Journal of Feline Medicine and Surgery (2009) **11**, 266–270
doi:10.1016/j.jfms.2008.06.007

jfms

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

Danielle B Bayliss DVM[1]*, **Arianne K Morris** BS[1], **Mauricio C Horta** DVM, PhD[2], **Marcelo B Labruna** DVM, PhD[2], **Steven V Radecki** PhD, Independent Statistical Consultant[3], **Jennifer R Hawley** BS[1], **Melissa M Brewer** BS[1], **Michael R Lappin** DVM, PhD, DACVIM[1]

[1]*Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado, United States*
[2]*Faculdade de Medicina Veterinária e Zootecnia; Universidade de São Paulo, São Paulo, SP, Brazil*
[3]*Fort Collins, CO, United States*

Date accepted: 25 June 2008

Rickettsia species antibodies have been detected in some cats but it is unknown whether infected cats develop clinical signs. The prevalence of *Rickettsia* species deoxyribonucleic acid (DNA) in blood from clinically ill cats has not been determined. The objective of this study was to determine if cats with fever (body temperature ≥102.5°F [39.2°C]) were more likely to have evidence of rickettsial infection than healthy, age-matched, control cats with a body temperature < 102.5°F. *Rickettsia* species polymerase chain reaction (PCR) assays were performed to detect rickettsial DNA extracted from blood (71 paired samples), indirect immunofluorescence assays (IFA) were performed to detect serum antibodies against *Rickettsia felis* (90 paired samples) and *Rickettsia rickettsii* (91 paired samples), and the results between pairs were compared. All samples were negative for *Rickettsia* species DNA. More cats with fever were seropositive for *R felis* or *R rickettsii* than control cats, but results were not statistically significant. Results of this pilot study failed to show an association between *Rickettsia* species DNA or *Rickettsia* species antibodies and fever.
© 2008 ESFM and AAFP. Published by Elsevier Ltd. All rights reserved.

Fever is a common finding in sick cats. Infection, immune-mediated diseases, and neoplasia are common causes of fever, with infectious disease thought to be the most common cause in cats.[1] A large number of viral, bacterial, rickettsial, and protozoal organisms have been associated with fever in cats. For some agents, the diagnosis may be easy to achieve because there are concurrent clinical syndromes that direct the diagnostic workup or the organism can be identified with widely available tests. Examples include feline leukemia virus, feline immunodeficiency virus, panleukopenia virus, and respiratory viruses.

Recently, fever in people has been attributed to infection with 'stealth' organisms.[2] These agents evade the immune system, cause subtle clinical signs, and often are not easily detected by traditional diagnostic methods. Examples in people that can also be associated with disease in cats include *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Bartonella* species.[3–5] Advances in serological and molecular diagnostic tests have aided in the diagnosis of these organisms, however, the tests are generally specific and so a particular agent must be suspected to trigger the veterinarian to choose the diagnostic test of choice. We believe there are previously unrecognized organisms of this type that infect cats and induce fever. *Rickettsia* species are possible causes but have not be studied extensively in cats.

Rickettsia felis deoxyribonucleic acid (DNA) has been amplified from clinically ill humans in Mexico, France, Brazil, Germany, Texas, California, and other places.[6–9] Fever was one manifestation of illness in these people. The cat flea, *Ctenocephalides felis*, has been identified as a host and biological vector of *R felis*.[7,10,11] Several infected humans have a history of flea exposure, suggesting that *C felis* may be capable of transmitting the organism to humans.[10] Cats are commonly infested with *C felis*, and *R felis* DNA was amplified from 62.4% of fleas collected from cats in the United States.[12] Antibodies against *R felis*, *R. rickettsii*, and *R. typhi* have been detected in cats in the United States, indicating that cats are exposed to rickettsial organisms.[8,13,14] Findings of these previous studies led to our hypothesis that *Rickettsia* species may be associated with fever in cats.

*Corresponding author. E-mail: danielle@colostate.edu

1098-612X/08/040266+05 $34.00/0
© 2008 ESFM and AAFP. Published by Elsevier Ltd. All rights reserved.

In a previous study, *R felis* DNA was not amplified from the blood of cats known to be infested with *R felis* infected fleas.[12] However, those cats were generally healthy other than their flea infestations. To the authors' knowledge, the prevalence of *Rickettsia* species DNA or *R felis* antibodies has not been reported in a population of clinically ill cats. The objective of this pilot study was to determine the prevalence of *Rickettsia* species DNA in blood, as well as the prevalence of *R felis* and *R rickettsii* antibodies in serum, of cats with and without fever.

