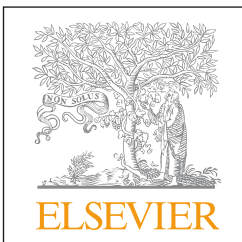


# Journal of **feline** medicine and surgery



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## JFMS 'classic' ... sample the pure

At the time the *Journal of Feline Medicine and Surgery* was launched, in 1999, interest in the cat as a veterinary species was growing worldwide, and the peculiar challenges presented by this complex species were well recognised. Against this backdrop, there was a clear vision for the journal: to provide a forum for the dissemination of information that impacts the way we undertake clinical practice, and which ultimately benefits the health and welfare of our feline patients and the bond between our patients and our clients.

A decade on, and the publication – journal of both the European Society of Feline Medicine and the American Association of Feline Practitioners – occupies a truly mainstream position in the veterinary literature. A 'journal of choice' for many authors who wish to reach those interested in clinical feline medicine and surgery, it presents a mix of original papers, case reports and state-of-the-art reviews in print and online at [www.elsevier.com/locate/jfms](http://www.elsevier.com/locate/jfms). *JFMS* is currently ranked 36th out of 133 titles in the Veterinary Sciences ISI Category, with an impact factor of 1.168\*, and these impressive credentials are down to the quality of the contributions we receive and a sceptical, but supportive, peer review process. This free sample issue previews one such paper, due for publication in print shortly – 'Prevalence of *Bartonella* species antibodies and *Bartonella* species DNA in the blood of cats with and without fever', by Professor Michael Lappin and colleagues in the USA – to give you a flavour of what we refer to as *JFMS* 'classic'. For 2009 and beyond we promise a wealth of quality 'classic' articles, such as this, and these will appear in the February, April, June, August, October and December issues each year – as has become traditional.

Building on that tradition, while moving forward with the original vision, the journal will become a monthly title from 2009. With the interests of those engaged in the 'front line' of first opinion practice firmly in mind, will be six 'clinical practice' issues of *JFMS* a year. Appearing in January, March, May, July, September and November, to alternate with *JFMS* 'classic', these issues will feature topical and timely information of direct relevance to clinical feline practice. This will largely comprise opinionated reviews from respected clinicians and experts in their field, including reviews where traditional thinking is challenged! The clinical issues will have a distinctive, engaging and entirely complementary look. But don't just take our word for it: flip over your sample issue and read on through to judge for yourself!

We're confident you'll agree that the two editions – 'classic' and 'clinical' – add up to one indispensable publication for the keen feline practitioner. So please join us as we mark our 10<sup>th</sup> anniversary and a very exciting milestone for the journal. The ESFM and AAFP, respectively, provide a host of benefits in addition to the journal, and look forward to welcoming new members. You will find full details on the membership forms included in this sample issue.

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## Aims and Scope\*

The *Journal of Feline Medicine and Surgery* is an international journal, and the official Journal of both the *European Society of Feline Medicine* and the *American Association of Feline Practitioners*. It is published monthly in two formats.

The 'classic' editions (published in February, April, June, August, October and December) contain high quality original papers on all aspects of feline medicine and surgery, including relevant basic research. Manuscripts can be submitted as full papers, short communications, case series, individual case reports or letters to the editor.

The 'clinical practice' editions (published in January, March, May, July, September and November) primarily contain commissioned opinionated review articles of direct relevance to feline clinical work, along with other relevant clinical articles such as case reports.

Offers of reviews and topics for consideration should be directed to the editors, via the editorial office (jfmsclinical@fabcats.org), for initial editorial approval. All submissions (including commissioned reviews and letters) are subject to peer review by the editors and selected referees. An international news section provides information about ESFM, AAFF and other feline veterinary meetings, society news, selected product and other developments, and relevant issues from other publications and meetings.

### Publication information

*Journal of Feline Medicine and Surgery* (ISSN 1098-612X). For 2009, Volume 11 (12 issues) is scheduled for publication. Subscription prices are available upon request from the publisher or from the Regional Sales Office nearest you or from this journal's website (<http://www.elsevier.com/locate/jfms>). Further information is available on this journal and other Elsevier products through Elsevier's website (<http://www.elsevier.com>). Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request. Claims for missing issues should be made within six months of the date of dispatch.

