing normal goat serum, Tween-20, and powdered nonfat dry milk to block nonspecific antigen binding sites and incubated on antigen slides. All available patient sera were screened at dilutions of 1 : 16 to 1 : 64. All sera that were reactive at a 1 : 64 dilution were further tested with 2-fold dilutions out to 1 : 8,192. A threshold titer of 1 : 64 was used to define a seroreactive (seropositive) antibody response against a specific *Bartonella* sp. antigen.

Statistical Analysis

Descriptive statistics were used to describe *Bartonella* spp. seroreactivity; number of cats with radiographic DJD and musculoskeletal pain in the appendicular and axial skeleton; summed severity for total DJD and total pain in each cat; physical examination findings related to pain and DJD; and demographic characteristics of the population, including owner assessment of whether or not they thought the cat had arthritis and the number of cats previously living with other cats. Comparisons were made to evaluate associations between each variable (including DJD score, pain scores, demographic characteristics, and clinical features) and *B. henselae*, *B. koehlerae*, and *B. visonii* subsp. *berkhoffii* seropositivity separately, in addition to a composite variable for seropositivity to any of the 3 *Bartonella* sp. antigens. Variables with >5% missing values were not included in the analyses to avoid potential exclusion biases for cats with missing data. Total DJD scores were grouped into approximate tercile categories: low (0–5), moderate (6–17), and high (18–41) scores. Similarly, total pain scores were

grouped into categories: low (0–2), moderate (3–10), and high (11–45) scores. Axial and appendicular pain and DJD scores were categorized also into approximate terciles: axial pain: low (0), moderate (1–2), and high (2–13) scores; appendicular pain: low (0–1), moderate (2–5), and high (6–33) scores; axial DJD: low (0), moderate (1–3), and high (4–23) scores; appendicular DJD: low (0–4), moderate (5–13), and high (14–34) scores. All terciles were created to correspond to approximate clinical designations of low, moderate and severe DJD or pain based on clinical experience. Univariate analysis was carried out using the chi-square or Fisher's exact test for 2 × 2 comparisons, the Fisher-Freeman-Halton test for 2 × 3 comparisons, and the Mann-Whitney-Wilcoxon test for continuous comparisons to assess associations between each variable and *Bartonella* spp. or sp. seropositivity status. The categorical variables of total, axial, and appendicular DJD and pain scores, were entered individually into logistic regression analysis while controlling for age (ie, DJD score and pain score were not entered into the model at the same time). Age was categorized into 3 levels: 0–4.99 years, 5–9.99 years, and ≥10 years. Four outcome variables included: *B. henselae* seroreactivity, *B. koehlerae* seroreactivity, *B. visonii* subsp. *berkhoffii* seropositivity, and seropositivity to any of the 3 *Bartonella* sp. antigens. Significance was set at $P \leq .05$. Statistical analyses were performed using SAS/STAT 9.2 for Windows.[b]

Results

One-hundred and twelve cats were included in this study; 18 cats were excluded from analysis because of missing values related to DJD score, pain score, or *Bartonella* spp. seropositivity status. A total of 94 cats, 55 (58.5%) spayed females and 39 (41.5%) neutered males, were included in the analysis. The median age in years was 9.26 (range, 1.03–19.89), and median body weight was 4.78 kg (range, 2.08–10.16). Median appetite score was 85.9 (range, 0–100). Temperament score distributions were: 0 in 43.6% of the cats, 1 in 19.2%, 2 in 13.8%, 3 in 21.3% to 4 in 2.1% of cats. Seventy-four (78.7%) cats had lived previously with other cats. Owners of 32 cats (34.0%) indicated they believed their cats had arthritis. Forty-one (43.6%) cats were given flea and tick preventative treatment on a regular basis. Forty-two (44.7%) cats were reported to have been vaccinated against rabies, whereas 11 (11.7%) and 33 (35.1%) were reported to have been vaccinated against FeLV and FVRCP. No cats (0.0%) tested positive for FeLV or FIV.

