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

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

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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

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.[1,2] Hemoplasma species have been extensively studied in cats and there is a strong association between hemolytic anemia in cats and *Mycoplasma haemofelis*, as well as a weaker association with 'Candidatus Mycoplasma haemominutum'.[3] Other vector-borne agents that are thought to be associated with anemia in cats include *Anaplasma phagocytophilum*, *Anaplasma platys*, *Babesia* species, *Bartonella* species, *Cytauxzoon felis* and *Ehrlichia canis*.[3,4] The route of transmission for these agents is primarily by *Amblyomma americanum* (*C felis*), *Ixodes* species (*A phagocytophilum*), *Rhipicephalus sanguineus* (*A platys*, *Babesia* species, *E canis*) or fleas (*Bartonella* species, hemoplasmas), and their geographic ranges are usually defined by their vector.[5–7]

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.[1,2,8] 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.[9] Others, like *C felis*, have a historical range that only includes the central and southeast USA, and therefore

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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* 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 *Ehrilichia 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 a 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 clarridgeiae* 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

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 genera	1 (1)	2007
Cytauxzoon felis	0	–
Babesia species	0	–

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.[14] 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.[22] However, cytauxzoonosis in cats is generally characterized by a severe and acute fever, with hemolytic anemia seen frequently in later stages.[23] 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 antiprotozoal drugs for best efficacy, such as a combination of atovaquone and azithromycin.[23]

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.[10,11] 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.[10] 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.[24] While neither agent was amplified in the blood of cats

in this study, clinicians in Colorado should continue to consider testing for these pathogens in anemic cats owing to the presence of *R sanguineus* in this state and the potential geographical expansion of *A americanum*.

Even with the use of additional DNA targets in the study described here, the prevalence of positive assay results for vector-borne agents (21.8%) was similar to anemic cats (24.7%) and healthy controls (23%) reported in a study performed over 10 years ago in this region.[1] As discussed elsewhere, as each agent tested for in this study can be amplified from healthy cats, the presence of specific DNA cannot prove disease causation.[3] 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.[3] 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.[25,26] 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.[25] 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.

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

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.

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