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For enquiries relating to commissioned articles for the 'clinical practice' editions, please contact the editorial office at [jfmsclinical@fabcats.org](mailto:jfmsclinical@fabcats.org)

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\* abbreviated version

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**Members of the European Society of Feline Medicine and the American Association of Feline Practitioners will receive the *Journal of Feline Medicine and Surgery* as part of their membership.**

## Prevalence of *Bartonella* species antibodies and *Bartonella* species DNA in the blood of cats with and without fever

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The purpose of this study was to determine whether there are associations between *Bartonella* species antibody (enzyme-linked immunosorbent assay (ELISA) and Western blot (WB)) and polymerase chain reaction assay results in cats with and without fever. Afebrile control cats (39/93; 42.0%) were more likely to have *Bartonella* species antibodies than cats with fever (29/93; 31.2%). The difference in prevalence of *Bartonella* species deoxyribonucleic acid (DNA) in blood of cats with fever (14/81; 17.3%) as compared to afebrile control cats (6/81; 7.4%) approached statistical significance ( $P = 0.0571$ ). *Bartonella* species ELISA or WB results frequently did not correlate to the presence or absence of *Bartonella* species DNA in blood. The results of this study indicate that in cats, *Bartonella* species antibody tests cannot predict whether fever is due to *Bartonella* species infection and should not be used to determine the *Bartonella* species infection status.

Date accepted: 23 June 2008

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*Bartonella henselae* is the cause of cat scratch disease as well as the most common cause of bacillary angiomatosis and of bacillary peliosis, disorders often found affecting people with acquired immune deficiency syndrome (AIDS).<sup>1-3</sup> Cats in North America are also infected with *Bartonella clarridgeiae* an organism found to be a potential cause of clinical disease in people.<sup>4</sup> Other *Bartonella* species known to infect cats less frequently include *Bartonella quintana*,<sup>5,6</sup> *Bartonella koehlerae*<sup>7</sup> and *Bartonella bovis*.<sup>8</sup> Based on prevalence studies in cats, exposure to *Bartonella* species is very common but varies by region in the United States.<sup>9-11</sup> The organism is known to be transmitted between cats by fleas.<sup>12</sup> Therefore, in a previous study, we collected fleas from 92 cats and attempted to amplify *Bartonella* species deoxyribonucleic acid (DNA) from flea digests as well as the blood of the cats.<sup>13</sup> The overall *Bartonella* species DNA prevalence rates in cats and their fleas were 47.8% and 62%, respectively. The *B. henselae* DNA prevalence rates in cats and their fleas were 34.8% and 40.2%, respectively. For *B. clarridgeiae*, these rates were 20.7% and 50%, respectively.

*Bartonella* species infection of experimentally inoculated cats<sup>14-18, 29</sup> or client-owned cats<sup>20-24</sup> has also been associated directly or indirectly with a variety of clinical manifestations including fever, lethargy, lymphadenopathy, uveitis, gingivitis, urinary tract disease, and neurological diseases. Blood culture and polymerase chain reaction (PCR) assays that amplify *Bartonella* species DNA from blood can be used to assess individual cats for *Bartonella* infection whereas serum antibody tests are used to implicate prior exposure. Because currently available tests for detection of exposure to a *Bartonella* species or infection with a *Bartonella* species can be positive in both healthy and clinically ill cats, the positive predictive values for different potential clinical manifestations are less than 100%. How often chronically infected bacteremic cats become ill as a result of their *Bartonella* species infection is unknown and so additional controlled studies are needed to help determine which clinical syndromes might be associated with bartonellosis in cats and to determine the optimal diagnostic tests that can be used by a veterinarian to support the diagnosis of bartonellosis in cats. If the presence of the organism can be epidemiologically linked to specific clinical syndromes, it would help veterinarians decide which clinically ill cats should be tested

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Please cite this article in press as: Michael R Lappin et al., Prevalence of *Bartonella* species antibodies and *Bartonella* species DNA in the blood of cats with and without fever, *J Feline Med Surg* (2008), doi:10.1016/j.jfms.2008.06.005

for *Bartonella* species infection. The purpose of this study was to determine if there are associations between *Bartonella* species serology and PCR results in febrile cats as compared to afebrile control cats that were sampled on the same day from veterinary clinics in North America.