The overall prevalence of DJD within the population was 91.5% (n = 86). The median total DJD score was 10 and ranged from 0 to 41. The appendicular skeleton was affected with DJD in 85 (90.4%) cats, whereas the axial skeleton was affected in 49 (51.1%) cats. The median DJD scores for the appendicular and axial skeletons were 7 (range, 0–34) and 1 (range, 0–23), respectively. Pain associated with orthopedic examination was detectable in 73 (77.6%) cats with a median total pain score of 4; range, 0–45. Pain in the appendicular skeleton was most commonly detected, and affected 65 (69.2%) cats; the median pain score for cats with appendicular involvement was 3 (range, 0–33). Thirty-nine (41.5%) cats had pain detectable in the axial skeleton; axial pain scores ranged from 0–12 (median, 0).

Based on IFA antibody testing, seropositivity to *B. henselae*, *B. koehlerae*, or *B. vinsonii* subsp. *berkhoffii* was identified in 33 (35.1%) cats. Titers ranged from <1 : 16 to 1 : 1024. Fourteen (14.9%) cats were *B. henselae* seropositive, 26 (27.6%) were *B. koehlerae* seropositive, and 20 (21.3%) were seropositive to *B. vinsonii* subsp. *berkhoffii* antigens. Of the 33 seropositive cats, 19 were seropositive to multiple *Bartonella* spp. antigens (57.6%).

Bartonella spp. seropositivity did not vary by age ($P = .13$), sex ($P = .89$), body weight (<4.78 kg versus ≥4.78 kg; $P = .27$), temperament ($P = .97$), appetite ($P = .09$), flea and tick prevention status ($P = .86$), or whether or not there were other cats in the household ($P = .78$).

Whether owners thought their cats had arthritis varied by *Bartonella* spp. seropositivity in univariate analysis ($P = .020$). Interestingly, in only 18.2% (n = 6) of *Bartonella* spp. seropositive cats did owners consider the cat had arthritis, compared to 42.6% (n = 26) of seronegative cats. After controlling for age, this association remained significant ($P = .027$), and indicated that cats with owners that considered them to have arthritis were 3.97 times more likely to be *Bartonella* spp. seronegative than cats with owners who did not think the cat had arthritis (OR, 0.25; CI, 0.07–0.85). Univariate analysis identified the following variables as significantly inversely associated with *Bartonella* spp. seropositivity: total DJD score ($P = .022$), appendicular pain score ($P = .0016$), and total pain score ($P = .0063$). Appendicular DJD score ($P = .19$), axial DJD score ($P = .12$), and axial pain score ($P = .13$) were not significantly associated with *Bartonella* spp. seropositivity (Table 1). After multivariate analysis controlling for age, total DJD score ($P = .042$), appendicular pain score ($P = .0011$), and total pain score ($P = .0045$) remained significantly inversely associated with *Bartonella spp*. seropositivity status (Tables 2, 3). Overall, our results from multivariate analysis indicated that cats with higher total DJD scores, appendicular pain scores or total pain scores were less likely to be *Bartonella* seropositive than cats with lower scores.

Significant inverse associations between DJD or pain scores and *Bartonella* spp. specific seropositivity also were found during univariate analysis for *B. henselae* seropositivity and axial pain ($P = .011$); *B. koehlerae* seropositivity and appendicular pain ($P = .043$), axial pain ($P = .019$), and total pain ($P = .028$); and, between *B. vinsonii* subsp *berkhoffi* seropositivity and appendicular pain ($P = .044$). Although some of these associations became nonsignificant after controlling for age in logistic regression models, indicating that age might have confounded the effect in univariate analysis, the inverse associations between *B. henselae* seropositivity and appendicular DJD ($P = .046$); *B. koehlerae* seropositivity and appendicular pain ($P = .033$), axial pain ($P = .037$) and total pain ($P = .017$); and, *B. vinsonii* subsp *berkhoffi* seropositivity and appendicular pain ($P = .024$) were significant while controlling for age (Tables 2, 3).

Table 1. *Bartonella* seroreactivity in association with total degenerative joint disease (DJD) scores and total pain scores after univariate analysis.

