Evaluation of the role of *Babesia* species and *Cytauxzoon felis* in feline anemia cases in Colorado, USA

Pierce K Chan, Jennifer R Hawley and Michael R Lappin

**Abstract**

**Objectives** The aim of this study was to evaluate the blood of cats in Colorado, USA, with suspected infectious causes of anemia for the presence of *Babesia* species and *Cytauxzoon felis* DNA. Results of PCR testing for other common vector-borne diseases potentially associated with anemia are also reported.

**Methods** Samples from 101 cats were tested using a PCR assay that coamplified the DNA of *C felis* and *Babesia* species mitochondrial DNA. PCR testing for DNA of hemoplasmas, *Bartonella* species, *Ehrlichia* species, *Anaplasma* species, *Neorickettsia risticii* and *Wolbachia* genera was also performed if not carried out previously.

**Results** Twenty-two cats (21.8%) were positive for DNA of an infectious agent. DNA from hemoplasma species were amplified from 14 cats (13.9%). *Bartonella* species DNA was amplified from four cats (4%) and *Ehrlichia canis*, *Anaplasma platys*, *Anaplasma phagocytophilum* and *Wolbachia* genera DNA were amplified from one cat each. *Babesia* species and *C felis* mitochondrial DNA were not amplified from any sample.

**Conclusions and relevance** Based on the results of this study, it does not appear that *Babesia* species or *C felis* are clinically relevant in anemic cats in Colorado, USA. For *C felis*, this suggests that the vector *Amblyomma americanum* is still uncommon in this geographic area.

**Keywords:** Anaplasma; Babesia; Bartonella; Cytauxzoon; Ehrlichia; hemoplasma

**Accepted:** 25 May 2021

**Introduction**

Cats with anemia in the USA are commonly evaluated for the DNA of vector-borne infectious causes by PCR assays, as primary immune-mediated hemolytic anemia in cats has, historically, been thought to be rare. There are few extensive studies of vector-borne agents in anemic cats. Most have been focused on retroviral infections and hemoplasmas, with some of the older studies not evaluating all agents. Owing to the suspected low prevalence in the USA, some infectious causes of hemolytic anemia in cats, such as *Babesia* species, were not typically included in PCR panels and are not routinely recommended in feline blood donor screening. Others, like *C felis*, have a historical range that only includes the central and southeast USA, and therefore...
may be missed in other regions if the range of *A. americanum* has expanded. Over time, molecular-based PCR panels have become more broadly available and sensitive, allowing for wider screening. For example, *Babesia vogeli* DNA was recently amplified from the blood of 1.4–39.5% of cats in Thailand.\(^ {10,11}\) The vector for this agent is thought to be *R. sanguineus*, a worldwide tick vector common throughout the USA that can also transmit *E. canis, A platys, Rickettsia rickettsii* species.

*Babesia* is common throughout the USA that can also transmit *E. canis, A platys, Rickettsia rickettsii* and *Babesia* species. In addition to the increased availability of molecular-based testing, the range of a number of infectious agents is changing over time.\(^ {12}\)

Historically, *C felis* has not been associated with anemia in cats in Colorado. In fact, researchers studying anemia in cats from the region previously did not evaluate for this agent.\(^ {1,2,8,13}\) Recently, a national tick surveillance program in the USA reported detection of *A. americanum* in Colorado.\(^ {14}\) Although the travel history of the host was not always documented, this could suggest expansion of the range of this tick. Furthermore, recent studies have noted or predicted that the range of *A. americanum* continues to expand in the USA.\(^ {15,16}\) Regardless, minimal data exist on the evaluation of cats in Colorado for DNA of *C. felis* or *Ehrlichia ewingii*, agents vectored by *A. americanum*. A study evaluating infectious agents with a known vector could be used as indirect proof that the vector is in the state.

The objective of this study was to identify, using PCR, vector-borne agents in the blood of cats with suspected infectious anemia in Colorado, USA, with a focus on pathogens not previously associated with disease in this state, such as *Babesia* species and *C felis*. We hypothesized that *Babesia* species and *C felis* would be amplified from the blood of anemic cats in Colorado.

### Materials and methods

#### Case selection

Records from the Specialized Infectious Diseases Laboratory division of the Veterinary Diagnostic Laboratory at Colorado State University were searched from June 2004 to December 2018, to identify cases for which an infectious cause of anemia was suspected. All cases were submitted by the referring veterinarian for PCR testing for the DNA of one or more vector-borne agents. Anemia was defined as a statement of ‘anemia’ on submission forms, or a packed cell volume or hematocrit of <30%. The submission record had to list a Colorado address for the owner or the referring clinic to be included in the study, and case history was limited to the original referring veterinarian submission form. Lastly, there had to be adequate DNA for performance of the PCR assays. Cases were excluded when it could be determined from the submission record that there was a known cause of non-infectious anemia, along with cases from research laboratories in which cats were inoculated with a pathogen.