## Materials and methods

### *Bartonella henselae* antigen preparation

*Bartonella henselae* strain Houston-1 was grown on 10 sheep blood agar plates at 35°C, 5% CO<sub>2</sub> for 11 days until colonies were plentiful (Vector Borne Disease Diagnostic Laboratory [VBDDL], North Carolina State University, Raleigh, NC, USA). Colonies were scraped from the agar and stored at -70°C in 0.01 M phosphate buffered saline solution (PBS) pH 7.2 until shipped to Colorado State University on dry ice. After thawing at 20°C room temperature, the bacterial suspension (2 ml) was sonicated for 90 s at 35% power and centrifuged at 10,000 × g for 10 min. The bacterial pellet was suspended in 1 ml distilled water to lyse the bacterial walls and the protein content measured (50 mg/dl).

### *Bartonella henselae* enzyme-linked immunosorbent assay (ELISA) optimization

An indirect ELISA was developed to measure the concentration of antibodies against *B henselae* lysates. Sera from four specific pathogen-free kittens collected prior to inoculation and after intravenous inoculation of *B henselae* (week 16) as well as sera with a range of *B henselae* titers determined by immunofluorescent assay (VBDDL) were selected for use in the initial ELISA titration experiments. Multiple concentrations of *B henselae* lysates, and secondary antibodies as well as various sources and formulations of buffers, blocking solutions and micro-ELISA plates were assessed during optimization of the ELISA.

In the optimized ELISA, 100 µl of a 1:2000 dilution of *B henselae* lysate in PBS (pH 7.2) was incubated in micro-ELISA plates (Immulon 2, Thermo Fisher Scientific, Waltham, MA, USA) for approximately 16 h at 4°C. Plates were washed three times with PBS solution containing 0.05% Tween 20 (PBS-Tween solution). One feline serum sample obtained prior to inoculation and one sample obtained at week 16 after inoculation with *B henselae* were selected randomly for use as the positive and negative controls for all experiments. A positive control sample, negative control sample, and the sera to be tested were diluted 1:64 in PBS-Tween solution, after which 100 µl was pipetted into triplicate wells of a micro-ELISA plate; the plate was incubated for 30 min at 37°C. Each well was then washed three times with 200 µl of PBS-Tween solution. One hundred microliters of a 1:1000 dilution of peroxidase-conjugated goat anti-cat immunoglobulin

G (IgG) heavy chain specific (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) in PBS-Tween solution was pipetted into appropriate wells. After a 30-min incubation period at 37°C, the plates were washed as described and 100 µl of substrate (SureBlue TMB 1-Component Microwell Substrate MB, Kirkegaard and Perry Laboratories, USA) was added to each well. The enzyme reaction was stopped after 10 min at approximately 20°C by pipetting 100 µl of 0.18 M H<sub>2</sub>SO<sub>4</sub> into each well. The optical density of each well (compared with a substrate control blank) was read at 450 nm with an automated micro-ELISA reader.

The mean absorbance value for the positive control sample, the negative control sample, and each test sample was calculated. The mean absorbance values were converted to %ELISA units by use of the following formula: (test sample mean absorbance minus the negative control sample mean absorbance)/(positive control sample mean absorbance minus the negative control sample mean absorbance) multiplied by 100. An individual cat was considered positive for *B henselae* antibodies if the %ELISA value was greater than the mean %ELISA value plus three standard deviations (SD) of the pre-inoculation samples for a group of 26 specific pathogen-free cats (10 kittens at 8 weeks of age and 16 cats at 3 years of age).

### *Bartonella* species ELISA validation

To determine the interassay variation of the optimized *B henselae* ELISA, the mean of the positive control wells was divided by the mean of the negative control wells for 11 ELISAs performed over 9 days and the coefficient of variation of these ratios calculated. In several previous experiments, we had documented *B clarridgeiae* DNA but not *B henselae* DNA in blood of eight cats based on appropriate band size in a PCR assay. Serum from these eight cats was assayed in the optimized *B henselae* ELISA to determine whether the assay was *B henselae* specific.

### *Bartonella henselae* Western blot (WB) immunoassay

To optimize the Western blot immunoassay, pre-inoculation and post-inoculation sera (16 weeks) from four *B henselae* inoculated kittens were assayed while assessing multiple *B henselae* lysate concentrations, buffers, blocking solutions, and secondary antibody concentrations.