Materials and methods

Cats

Samples selected for this study were initially collected for a study of *Bartonella* species IgG (immunoglobulin G) and DNA in the blood of cats with and without fever.[15] Samples were requested from participating veterinarians by announcements made at continuing education meetings throughout the United States and via email and phone consultations with one of the authors (MRL). The test group included cats with an elevated body temperature of $\geq$102.5°F (39.2°C), for which a cause could not be determined on physical examination, and for which the veterinarian felt was related to fever, rather than stress or environmental factors. The control group consisted of cats with a body temperature $<$ 102.5°F (39.2°C) and no detectable medical problems, which were examined in the same clinic on the same day. Information requested for all cats included state of origin and age. States were classified as low flea-risk (Alaska, Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, Wyoming) or high flea-risk (all other states) based on a previous study.[16] Upon receipt at Colorado State University, DNA was extracted from blood stored in ethylenediaminetetraacetic acid (EDTA) and assayed for *Bartonella* species DNA by polymerase chain reaction (PCR) and sera were assayed for *Bartonella* species IgG by enzyme-linked immunosorbent assay (ELISA). The DNA extracts and sera were saved at −20°C or −70°C until used in this study.[15]

Immunofluorescence assays (IFA) slides

Slides coated with *R felis* infected cells, for use with indirect IFA, were obtained from The University of São Paulo, Department of Veterinary Medicine and Animal Health. *R felis* was cultivated in C6/36 cells as previously described, except that infected cells were incubated at 28°C. Cells were harvested when nearly 100% of the cells were infected.[17] Infected cells were centrifuged at $4000 \times g$ for 10 min. The pellet was washed in 0.1 M phosphate buffered saline (PBS, pH 7.4), centrifuged again, and re-suspended in PBS containing 1% bovine calf serum and 0.1% sodium azide. Ten microliters of *R felis* infected cell suspension were applied on to each well of 12-well antigen slides (IFA slides, Precision Lab Products, LLC Middleton, WI). Cell suspensions on the slides were air-dried, then fixed in acetone for 10 min. Slides were stored at −80°C until shipped overnight with cold packs to Colorado State University. Slides coated with *R rickettsii*-infected cells were obtained from a commercial supplier (*Rickettsia rickettsii* IFA slides, Prototek International, St Paul, MN).

IFA assays

The negative control used in all assays was sera collected and pooled from laboratory-reared, specific pathogen-free cats housed without potential for exposure to arthropods For the *R felis* assay, known positive serum samples from an infected cat and an infected dog were used as positive controls. The control cat serum was determined to be positive based on a reaction with the *R felis* antigen at a titer of 1:128, as well as reactions to *R rickettsii* and *Rickettsia parkeri* at titers $>$ 1:128. Canine serum that was positive for *R rickettsii* antibodies was used as a positive control in the *R rickettsii* assay. IFA slides control sera were stored at −20°C until used.

Slides and sera were removed from the freezer and allowed to come to room temperature. Suspect samples and the negative control sample were diluted 1:20 with 0.075% Tween in PBS-Tw + 1% bovine serum albumin (BSA) solution. The feline *R felis* positive control sample and the canine *R rickettsii* positive control sample were diluted at 1:10 in 0.075% PBS-Tw + 1% BSA solution. Aliquots (25 μl) of the appropriate positive and negative controls and suspect sera were used to coat individual IFA wells with care taken to control cross-contamination. Slides were incubated in a pre-warmed humidity chamber at 37°C for 30 min and then washed briefly using PBS-Tw. Slides were washed on a rocker in PBS for 5 min followed by an additional 5 min in deionized water (dH_2O).

Fluorescein-conjugated goat anti-cat heavy chain IgG (Kirkegard and Perry Laboratories, Gaithersburg, MD) and fluorescein-conjugated goat anti-dog heavy chain IgG (Kirkegard and Perry Laboratories) (*R rickettsii* positive control wells only) were diluted at 1:40 in 0.25% Evan's blue and 25 μl was pipetted on to appropriate wells of the IFA slides for the respective *Rickettsia* species. Slides were incubated at 37°C for 30 min in the humidity chamber and then washed again as described previously. After the final wash, two drops of mounting fluid (nine parts glycerol to one part PBS) were applied, and a cover slip was placed on each slide.