	DJD Total				Pain Total			
Seroreactivity	Low	Moderate	High	*P*-Value	Low	Moderate	High	*P*-Value
Bartonella spp. composite sero+	14 (42.2)	14 (42.2)	5 (15.2)	.022	18 (54.5)	13 (39.4)	2 (6.1)	.0063
Bartonella spp. composite sero−	19 (31.2)	15 (24.6)	27 (44.3)		18 (29.5)	24 (39.3)	19 (31.2)	
B. henselae sero+	7 (50.0)	5 (35.7)	2 (14.3)	.22	8 (57.2)	5 (35.7)	1 (7.2)	.19
B. henselae sero−	26 (32.5)	24 (30.0)	30 (37.5)		28 (35.0)	32 (40.0)	20 (25.0)	
B. koehlerae sero+	10 (38.5)	11 (42.3)	5 (19.2)	.14	15 (57.7)	9 (34.6)	2 (7.7)	.028
B. koehlerae sero−	23 (33.8)	18 (26.5)	27 (39.7)		21 (30.8)	28 (41.2)	19 (27.9)	
B. vinsonii subsp. *berkhoffii* sero+	9 (45.0)	8 (40.0)	3 (15.0)	.13	10 (50.0)	9 (45.0)	1 (5.0)	.082
B. vinsonii subsp. *berkhoffii* sero−	24 (32.4)	21 (28.4)	29 (39.2)		26 (35.2)	28 (37.8)	20 (27.0)	

Numbers indicate the number of cats in each DJD or Pain designation that were seropositive (sero+) or seronegative (sero−) for each *Bartonella* sp., and for any of the three *Bartonella* spp. (composite). Numbers indicate the number of cats and numbers in brackets indicate the percentage distribution within the designation of sero+ or sero− for either DJD or Pain.

Table 2. *Bartonella* seroreactivity in association with total degenerative joint disease (DJD) scores, appendicular DJD scores, and axial DJD scores after multivariate analysis.

Seroreactivity	Total DJD OR (95% CI; *P*-Value)	Appendicular DJD	Axial DJD
B. henselae	0.46 (0.19–1.11; $P = .085$)	0.41 (0.17–0.98; $P = .046$)	0.59 (0.26–1.41; $P = .24$)
B. koehlerae	0.75 (0.38–1.48; $P = .42$)	0.86 (0.45–1.63; $P = .64$)	0.55 (0.28–1.11; $P = .095$)
B. vinsonii subsp. *berkhoffii*	0.54 (0.25–1.16; $P = .11$)	0.75 (0.37–1.52; $P = .42$)	0.77 (0.37–1.06; $P = .48$)
Bartonella spp. composite	0.51 (0.26–0.97; $P = .042$)	0.72 (0.40–1.32; $P = .29$)	0.53 (0.86–1.03; $P = .062$)

Odds ratios (and 95% confidence intervals) are shown in relation to seroreactivity to *B. henselae*; *B. koehlerae*; and *B. vinsonii* subsp. *berkhoffii*; and a composite *Bartonella* variable representing seroreactivity to any of the three species.

Table 3. *Bartonella* seroreactivity in association with total pain, appendicular pain, and axial pain scores after multivariate analysis.

Seroreactivity	Total Pain OR (95% CI; *P*-Value)	Appendicular Pain	Axial Pain
B. henselae	0.42 (0.16–1.09; $P = .075$)	0.65 (0.29–1.42; $P = .27$)	0.45 (0.29–1.42; $P = .081$)
B. koehlerae	0.39 (0.18–0.84; $P = .017$)	0.49 (0.26–0.95; $P = .033$)	0.50 (0.26–0.96; $P = .037$)
B. vinsonii subsp. *berkhoffii*	0.49 (0.22–1.09; $P = .083$)	0.42 (0.20–0.89; $P = .024$)	0.82 (0.44–1.53; $P = .54$)
Bartonella composite	0.35 (0.17–0.72; $P = .0045$)	0.33 (0.17–0.65; $P = .0011$)	0.62 (0.35–1.07; $P = .087$)

Odds ratios (and 95% confidence intervals) are shown in relation to seroreactivity to *B. henselae*; *B. koehlerae*; and *B. vinsonii* subsp. *berkhoffii*; and a composite *Bartonella* variable representing seroreactivity to any of the three species.