### PCR assays

Remnant DNA samples were stored at −80°C until assayed in this study. A previously published quantitative PCR assay that coamplifies the mitochondrial DNA of *C. felis* and *Babesia* species was used to evaluate all samples.\(^ {17}\) Results of other PCR assays that were performed at the time of the initial submission were noted from the laboratory records. For samples with incomplete results, the stored DNA was also assayed for *Bartonella* species and hemoplasmas (*M. haemofelis*, ‘*Candidatus Mycoplasma turicensis*’ and ‘*Candidatus M. haemominutum*’).\(^ {18,19}\) Samples were also retested if the final results were unclear from the submission sheet. Lastly, a conventional PCR assay that amplifies the DNA of *Ehrlichia* species, *Anaplasma* species, *Neorickettsia* species and *Wolbachia* species was performed on all samples, and the genus and species in positive samples was confirmed by sequencing.\(^ {20}\) DNA was extracted in our research laboratories in the Center for Companion Animal Studies and genetic sequencing completed by a commercial service (Genewiz). All sequenced products were analyzed for homology by comparison to sequence data available in NCBI GenBank using the Nucleotide BLAST database.\(^ {21}\)

### Results

Adequate DNA for testing was available from 101 anemic cats that were classified as living in Colorado at the time of sample submission. The cats ranged in age from 6 weeks to 19 years (median 6 years). The sample set comprised 44 spayed females, three intact females, one intact male and 51 castrated males, as well as one male and one female with unknown sterilization status. The majority of samples (n = 93 [92.1%]) were initially submitted for evaluation of hemoplasma species DNA.

Overall, 22 cats (21.8%) had DNA of an infectious agent amplified from blood (Table 1). DNA from hemoplasma species were most commonly amplified (13.9%), with two cats having *M. haemofelis*/*Candidatus M. turicensis*’ DNA. The exact species detected in these two cats was not differentiated at the time, while the other 12 cats had ‘*Candidatus M. haemominutum*’ DNA amplified. One cat each had *E. canis, A platys, A phagocytophilum* or *Wolbachia* genera DNA amplified. None of these positive samples was originally tested for these agents. *Bartonella* species DNA was amplified from four cats (4%), *Bartonella henselae* from three cats and *Bartonella claridgeiae* from one cat. *Babesia* species and *C felis* mitochondrial DNA were not amplified from any sample. Coinfections were not detected for any of the cats.

### Discussion

*C felis* was not detected in 101 anemic cats residing in Colorado – a finding that did not support our primary hypothesis. These data suggest that *A. americanum* may not be established in Colorado, a perception that is further supported by the failure to amplify another
common *A americanum* transmitted pathogen, *E ewingii*, from our samples. Although *A americanum* has been reported to be present in the state, the travel history of those hosts was not always known, and out-of-state transmission cannot be ruled out. In addition, one study showed no amplification of *C felis* from bobcats (the reservoir host of *C felis*) in Colorado from 1999 to 2010, providing further evidence of a low prevalence of *A americanum* in Colorado. However, cytauxzoonosis in cats is generally characterized by a severe and acute fever, with hemolytic anemia seen frequently in later stages. Veterinarians in endemic areas are well versed in evaluating for *C felis*, but it is still prudent to consider this differential in cats living near the known range. Owing to the quick clinical progression of cytauxzoonosis, diagnosis and treatment of these cats needs to be made with haste. Unlike many of the other vector-borne agents, *C felis* requires antiparasitic drugs for best efficacy, such as a combination of atovaquone and azithromycin.

*B vogeli* DNA was recently amplified from cats in Thailand, with two studies revealing that between 1.4% and 39.5% of the stray cat population had molecular evidence of the agent. In contrast, light microscopy observed merozoites in only 0.13% of samples, which suggests that PCR assays should be used to evaluate for this agent, if available. In the study described here, *E canis* or *A platys* DNA was amplified from one cat each, suggesting exposure to *R sanguineus*. However, although *B vogeli* is also suspected to be transmitted to cats by *R sanguineus*, *B vogeli* DNA was not amplified from the blood of any cat in this study. Our laboratory calculated that the assay used to amplify DNA of *C felis* and *B vogeli* has a detection limit of 28.1 fg/µl and therefore false-negative results are unlikely. However, the DNA had been stored for months to years, which could have resulted in false-negative results from degradation. In addition, blood-borne infectious agents can have fluctuating levels of DNA and so it is possible that *B vogeli* or *C felis* were missed as only one sample from each cat was assessed.