Slides were read by two examiners independently on a fluorescent microscope. Discordant samples were assayed a second time, and if still discordant, were read by a third examiner. Discordant samples were considered positive if, after the second assay, at least two of the three examiners reported it as positive. All samples that were positive at 1:20 were evaluated serially in two-fold

dilutions to the end point. The reported titer was the last dilution to give positive fluorescence.

Rickettsia species PCR assay

The DNA extracts were thawed at room temperature and *Rickettsia* species PCR assays were performed using previously described protocols.[12,18] Oligonucleotide primers for the citrate synthase gene *(gltA)* and outer membrane protein B *(ompB)* gene were utilized. The sensitivities of the *gltA* and *ompB* PCR assays were 1.10 fg and 0.110 fg, respectively. On each run for both PCR assays, a negative control, consisting of PCR reagents and PCR water as template (no DNA template added), and positive controls consisting of purified *R felis* DNA and *R rickettsii* DNA, were included.

Statistical analyses

To determine the association between *R felis* or *R rickettsii* antibodies and fever, the distribution of the paired sample results were evaluated by logistic regression to determine odds ratios and 95% confidence intervals. Significance was defined as $P < 0.05$.

Results

Cases were included if sufficient sample volume existed for performance of individual assays (*Rickettsia* species PCR assays, *R felis* IFA, and *R rickettsii* IFA) on the sample from both the febrile cat and the paired control cat. There were 71, 90, and 91 paired samples available for testing by *Rickettsia* species PCR assays, *R felis* IFA, and *R rickettsii* IFA, respectively. Sample pairs came from the following states; Alabama (one), Arkansas (one), California (four), Colorado (25), Connecticut (one), Florida (one), Georgia (seven), Illinois (three), Indiana (two), Kansas (three), Louisiana (two), Massachusetts (five), Michigan (two), Minnesota (four), Mississippi (two), Missouri (two), Nebraska (one), New Jersey (six), New Hampshire (two), North Carolina (three), Ohio (one), Pennsylvania (one), South Carolina (two), Tennessee (two), Texas (one), Utah (one), Virginia (one), and Wisconsin (one). The state of origin was not recorded for three sample pairs. Of the represented states, only Colorado and Utah were considered low flea-risk, so at least 65 of 91 sample pairs (71.4%) were from high flea-risk states.

It was previously determined that many of the cats assessed in this study had *Bartonella* species IgG in serum or *Bartonella* species DNA amplified from blood, suggesting that many of the cats had been exposed to fleas.[15] However, *Rickettsii* species DNA was not amplified from any of the blood samples by use of either PCR assay.

Antibodies against *R felis* were detected in serum from five cats with fever (5.6%: titers; 1:20, 1:20, 1:40, 1:40, 1:80) and two control cats (2.2%: titers; 1:20, 1:20), but were not concurrently detected in the serum of any paired sample. A greater number of cats with fever were *R felis* seropositive compared to control cats, but results were not statistically significant (odds ratio = 2.5; 95% CI = 0.485, 12.886; $P = 0.2734$). The *R felis* seropositive cats were from Georgia (two), North Carolina (one), California (one), Ohio (one), Nebraska (one), and New Jersey (one). These states are considered to have a high flea-risk. Of the *R felis* seropositive cats, three cats with fever and both control cats were positive for *Bartonella* species antibodies, and one cat with fever and one control cat were PCR assay positive for *Bartonella henselae* DNA.

Antibodies against *R rickettsii* were detected in serum from six cats with fever (6.6%: titers; 1:20, 1:20, 1:40, 1:40, 1:40, 1:80) and two control cats (2.2%: titers; 1:20, 1:20), but were not concurrently detected in the serum of any paired sample. A greater number of cats with fever were *R rickettsii* seropositive compared to control cats, but results were not statistically significant (odds ratio = 3.0, 95% CI = 0.606, 14.86, $P = 0.1785$). The *R rickettsii* seropositive cats were from Georgia (two), Colorado (two), Arkansas (one), California (one), Connecticut (one), and New Jersey (one). Of the *R rickettsii* seropositive cats, one cat with fever and one control cat were positive for *Bartonella* species antibodies. Of the seven *R rickettsii* seropositive cats with samples available for *Bartonella* PCR assay, one cat with fever was PCR assay positive for *B henselae* DNA.