Discussion

In this study, we found that cats with higher DJD and pain scores were less likely to be *Bartonella* seropositive than cats with lower scores, thus rejecting our hypothesis that *Bartonella* exposure is associated with more severe DJD and a greater burden of musculoskeletal pain. Even after controlling for age, the association between *Bartonella* seronegativity and increased DJD and pain scores remained significant. However, it is possible that unmeasured confounders such as immune status, arthropod exposure and other infections could impact the observed associations. These findings are surprising and require additional study.

Radiographic evidence of DJD and pain responses on manipulation of the skeleton are not necessarily measures of the same thing. Previous work by our group has indicated that the detection of joint pain had poor sensitivity for the detection of radiographic DJD, and also had poor positive predictive value.[8] This is not surprising given that clinical signs and radiographic severity are not closely related in humans with DJD.[19,20] Radiographic signs of DJD relate to 1 aspect of the disease, and pain scores relate to another (ie, to the current clinical impact of the disease). Obviously, there must be a relationship between the 2, but at a given point in time the burden of radiographic disease in a given joint might not match the burden of pain. Thus, in order to more completely assess the relationship between *Bartonella* seropositivity and DJD in cats, we, a priori, set out to look at both pain and radiographic DJD. Appendicular DJD refers to synovial joints, and axial DJD to a combination of some synovial joints (facets) and intervertebral disk joints.[9] Overall, appendicular and axial DJD could be considered to represent 2 different pathologies, but the

pathogenesis of appendicular and axial DJD in the cat is largely unknown.[9] Interestingly, our data did not indicate any association between *Bartonella* seropositivity and either appendicular DJD or axial DJD (except for some individual *Bartonella* sp. associations), only with the total DJD score. On the basis of the consistency of the significant associations we found across our data, our results appeared to indicate a more robust association between *Bartonella* seropositivity and lower pain scores than between seropositivity and lower radiographic DJD scores.

Our results were opposite of what we expected to find, and it is important to consider all explanations. Firstly, although these findings are provocative, the results are from 1 study, and these results should be replicated in order to be more certain they do not reflect Type I error. Importantly, we did not correct for multiple comparisons within the factors being evaluated (eg, DJD, pain) and this increases the likelihood of finding significant associations. We believe our approach was appropriate for an exploratory study. Other factors we did not consider might be important confounding variables. For example, the presence of clinical DJD might have altered the cats' behavior, making them less likely to be exposed to *Bartonella*. Conversely, a lack of pain might have altered the cats' behavior making them more likely to roam and become exposed to *Bartonella*.

Other investigators have found surprising associations between *Bartonella* spp. seropositivity in cats and disease states. In a study investigating *B. henselae* seroprevalence in cats with clinical signs of neurologic disease,[22] the authors found that the prevalence of *Bartonella* spp. antibodies was significantly lower in the group of cats with neurologic manifestations than in healthy cats and clinically ill cats without neurologic signs. The authors discussed various explanations for this finding, including the possibility that antibodies might not accurately indicate exposure to or infection with *B. henselae*. Because neurobartonellosis is a well recognized entity in human patients,[23] the authors suspected an association between neurologic disease and bartonellosis. In another study, the prevalence of *Bartonella* spp. antibody titers in cats with gingivitis and stomatitis [37/70 (52.9%)] was slightly lower than in the healthy control cats [36/61 (59.0%)], but this difference was not significant.[24] In a different study, in which, both antibody status and bacteremia were assessed, only bacteremia was significantly associated with gingivitis and stomatitis.[25] In a study evaluating *Bartonella* spp. seroprevalence in cats with or without uveitis, the investigators found that healthy cats were significantly more likely to be *Bartonella* spp. seropositive than cats with uveitis and healthy cats were more likely to have higher titers than cats with uveitis and cats with nonocular disease.[26] Collectively, in conjunction with the results of this study, there is a body of observational evidence indicating that seropositivity to *Bartonella* spp. appears to be associated with decreased radiographic DJD, musculoskeletal pain, neurologic disease, gingivitis, stomatitis and uveitis. These studies do not indicate a cause-and-effect relationship, simply an inverse relationship between *Bartonella* spp. seropositivity and certain diseases. If there is a cause-and-effect relationship, establishing the mechanisms ultimately could lead to substantial preventive care, medical treatment, or both.

