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Prevalence of *Mycoplasma haemofelis*, 'Candidatus *Mycoplasma haemominutum*', *Bartonella* species, *Ehrlichia* species, and *Anaplasma phagocytophilum* DNA in the blood of cats with anemia

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Hemoplasmas are known causes of anemia in some cats and some *Bartonella* species have been associated with anemia in people and in dogs. In this retrospective study, we used polymerase chain reaction (PCR) assays to determine the prevalence rates of *Mycoplasma haemofelis*, 'Candidatus *Mycoplasma haemominutum*', *A. phagocytophilum*, *Ehrlichia* species, and *Bartonella* species DNA in the blood of cats with anemia and a control group of healthy cats. DNA of the organisms was amplified from 22 of 89 cats with anemia (24.7%) and 20 of 87 healthy cats (23.0%). DNA of a hemoplasma was amplified from 18 of 89 cats with anemia (20.2%) and 13 of 87 healthy cats (14.9%); DNA of a *Bartonella* species was amplified from five of 89 cats with anemia (5.6%) and seven of 87 healthy cats (8.0%). There were no statistically significant differences detected between groups.

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Both regenerative and non-regenerative anemias are common in cats (Klaser et al 2005). Non-regenerative anemia is associated with a number of infectious agents, including feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and coronaviruses associated with feline infectious peritonitis (FIP), as well as a variety of non-infectious diseases including neoplasia, renal disease, endocrinopathies such as diabetes mellitus, and bone marrow diseases. Regenerative anemia usually develops after blood loss or hemolysis of red blood cells. Hemolytic anemia is frequently associated with oxidative damage to red blood cells, infectious agents, neoplasia, and primary immune-mediated syndromes (Kohn et al 2006). Infectious agents associated with hemolytic anemia in cats in the United States include *Mycoplasma haemofelis*, 'Candidatus *Mycoplasma haemominutum*', and *Cytauxzoon felis* (George et al 2002, Tasker and Lappin 2002, Messick 2004, Birkenheuer et al 2006a,b). It is

currently unknown if 'Candidatus *M. turicensis*', another hemoplasma detected in some cats in Europe, infects cats in the United States (Willi et al 2005, 2006).

Most causes of anemia in cats can be excluded by evaluation of the history, physical examination, and results of a complete blood cell count, serum biochemical panel, coagulation tests, diagnostic imaging for neoplasia or other mass lesions such as abscessation, FeLV antigen test, FIV antibody test, polymerase chain reaction (PCR) assay for hemoplasmas, and bone marrow examination. When associated with anemia, the hemoplasmas and *C. felis* are sometimes cytologically apparent. In cases where the agent is not identified, results of PCR assays can be used to confirm the presence of organismal DNA in blood (Jensen et al 2001, Tasker and Lappin 2002, Birkenheuer et al 2006b). Our research laboratory provides a number of infectious disease tests and consultations to veterinarians. We have observed that for many cats with anemia, the cause cannot be identified by use of the diagnostic workup described previously, leading to the hypothesis that there may be other

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previously unrecognized infectious causes of anemia in cats or a higher prevalence of primary immune-mediated anemia in cats than previously thought.

Bartonella species are small, Gram-negative bacteria that infect a variety of animals including cats and people. *Bartonella henselae* is the most common cause of cat scratch disease in people; approximately 25,000 people are affected annually in the United States (Jackson et al 1993). Both *B bacilliformis* (Spach and Koehler 1998) and *B henselae* (Van Audenhove et al 2001) infection have been associated with hemolytic anemia in people and *B vinsonii* infection has been associated with hemolytic anemia in dogs (Breitschwerdt et al 2004). Some *Bartonella* species are transmitted by *Ctenocephalides felis* and so infection can be common in cats. For example, in one study, *B henselae* or *B clarridgeiae* DNA was amplified from 47.8% and 60.9% of cats and their fleas, respectively (Lappin et al 2005). *Bartonella* species infections have been associated with a variety of clinical syndromes in cats including uveitis, fever, gingivitis, and lymphadenopathy (Guptill 2005). When sick cats in North Carolina were assessed, cats with *B henselae* antibodies in their serum were no more likely to be anemic than cats that were *B henselae* seronegative, suggesting that *B henselae* infection is not associated with hemolytic anemia in cats (Breitschwerdt et al 2005). However, to our knowledge, the *Bartonella* species prevalence rates as determined by PCR assay results have not been determined in cats with anemia and compared to results from healthy cats.