One control cat (*R felis* titer = 1:20; *R rickettsii* titer = 1:20) and one cat with fever (*R felis* titer = 1:40; *R rickettsii* titer = 1:40) were antibody positive for both organisms.

Discussion

The cases included in this study represented 28 states in the United States, 26 of which are considered to have a high flea-risk. *Bartonella* species IgG antibodies and *Bartonella* species DNA were detected in > 30% of the samples, indicating that many of cats included in this study were exposed to fleas. However, despite a previously reported prevalence rate of 62.4% for *R felis* infection in *C felis* collected from cats in the United States, the results of this study did not show an association between fever in cats and *Rickettsia* species DNA in blood or serologic evidence of exposure to *R felis*.[12] These findings indicate that *R felis* is not a common cause of fever in cats or merely reflects the sample population tested.

The prevalence of *R felis* antibodies in cats described here (seven of 180 cats tested; 3.9%) is lower than the seroprevalence reported in two of three previous studies performed in the United States. In one study, the authors refer to unpublished data that showed a *R felis* seroprevalence rate of 8% in febrile cats in the northeastern United States.[8] In another study, the *R felis* seroprevalence rate amongst pet, shelter, and feral cats in California and Wisconsin was 11.1%.[13] The third study evaluated for associations between *R typhi* antibodies and clinical disease in cats in North Carolina; the

authors detected *Rickettsia* species antibodies in nine of 436 cats (2.1%) that were likely a species other than *R typhi* and so suspected to be *R felis*.[14]

It is possible that studies with higher prevalence rates are related to higher flea burdens in the study populations, as the northeastern United States, California, and Wisconsin are all considered to have a high flea-risk.[13] In addition, in the study with 11.1% *R felis* seroprevalence rate, 150 of 170 cats (88.2%) were shelter cats or feral cats. These cats may be more likely to have flea infestations, and may be more susceptible to infection due to concurrent diseases, stress, or sub-optimal housing when compared to client-owned cats. In the current study, all of the samples were from client-owned cats and 26 pairs were from Colorado or Utah which may have contributed to the lower number of *R felis* seropositive cats reported in this study.

While a statistically significant association between fever in cats and antibodies against *R felis* was not found in this study, numerical differences were noted between groups. When discordant samples for *R felis* antibodies within pairs were examined, there were more discordant pairs which included a seropositive cat with fever and a seronegative cat without fever than pairs which included a seronegative cat with fever and a seropositive cat without fever. In addition, the highest titers reported for *R felis* antibodies in this study (1:80 for both) were detected in the serum of febrile cats. These results suggest that *R felis* may be associated with fever in some cats and that a larger study should be performed.

Similar to the *R felis* results, there was no statistically significant association between fever in cats and presence of *R rickettsii* antibodies in serum, and the prevalence of *R rickettsii* antibodies was low (eight of 182 cats; 4.4%). These findings indicate that *R rickettsii* is not a common cause of fever in cats or may relate to the population of cats tested. None of the cats in this study were known to be exposed to known *R rickettsii* vectors and the majority of states of origin are not considered endemic for *R rickettsii* infection. An *R rickettsii* seroprevalence rate of 17.2% was reported in the study of pet, shelter, and feral cats in California and Wisconsin.[13] As discussed for *R felis*, inclusion of a large number of shelter and feral cats may have contributed to the higher *R rickettsii* seroprevalence rate reported in that study. A future study should be performed comparing *R rickettsii* antibody and *Rickettsia* species PCR assay results between cats with and without clinical illness in a state that is endemic for *R rickettsii*, such as North Carolina or Georgia.

Failure to amplify *Rickettsia* species DNA from the blood of these cats may simply reflect lack of exposure as discussed for *R felis* and *R rickettsii* serology results. However, in a previous study, when *R felis* infected fleas were allowed to feed on cats, 5/16 cats were transiently PCR positive for *R felis* DNA in blood.[19] Thus, it is possible that the negative PCR test results in the cats of the study described here merely related to the timing of sample collection; the microbial DNA may have been cleared from the blood by a rapid and effective immune response by the time the cats were tested. The finding that some cats were *R felis* and *R rickettsii* seropositive but PCR negative supports this hypothesis. In future studies, it would be optimal to collect samples from clinically ill cats more than one time. Lastly, it is also possible that the organisms were present in some cats but sequestered in other tissues like the skin or spleen.