In the optimized WB, the *B henselae* lysate was separated under reducing conditions on a gel in a discontinuous buffer system according to manufacturer instructions (4–12% Bis Tris Nupage; Invitrogen, Carlsbad, CA, USA). A total protein concentration of 20 µg of the *B henselae* lysate was mixed with the appropriate amount of sample buffer (NuPage 4× LDS Sample Buffer; Invitrogen) and reducing agent (NuPage 10× Reducing Agent; Invitrogen), denatured

and loaded on the gel. A molecular mass standard (MultiMark; Invitrogen) was loaded and the gel electrophoresed at 200 V constant for 35 min. The proteins were transferred to a polyvinylidene difluorure membrane (XCell II Blot Module PVDF; Invitrogen) at 30 V constant for 90 min in a transfer buffer (NuPage 20× Transfer Buffer; Invitrogen) and 20% methanol.

Following protein transfer, the membranes were blocked overnight on a rocker at room temperature with 10% (w/v) non-fat dry milk (50 mM Tris (pH 7.4), 150 mM NaCl, 1.0 mM ethylenediaminetetraacetic acid (EDTA); milk-tris (hydroxymethyl) aminomethane, sodium chloride, ethylenediaminetetraacetic solution (TNE) and then washed with 0.05% Tween 20 (v/v) TNE (TNE-TW) for 20 min. The membranes were incubated for 1 h at room temperature in serum diluted 1:10 in milk-TNE (Miniblotter 16; Immunetics, Cambridge, MA, USA). After another 20 min TNE-TW wash, peroxidase-conjugated goat anti-cat IgG, heavy chain specific (Kirkegaard and Perry Laboratories) was diluted to an optimal dilution of 1:1000 in milk-TNE, and the membranes were incubated for an additional hour at room temperature. The wash was repeated as before, and then the membranes were incubated with 4-chloro-1-naphthol/3,3'-diamino benzidine tetrahydrochloride in peroxide substrate buffer (CN/DAB, Pierce Laboratories, Rockford, IL, USA) until a color reaction was visible. The reaction was terminated by rinsing the membranes in ddH<sub>2</sub>O.

### Immunodominant antigen determination

To determine the immunodominant *B henselae* antigen molecular masses recognized by cat sera when used in this WB, samples from three specific pathogen-free cats that had been experimentally infected with *B henselae* (pre-inoculation and weeks 2, 12, and 20 post-inoculation) were evaluated in the optimized assay as described. Molecular mass standards, a positive control sample, and a negative control sample were included on each WB. To determine the apparent molecular masses of bands that developed on each WB, the membrane was first scanned as a digital image (Gel Doc 1000 System with Quantity One software; Bio-Rad Laboratories, Hercules, CA, USA). The image was then analyzed using the system software. Each WB was individually analyzed by one investigator (JH) and any bands that were missed or falsely added by the software were added or removed manually. Apparent molecular masses in the positive control samples and suspect samples were determined by comparing to molecular weight standards performed with each WB.

### Clinical samples

Announcements for the study were made by one of the authors (MRL) at multiple national, state, and regional continuing education meetings in the United States and via email and phone consultations.

Interested veterinarians were provided a standard operating procedure, submission forms, and history sheets. The test group was cats with an elevated body temperature >102.5°F (39.2°C) for which a cause could not be determined on physical examination and for which the referring veterinarian felt the increased body temperature was not due to stress or environmental factors. The control group consisted of samples from cats examined in the same clinic on the same day that had a body temperature <102.5°F (39.2°C) and no detectable medical problems. Information requested from the veterinarians of all cats on submission included age, state of residence, history of flea exposure (yes, no, or unknown), and housing status (always indoors or access to outdoors). Blood was collected by the referring veterinarians and an aliquot placed in an EDTA tube; the remainder was allowed to clot and serum collected. The blood in EDTA and serum were either stored at 4°C (maximum of 3 days) or 20°C until shipped to Colorado State University by overnight express on a cold pack. On arrival, DNA was extracted from the blood sample<sup>25</sup> and the DNA, remaining blood, and serum were stored at -70°C until assayed.