Bartonella spp. have been associated with various serious diseases in cats (eg, endocarditis,[27] osteomyelitis,[28] and myocarditis[29]), and there is continued discussion on what a "*Bartonella* sp. seropositive" result actually means in terms of prior exposure or ongoing infection.

The flea, the cat, and *Bartonella* have co-existed for so long that it is possible *Bartonella* spp. and the cat have co-evolved over time and there is some benefit to both species of this co-existence. Most studies of *Bartonella* in cats refer to cats as the natural reservoir of *B. henselae and B. clarridgeiae*, and discuss medical implications of infection in cats with other *Bartonella* spp. Recent evidence indicates that there is variation in virulence among *B. henselae* strains, with most strains found in cats differing genetically from the strains that induce cat scratch disease in humans, solely or predominantly caused by *B. henselae*. Although the prevalence of *Bartonella* spp. bacteremia (most often because of *B. henselae* or *B. clarridgeiae)*, can be ≥50% or in feral cats or cats with extensive arthropod exposure. The majority of these cats do not have any clear disease associated with a Bartonella. However, it is clear that some strains of *B. henselae* are highly pathogenic in cats.[29]

If this finding of an association between seropositivity to *Bartonella* spp. and decreased burden of DJD and musculoskeletal pain is eventually proven to be a causative association, it is likely a complex immune response occurring after *Bartonella* spp. exposure that will be the modulating factor in the development of DJD and musculoskeletal pain. Unfortunately, very little is known about the etiology of DJD in cats,[9] or the mechanisms of long-term musculoskeletal pain in cats, and it is too early to postulate what these mechanisms might be. It was recently shown that *B. quintana* lipopolysaccharide (LPS) is a potent Toll Like Receptor-4 (TLR-4) antagonist[21] suggesting *B. quintana* LPS might prove useful as a potent anti-TLR-4 agent with therapeutic potential in both infections and autoimmune inflammation. The same group found that inhibition of TLR-4 suppresses the severity of arthritis in an experimental model of an immune-based arthritis (ie, collagen-induced arthritis) and resulted in lower IL-1 expression in arthritic joints, and they suggested that TLR-4 might be a novel target in the treatment of rheumatoid arthritis.[30] However, this rodent model was an experimental immune-based arthritis, and might not reflect naturally occurring DJD in cats. With regard to pain, there is increasing evidence that TLRs and their associated signaling components contribute to pain hypersensitivity, and that blockade of TLR signaling can decrease pathologic pain and hypersensitivity,[31] including that in rodent models of immune-mediated arthritis.[32] However, these rodent

models of immune-mediated arthritis may not reflect DJD in cats.

Additional work is needed to understand the mechanisms of DJD and long-term musculoskeletal pain in cats, and to evaluate whether or not there are immunologic differences between seroreactive and nonseroreactive cats and whether these differences might relate to the observations seen in this study.

One of the limitations of this study is that we used cats from a restricted geographic area, and the majority of the cats in our study were under the care of an exclusively feline only practice. The seroprevalence of *Bartonella* spp. varies across different regions of North America,[33] and it would be useful to repeat this study in different geographical areas and determine if the relationships remain. Additionally, this work should be repeated in a more diverse local population of cats.

The results of our study add to a small body of work reporting associations between *Bartonella* seropositivity and decreased disease burden across several diseases. Breitschwerdt and Lappin[34] wrote "comprehensive, sequential, long term studies will be necessary to establish whether cats pay a 'biologic price' when chronically bacteremic with a *Bartonella* species." Although this is true, we suggest that some cats may gain a "biologic benefit" from *Bartonella* spp seropositivity.

Footnotes

[a] Thermo Fisher Scientific, Rockford IL
[b] SAS Institute Inc, Cary, NC

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Conflict of Interest Declaration: In conjunction with Dr. Sushama Sontakke and North Carolina State University, Dr. Breitschwerdt holds U.S. Patent No. 7,115,385, Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is the chief scientific officer for Galaxy Diagnostics, a newly formed company that provides advanced diagnostic testing for the detection of *Bartonella* species infection in animals.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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