In summary, we failed to find an association between *R felis* and *R rickettsii* and fever in cats of this study but sample sizes and distribution of cases may have influenced the results. Larger study populations of sick and healthy cats are needed to further assess for associations between *Rickettsia* species and clinical diseases in cats.

Acknowledgement

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

References

1. Miller JB. Hyperthermia and fever of unknown origin. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine. 6th edn. St Louis: Elsevier Saunders, 2005: 9–13.
2. Merrel DS, Falkow S. Frontal and stealth attack strategies in microbial pathogenesis. *Nature* 2004; **430**: 250–6.
3. Bakken JS, Dumler JS. Clinical diagnosis and treatment of human granulocytotropic anaplasmosis. *Ann N Y Acad Sci* 2006; **1078**: 236–47.
4. Breitschwerdt EB, Abrams-Ogg AC, Lappin MR, et al. Molecular evidence supporting *Ehrlichia canis*-like infection in cats. *J Vet Intern Med* 2002; **16**: 642–9.
5. Kordick DL, Breitschwerdt EB. *Bartonella* infections in domestic cats. In: Bonagura J, ed. Kirk's Current Veterinary Therapy XIII. Philadelphia: WB Saunders, 2000: 302–7.
6. Zavala-Velazquez JE, Ruiz-Sosa JA, Sanchez-Elias RA, Becerra-Carmona G, Walker DH. *Rickettsia felis* Rickettsiosis in Yucatan. *Lancet* 2000; **356**: 1079–80.
7. Raoult D, La Scola B, Enea M, et al. A flea associated Rickettsia pathogenic for humans. *Emerg Infect Dis* 2001; **7**: 73–81.
8. Higgins JA, Radulovic S, Schriefer ME, Azad AF. *Rickettsia felis*: a new species of pathogenic Rickettsia isolated from cat fleas. *J Clin Microbiol* 1996; **34**: 671–4.
9. Brouqui P, Raoult D. Arthropod-borne diseases in homeless. *Ann N Y Acad Sci* 2006; **1078**: 223–35.
10. Oliviera RP, Galvao MA, Mafra CL, et al. *Rickettsia felis* in *Ctenocephalides* spp. fleas, Brazil. *Emerg Infect Dis* 2002; **8**: 317–9.
11. Zavala-Velazquez JE, Zavala-Castro JE, Vado-Solis I, et al. Identification of *Ctenocephalides felis* fleas as a host of

Rickettsia felis, the agent of a spotted fever rickettsiosis in Yucatan, Mexico. *Vector Borne Zoonotic Dis* 2002; **2**: 69−75.
12. Hawley JR, Shaw S, Lappin M. Prevalence of *Rickettsia felis* in the blood of cats and their fleas in the United States. *J Feline Med Surg* 2007; **9**: 258−62.
13. Case JB, Chomel B, Nicholson W, Foley JE. Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. *J Feline Med Surg* 2006; **8**: 111−7.
14. Breitschwerdt EB, Levine JF, Radulovik S, Hanby SB, Kordick DL, La Perle KMD. *Bartonella henselae* and *Rickettsia* seroreactivity in a sick cat population from North Carolina. *Int J Appl Res in Vet Med* 2005; **3**: 287−302.
15. Lappin MR, Breitschwerdt EB, Brewer M, Hawley J, Hegarty B, Radecki S. Prevalence of *Bartonella* species antibodies and *Bartonella* species DNA in the blood of cats with and without fever. *J Feline Med Surg* 2009; **11**: 141−8.
16. Jameson P, Greene C, Regnery R, et al. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. *J Infect Dis* 1995; **172**: 1145−9.
17. Horta MC, Labruna MB, Durigon EL, Schumaker TT. Isolation of *Rickettsia felis* in the mosquito cell line C6/36. *Appl Environ Microbiol* 2006; **72**: 1705−7.
18. Shaw SE, Kenny MJ, Tasker S, Birtles RJ. Pathogen carriage by the cat flea *Ctenocephalides felis (Bouché)* in the United Kingdom. *Vet Microbiol* 2004; **102**: 183−8.
19. Wedincamp J, Foil LD. Infection and seroconversion of cats exposed to cat fleas (*Ctenocephalides felis* Bouche) infected with *Rickettsia felis*. *J Vect Ecol* 2000; **25**: 123−6.

Available online at www.sciencedirect.com