### Assays

Each serum sample was tested for feline leukemia virus antigen (Snap FeLV/FIV, Idexx Laboratories, Portland, ME, USA), antibodies against feline immunodeficiency virus (Snap FeLV/FIV, Idexx Laboratories), and *Bartonella* species antibodies by ELISA. The *Bartonella* species antibody titer was estimated by comparing the %ELISA value of the sample to a standard curve generated from positive and negative control sera assayed on each plate. Each DNA extract was tested in a PCR assay capable of amplifying multiple *Bartonella* species, including *B henselae* and *B clarridgeiae*.<sup>25</sup> Only samples giving the appropriate band size were considered positive.<sup>26</sup> Sera from 20 cats with fever and 19 corresponding afebrile control cats were sequentially selected from the sample set for assessment by the WB immunoassay based on state of origin (high flea states to maximize the chance positive results would be detected), availability of adequate serum, and availability of PCR assay results. All samples assessed using the PCR assay were known to contain DNA based on results of spectrophotometry.

### Statistical evaluation

To determine the association between *Bartonella* species antibodies and fever or *Bartonella* species DNA and fever, the distribution of the paired sample results was evaluated by logistic regression to determine odds ratios and 95% confidence intervals (CI). Logistic regression was also used to determine associations between *Bartonella* species ELISA or PCR results, health status (fever or no fever), age, flea risk as determined

by state, reported flea exposure, and housing status. The state of origin was classified as low flea risk (Alaska, Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming) or high flea risk (all other states) based on a previous study.<sup>9</sup> Wilcoxon's rank sum test was used to determine whether *Bartonella* ELISA titer magnitude was associated with presence of fever. Significance was defined as  $P < 0.05$ .

Presence of DNA of any *Bartonella* species in blood was considered evidence of current infection and then positive and negative predictive values of *Bartonella* species ELISA and WB results for prediction of bacteremia were calculated. It is unknown whether the *Bartonella* species ELISA or WB is the gold standard and so sensitivity and specificity of the two assays could not be calculated. Thus, results of the ELISA and WB were compared by calculating percentage agreement using the following formula:

$$\begin{aligned} & (\text{ELISA}+, \text{WB}+) \\ & + (\text{ELISA}-, \text{WB}-) / (\text{ELISA}+, \text{WB}+) \\ & + (\text{ELISA}-, \text{WB}-) + (\text{ELISA}+, \text{WB}-) \\ & + (\text{ELISA}-, \text{WB}+) \times 100. \end{aligned}$$

## Results

### *Bartonella* ELISA validation studies

The coefficient of variation of the ratio of positive to negative control absorbance for 11 ELISAs performed on nine different days was 13.7%. Of the eight cats from which only *B clarridgeiae* DNA was amplified from blood, seven were positive in the *Bartonella* species ELISA described here.

### Immunodominant antigens recognized by experimentally inoculated cats

All three experimentally inoculated cats recognized multiple antigens over time (Fig 1). Up to 17 distinct antigens were detected at individual time points. Based on band density and numbers of samples recognizing the individual antigens, we subjectively defined antigens with apparent molecular masses of 8, 20, 39, 48, 57, 62, 69, 73, and 82 kDa to be immunodominant.

### Client-owned cats results

**Clinical information.** Serum was available from 93 paired cases (ie, a febrile cat and a non-febrile control cat from the same practice, sampled on the same day) for *Bartonella* species ELISA testing and blood in EDTA was available from 81 of these paired cases for testing by the *Bartonella* species PCR assay. The states of origin for the cases were Colorado (23 pairs),