### Table 1 Prevalence rates of infectious organisms in anemic cats (n = 101) in Colorado, USA

| Infectious agent                  | n (%)   | Year of submissions  |
|-----------------------------------|---------|----------------------|
| Overall                           | 22 (21.8) | 2004–2018            |
| Hemoplasma species                | 14 (13.9) | 2004–2018            |
| *Bartonella* species              | 4 (4)   | 2006, 2009, 2015, 2017|
| *Ehrlichia canis*                 | 1 (1)   | 2006                 |
| *Anaplasma phagocytophilum*       | 1 (1)   | 2005                 |
| *Anaplasma platys*                | 1 (1)   | 2005                 |
| *Wolbachia* species               | 1 (1)   | 2007                 |
| *Cytauxzoon felis*                | 0       | –                    |
| *Babesia* species                 | 0       | –                    |

Regardless, any recent positive infection should be treated as a potential underlying cause of anemia. If clinicians suspect the vector-borne pathogen is the cause of immune-mediated hemolytic anemia, appropriate antimicrobial treatment is recommended alone or in conjunction with immunosuppressive therapy depending on the condition of the animal. Doxycycline is the treatment of choice for hemoplasmosis, ehrlichiosis and anaplasmosis, therefore if there is concern for these infections, empirical treatment may be indicated. The results described here provide more information that some agents thought to be rare in cats, such as *E canis* and *A platys*, might be more common than previously believed and support the use of vector-borne disease panels when assessing cats with fever or cytopenias such as anemia.

Whether *Bartonella* species or *A phagocytophilum* infections are associated with anemia in cats is still being evaluated. Recently, an association between *Bartonella* species DNA in blood and anemia was not recognized (Williams et al, submitted). Similarly, thrombocytopenia – not anemia – is usually associated with *A phagocytophilum* in cats recognized to date. The cat described here with *Wolbachia* DNA amplified from blood was likely infected with *Dirofilaria immitis* or infested with fleas. *Ixodes* species are the vector for *A phagocytophilum* and since these ticks are endemic to the northwest, midwest and western USA, but not to Colorado, it is likely the positive cat had a travel history or recently moved to the state. Regardless, the
data presented here support the recommendation for providing flea, tick and *D. immitis* preventives to cats regardless of the region.\(^{27}\)

**Limitations**

One limitation of this study was the lack of complete histories, which was due to information being pulled from submission forms only. There was not enough information to classify the cause of anemia in our study, and other common causes of anemia, such as primary bone marrow disease or renal insufficiency, could have been missed. Another limitation of the study is that the regenerative status of the anemia was not always listed. However, as all samples were submitted for infectious agent testing, with the majority being assayed for hemoplasma species, many cats may have had a regenerative anemia suspected to be caused by infection. Other limitations stemming from incomplete histories include a lack of information about travel history and use of flea and tick control.

As discussed, another major limitation of this study was the fact that assays were performed on stored DNA, which could have led to false-negative results. For example, one sample in this study was positive for ‘*Candidatus M. haemominutum*’ DNA on initial assay but was negative when retested, likely a false-negative due to DNA degradation.

**Conclusions**

Although 21.8% of samples tested positive for an infectious agent using PCR assays, *C. felis* and *Babesia* species DNA were not amplified in any sample. Based on the results of this study, it does not appear that *Babesia* species or *C. felis* are clinically relevant in anemic cats in Colorado, and the range of *C. felis* has not expanded to include Colorado. Other agents vectored by *R. sanguineus*, such as *E. canis* and *A. platys*, are endemic to Colorado.

**Author note** This paper was presented, in part, at the 2020 ACVIM Forum On Demand as an abstract.

**Conflict of interest** The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding** The study was funded by the Center for Companion Animal Studies at Colorado State University.

**Ethical approval** This work involved the use of non-experimental animals only (including owned or unowned animals and data from prospective or retrospective studies). Established internationally recognized high standards (‘best practice’) of individual veterinary clinical patient care were followed. Ethical approval from a committee was therefore not necessarily required.

**Informed consent** Informed consent (either verbal or written) was obtained from the owner or legal guardian of all animal(s) described in this work for the procedure(s) undertaken. No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