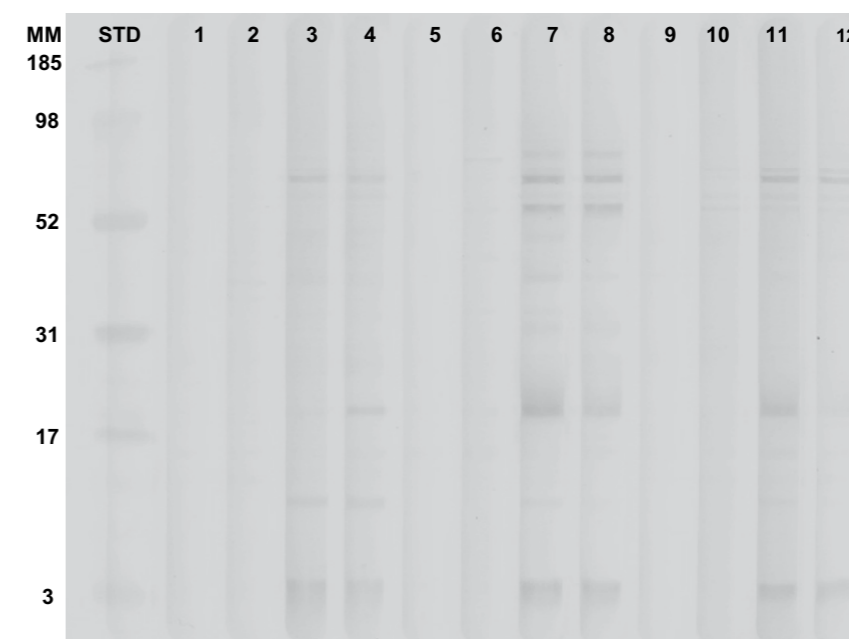
Georgia (seven pairs), Massachusetts (seven pairs), New Jersey (six pairs), Minnesota (five pairs), Illinois (four pairs), California (four pairs), Kansas (three pairs), New Hampshire (three pairs), Indiana (two pairs), Louisiana (two pairs), Michigan (two pairs), Mississippi (two pairs), Missouri (two pairs), South Carolina (two pairs), North Carolina (two pairs), Tennessee (two pairs), Texas (two pairs), Arkansas (one pair), Connecticut (one pair), Florida (one pair), Nebraska (one pair), Ohio (one pair), Pennsylvania (one pair), Utah (one pair), Virginia (one pair), Wisconsin (one pair), and the state was not reported (four pairs). Flea exposure history was available for 41 cats with fever (flea exposure = 20 cats; no flea exposure = 21 cats) and for 35 control cats (flea exposure = 16 cats; no flea exposure = 19 cats). Age was known for 89 cats with fever which ranged from 3 months to 14 years (median = 3 years; mean = 4.1 years). Age was known for 63 control cats which ranged from 4 months to 17 years (median = 2 years; mean = 3.7 years). Housing status was known for 67 cats with fever (25 cats housed indoors; 42 cats allowed outdoors) and 42 control cats (23 cats housed indoors; 19 cats allowed outdoors).

***Bartonella* species IgG prevalence rates by ELISA.** Based on ELISA, the overall *Bartonella* species IgG seroprevalence rates for cats with fever and control cats were 31.2% (29/93 cats) and 42.0% (39/93 cats), respectively. Control cats were more likely to have *Bartonella* IgG detected in serum by ELISA than cats with fever ( $P = 0.0443$ ; odds ratio = 2.09; 95% CI = 0.233, 0.981). Estimated positive antibody titers ranged from 1:64 to 1:512 and from 1:64 to 1:2048 in cats with and without fever, respectively. The magnitude of IgG titer was not associated with the presence or absence of fever ( $P > 0.05$ ). In the logistic regression model containing age, housing, flea risk by state, flea exposure, and housing status, presence of *Bartonella* species IgG was associated with age ( $P = 0.0303$ ) and flea exposure ( $P = 0.0467$ ).

***Bartonella* species DNA prevalence rates.** Of the 81 sample pairs available for PCR testing, 14 cats (17.3%) with fever and six (7.4%) of the afebrile control cats were positive. However, this numerical difference did not achieve statistical significance ( $P = 0.0571$ ).

The distribution of *Bartonella* IgG and *Bartonella* PCR results in the 81 paired samples evaluated in both assays is listed in Table 1. Of the 20 PCR assay positive cats, each was infected with *B henselae* and two cats were co-infected with *B clarridgeiae*. Of the two cats with *Bartonella* species antibodies and both *B henselae* and *B clarridgeiae* DNA in blood, both were healthy.

**FeLV and FIV prevalence rates.** FeLV antigen was detected in one febrile cat (*Bartonella* species ELISA positive; PCR negative). FIV antibodies were detected in one afebrile cat (*Bartonella* species ELISA positive; PCR negative) and three febrile cats (Two cats were *Bartonella* species ELISA negative; PCR negative and



**Fig 1.** *Bartonella henselae* antigen recognition patterns from experimentally inoculated cats over time. MM = molecular mass standards (STD). The 12 lanes are individual samples from three cats collected pre-inoculation and then experimentally inoculated with *B henselae* and assessed on weeks 2, 10, and 12 post-inoculation. Cat 1 = lanes 1–4; Cat 2 = lanes 5–8; and Cat 3 = lanes 9–12.

one cat was *Bartonella* species ELISA positive; PCR positive).

***Bartonella* species antigen recognition patterns in cats with and without fever.** Results of the serum WB were considered positive if two or more of the nine immunodominant antigens were recognized and were greater than a predetermined optical density. WB results were positive in 10 of 20 cats (50%) with fever and 6/19 afebrile cats (31.6%; Table 2) but this difference was not significant. Three or more antigens were recognized by 5/10 (50%) WB positive cats with fever and 5/6 (83.3%) WB positive control cats. Antigens with the apparent molecular masses of 48, 57, 62, 69, and 82 kDa were each recognized by at least four cats but there was no antigen recognition pattern that was detected only in cats with or without fever.

**Percentage agreement between *Bartonella* species ELISA and WB results.** The distribution of results for the *Bartonella* species ELISA and WB was: WB positive, ELISA positive (15 cats); WB positive, ELISA negative (one cat); WB negative, ELISA positive (22 cats); and WB negative, ELISA negative (one cat) giving a percentage agreement of 43.6%.

**Predictive values of *Bartonella* ELISA and WB results.** The distribution of results for the *Bartonella* species ELISA and PCR was: ELISA positive, PCR positive (13 cats); ELISA positive, PCR negative (55 cats); ELISA negative, PCR positive (seven cats); and ELISA negative, PCR negative (87 cats). The positive predictive value and negative predictive value of *Bartonella* species ELISA results for predicting presence of *Bartonella* species DNA in blood were 19.1% and 92.6%,

respectively. The distribution of results for the *Bartonella* species WB and PCR was: WB positive, PCR positive (three cats); WB positive, PCR negative (13 cats); WB negative, PCR positive (three cats); and WB negative, PCR negative (20 cats). The positive predictive value and negative predictive value of *Bartonella* species WB for predicting presence of *Bartonella* species DNA in blood were 18.8% and 86.9%, respectively.

## Discussion

The *Bartonella* species seroprevalence results in the cats described here are similar to those in other studies.<sup>27</sup> The association between *Bartonella* species antibodies and age in the cats of this study is likely to reflect increased chance of exposure over time. The association between *Bartonella* species antibodies and flea exposure is likely to reflect the fact that *Ctenocephalides felis* is considered the primary vector for *B henselae* and *B clarridgeiae*.

*Bartonella henselae* infection is associated with both acute and chronic fever in some infected people.<sup>28</sup> In addition, experimental intradermal or intravenous inoculation of cats with some strains of culture derived *B henselae* resulted in fever in some cats, generally in the acute phase of infection.<sup>14–19,29</sup> Based on these findings, it seems reasonable that *B henselae* infection of naturally exposed cats occasionally would be associated with fever. However, to our knowledge, fever was not detected in cats infected after exposure to *B henselae* infected *C felis* in a controlled study.<sup>12</sup>

**Table 1.** *Bartonella* species IgG ELISA and PCR assay results from cats with fever and afebrile cats collected in pairs ( $n=81$ ) from veterinary clinics in North America

	Fever (%)	Control (%)
ELISA positive, PCR positive	8 (9.9)	5 (6.2)
ELISA positive, PCR negative	21 (25.9)	34 (42.0)
ELISA negative, PCR positive	6 (7.4)	1 (1.2)
ELISA negative, PCR negative	46 (56.8)	41 (50.6)
Any ELISA positive	29 (35.8)	39 (48.1)
Any PCR positive	14 (17.3)	6 (7.4)

In the study described here, pair-matched afebrile cats were more likely to have *Bartonella* species antibodies as detected by ELISA than cats with fever. *Bartonella* species titer magnitude also was not associated with the presence of fever. In addition, we failed to detect a *Bartonella* species antigen recognition pattern by WB that correlated with the presence of fever. These results are similar to those in a study of cats in North Carolina where fever was not associated with the presence of *Bartonella* species antibodies as detected by immunofluorescent antibody assay (IFA).<sup>24</sup> The results of these studies do not exclude the possibility that *Bartonella* species induce fever in naturally exposed cats but may just reflect the difficulty in confirming disease associations when evaluating serological test results obtained from a diverse cat population, as was used in this study. Confirming disease causation is a particular problem with agents like *Bartonella* species that are very prevalent among healthy as well as sick cat populations and are often associated with subclinical infections.<sup>11</sup> The *B henselae* antigen based ELISA described here detected antibodies in serum of 7/8 cats with only *B clarridgeiae* DNA in blood suggesting the assay is not specific for *B henselae* antibodies or that these cats were