**References**

1. Ishak AM, Radecki S and Lappin MR. Prevalence of *Mycoplasma haemofelis, Candidatus Mycoplasma haemominutum*, *Bartonella* species, *Ehrlichia* species, and *Anaplasma phagocytophilum* DNA in the blood of cats with anemia. *J Feline Med Surg* 2007; 9: 1–7.
2. Korman RM, Hetzel N, Knowles TG, et al. A retrospective study of 180 anaemic cats: Features, aetiologies and survival data. *J Feline Med Surg* 2013; 15: 81–90.
3. Garden OA, Kidd L, Mexas AM, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *J Vet Intern Med* 2019; 33: 313–334.
4. Winzelberg Olson S and Hohenhaus AE. Feline non-regenerative anemia: diagnostic and treatment recommendations. *J Feline Med Surg* 2019; 21: 615–631.
5. Solano-Gallego L and Baneth G. Babesiosis in dogs and cats – expanding parasitological and clinical spectra. *Vet Parasitol* 2011; 181: 48–60.
6. Pennisi MG, Hofmann-Lehmann R, Radford AD, et al. *Anaplasma, Ehrlichia* and *Rickettsia* species infections in cats: European guidelines from the ABCD on prevention and management. *J Feline Med Surg* 2017; 19: 542–548.
7. Qurollo B. Feline vector-borne diseases in North America. *Vet Clin North Am Small Anim Pract* 2019; 49: 687–702.
8. Kobn B, Weingart C, Eckmann V, et al. Primary immune-mediated hemolytic anemia in 19 cats: diagnosis, therapy, and outcome (1998–2004). *J Vet Intern Med* 2006; 20: 159–166.
9. Wardrop KJ, Birkenheuer A, Blais MC, et al. Update on canine and feline blood donor screening for blood-borne pathogens. *J Vet Intern Med* 2016; 30: 15–25.
10. Simking P, Wongnakphet S, Stich RW, et al. Detection of *Babesia vogeli* in stray cats of metropolitan Bangkok, Thailand. *Vet Parasitol* 2010; 173: 70–75.
11. Do T, Kamyingkird K, Chimnoi W, et al. Evaluation of hematological alteration of vector-borne pathogens in cats from Bangkok, Thailand. *BMC Vet Res* 2021; 17: 28. DOI: 10.1186/s12917-020-02737-1.
12. Rochlin I and Toledo A. Emerging tick-borne pathogens of public health importance: a mini-review. *J Med Microbiol* 2020; 69: 781–791.
13. Swann JW, Szladovits B and Glanemann B. Demographic characteristics, survival and prognostic factors for mortality in cats with primary immune-mediated hemolytic anemia. *J Vet Intern Med* 2016; 30: 147–156.
14. Mather T. Tick Encounter Resource Center. https://tick-encounter.org/ (accessed September 3, 2020).
15. Monzón JD, Atkinson EG, Henn BM, et al. Population and evolutionary genomics of *Amblyomma americanum*, an expanding arthropod disease vector. *Genome Biol Evol* 2016; 8: 1351–1360.
16 Scott Dahlgren F, Paddock CD, Springer YP, et al. Expanding range of Amblyomma americanum and simultaneous changes in the epidemiology of spotted fever group ricketsiosis in the United States. *Am J Trop Med Hyg* 2016; 94: 35–42.

17 Qurollo BA, Archer NR, Schreeg ME, et al. Improved molecular detection of *Babesia* infections in animals using a novel quantitative real-time PCR diagnostic assay targeting mitochondrial DNA. *Parasit Vectors* 2017; 10: 128–141.

18 Jensen WA, Fall MZ, Rooney J, et al. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. *J Clin Microbiol* 2000; 38: 1717–1722.

19 Jensen WA, Lappin MR, Kamkar S, et al. Use of a polymerase chain reaction assay to detect and differentiate two strains of *Haemobartonella felis* in naturally infected cats. *Am J Vet Res* 2001; 62: 604–608.

20 Lappin MR, Breitschwerdt EB, Jensen WA, et al. Molecular and serologic evidence of *Anaplasma phagocytophilum* infection in cats in North America. *J Am Vet Med Assoc* 2004; 225: 893–896.

21 National Center for Biotechnology Information. BLAST. https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed March 26, 2021).

22 Shock BC, Murphy SM, Patton LL, et al. Distribution and prevalence of *Cytauxzoon felis* in bobcats (*Lynx rufus*), the natural reservoir, and other wild felids in thirteen states. *Vet Parasitol* 2011; 175: 325–330.

23 Sherrill MK and Cohn LA. Cytauxzoonosis: diagnosis and treatment of an emerging disease. *J Feline Med Surg* 2015; 17: 940–948.

24 Kidd L, Qurollo B, Lappin M, et al. Prevalence of vector-borne pathogens in southern California dogs with clinical and laboratory abnormalities consistent with immune-mediated disease. *J Vet Intern Med* 2017; 31: 1081–1090.

25 Savidge C, Ewing P, Andrews J, et al. *Anaplasma phagocytophilum* infection of domestic cats: 16 cases from the northeastern USA. *J Feline Med Surg* 2016; 18: 85–91.

26 Hoyt K, Chandrashekar R, Beall M, et al. Evidence for clinical anaplasmosis and borreliosis in cats in Maine. *Top Companion Anim Med* 2018; 33: 40–44.

27 Companion Animal Parasite Council. Parasite prevalence maps. https://capcvet.org/maps/#2020/all/ehrlichiosis/dog/united-states/colorado/ (2020, accessed October 19, 2020).