**Table 2.** Antigen recognition patterns by *Bartonella* species positive cats with fever and paired afebrile cats fever

Fever cats	Antigens	Afebrile cats	Antigens
S18	82, 69, 62, 57	C18	82, 62, 20
S45	69, 62	C29	48, 39
S54	62, 57	C54	57, 48, 20
S55	73, 57	C55	73, 48, 20
S59	57, 48	C97	82, 69, 62, 48, 39
S91	82, 69, 62, 48, 39	C113	82, 69, 62, 39
S96	82, 69, 62, 48, 39		
S97	82, 69, 62, 57, 39, 20		
S102	82, 69, 62, 57		
S113	62, 48		

previously exposed to both *Bartonella* species but only *B clarridgeiae* DNA was detected by PCR amplification at the time of sample collection. It is possible that the results may have been different if a different *B henselae* strain or genotype had been used as the antigen source in the ELISA or WB assay, however, this hypothesis seems unlikely as previous studies have shown the immunological responses of cats infected with different *B henselae* genotypes and strains to be similar.<sup>18,19</sup>

While the same *B henselae* antigens were used in both assays, ELISA and WB results varied considerably. Most discordant samples were ELISA positive, WB negative which is likely only to reflect a decreased sensitivity of the WB that relates to the criteria we used to define a positive test result in this study. These results emphasize that it can be difficult to compare results of *Bartonella* species antibody test results between laboratories, particularly as assay validation can vary substantially among laboratories.

More cats with fever were positive for *B henselae* or *B clarridgeiae* DNA in blood than control cats and the difference between groups approached statistical significance ( $P=0.0571$ ). The results suggest that *Bartonella* species infection of naturally exposed cats can be associated with fever. However, the results also document that the detection *Bartonella* species DNA in blood samples from an individual cat cannot be used as the sole criteria supporting the agent as the cause of the clinical syndrome.<sup>27</sup> Similar to other highly adapted infectious agents like *Toxoplasma gondii* and the haemoplasmas, *Bartonella* species infection may represent a co-factor in the development of fever in an individual patient. In this study, we chose to use a conventional PCR assay; it is possible that the use of more sensitive real-time PCR assays or a novel pre-enrichment culture/PCR approach may have led to different results.<sup>26,30</sup> The failure of both the ELISA and WB assays to accurately predict presence or absence of *Bartonella* species DNA in blood is similar to previous reports and emphasizes that antibody test results should not be used to infer active infection in patient populations.<sup>27</sup>

Whether individuals develop clinical disease from infection with a *Bartonella* species appears to be determined by complex interactions. These may involve strain variation in pathogenicity or individual host susceptibilities which are potentially related to immunocompetence or concurrent disease.<sup>3,31,32</sup> In this study, dual infection with *B henselae* and *B clarridgeiae* or a *Bartonella* species and FeLV or FIV was detected in some cats but there was no obvious association with the presence of fever. In dogs, co-infection with multiple vector-borne pathogens is likely to be associated with increased risk of illness in some individuals.<sup>33</sup> The failure to make an association between fever and the detection of antibodies or organism specific DNA sequences in the blood may merely reflect the small sample size and failure to have a complete diagnostic evaluation for all cats. In addition, because the

study design involved a large number of practices throughout the United States, it cannot be definitively proven that the cats in this study truly had fever or alternatively had hyperthermia because of stress which may have influenced the interpretation of our results. Lastly, while the cats used as controls were reportedly afebrile and healthy, it is possible that some had undetected *Bartonella* species associated illness which may have affected the results of the statistical comparisons. In conclusion, the results of this study indicate that in cats, *Bartonella* species serum antibody tests cannot predict whether fever is due to *Bartonella* species infection and should not be used to determine the *Bartonella* species infection status.

### Acknowledgment

Funding for this study was provided by donations to the Center for Companion Animal Studies at Colorado State University ([www.csuvels.colostate.edu/companion](http://www.csuvels.colostate.edu/companion)).

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