Ehrlichia canis-like DNA and *A phagocytophilum* DNA have been amplified from naturally exposed cats by use of PCR assays (Bjoersdorff et al 1999, Breitschwerdt et al 2002, Lappin et al 2004). In addition, *Ehrlichia*-like morula has been detected in mononuclear cells or neutrophils of naturally exposed cats in the United States, Kenya, Brazil, France, Sweden, and Thailand and a number of cats have been shown to be seropositive to *E canis* antigens (Stubbs and Reif 2000, Neer et al 2002). Cats with presumed ehrlichiosis or anaplasmosis have a wide variety of clinical and laboratory abnormalities; anemia has been reported in some cats with presumed ehrlichiosis (Breitschwerdt et al 2002). However, to our knowledge, *Ehrlichia* species and *A phagocytophilum* prevalence rates as determined by PCR assay results have not been determined in cats with anemia and compared to results from healthy cats.

The purpose of this study was to determine whether there were differences in prevalence rates of *M haemofelis*, '*Candidatus M haemominutum*', *A phagocytophilum*, *Ehrlichia* species, and *Bartonella* species DNA in the blood of healthy cats and retrovirus-negative cats with anemia that did not have an apparent non-infectious (ie, blood loss, chronic disease) cause for their anemia.

Materials and methods

Case selection

The records of the Colorado State University Infectious Disease Laboratory between January 2001 and November 2004 were reviewed for feline submissions from United States veterinarians for which the primary presenting complaint included anemia and for which sufficient blood in EDTA or DNA from the blood digest were available for further testing. The majority of the samples had been submitted because the referring veterinarian suspected hemoplasmosis and requested assessment with a previously published hemoplasma PCR assay (Jensen et al 2001) offered as a clinical service at Colorado State University. The referring veterinarian was contacted for permission to review the medical record. Information gathered included results of complete blood count, serum biochemical panel, FeLV antigen test, FIV antibody test, age, state of origin, recent antibiotic history, recent vaccination history, clinical history and final diagnosis. Anemia was defined as a packed cell volume (PCV) or hematocrit (HCT) of <30%. Cases with a known cause of anemia were excluded from further study. In addition, cats known to have been administered vaccines within the previous 1 month or treated with antibiotics within 10 days of the presentation for evaluation of anemia based on the history listed on the submission forms were excluded. Anemia was classified as regenerative (absolute reticulocyte count >60,000 reticulocytes/ μ l or a corrected reticulocyte percent >1.5%) or non-regenerative (absolute reticulocyte count <60,000 reticulocytes/ μ l or a corrected reticulocyte percent <1.5%). Because very few of the records or submission forms stated the presence or absence of fleas, the state of origin of the case was used to classify each cat as high risk or low risk of exposure to *Ctenocephalides felis* (Jameson et al 1995). Samples were categorized as being from areas

with low (Alaska, Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming) or high flea prevalence (all other states). In a separate study of fever of unknown origin in cats, referring veterinarians from around the United States were requested to submit blood from a clinically healthy cat for use as a control sample for each case with fever (Lappin 2002). While the PCV of these cats was unknown, there were no history or physical examination findings consistent with anemia. DNA digests were selected from this group to serve as a control group solely based on sample availability.

Assays

The samples had been stored at -20°C or -80°C until assayed. Previously published PCR assays for amplification of DNA of *Bartonella* species (Jensen et al 2000), *Ehrlichia* species and *A phagocytophilum* (Lappin et al 2004), and *M haemofelis* and '*Candidatus M haemominutum*' (Jensen et al 2001) were performed on each sample. The *Bartonella* species PCR assay amplifies the DNA of *B henselae*, *B bacilliformis*, *B elizabethae*, *B clarridgeiae*, *B vinsonii* subspecies *berkhoffii*, and *B quintana*.

Statistical analysis

PCR assay results were stratified by the presence or absence of anemia, the form of anemia, and flea risk based on state of origin. When sample size allowed, prevalence rates for some PCR assay results were compared between groups by the Fisher's exact test. Significance was defined as $P < 0.05$. Because of small sample size and numbers of positive test results, logistic regression analyses to assess the influence of age or risk of flea exposure could not be performed.

Results

During the study period, we identified 133 cats for which the laboratory submission form stated that anemia was a presenting complaint and blood or DNA was available for further testing. After contacting the referring veterinarian and review of the medical records, we excluded 14 cats that were not anemic at the time of sample submission (most of the cats were anemic previously but were now normal), 22 cats that had insufficient medical record information for review, four cats with FeLV infection, two cats with FIV infection, and two cats that had other obvious

causes of anemia identified during the diagnostic workup (lymphoma of the liver and spleen in one cat, and metastatic cancer on necropsy on the other). Of the 89 cats with anemia that qualified for the study, 27 had regenerative anemia, 27 had non-regenerative anemia, and 35 had unclassified anemia (Table 1).

DNA of *M haemofelis*, '*Candidatus M haemominutum*', *B henselae*, or *B clarridgeiae* was amplified from many of the 89 cats with anemia and the 87 healthy cats (Table 1). Overall, DNA of one or more of the organisms was amplified from 22 of the 89 cats with anemia (24.7%) and 20 of the 87 healthy cats (23.0%); DNA of a hemoplasma was amplified from 18 of the 89 cats with anemia (20.2%) and 13 of the 87 healthy cats (14.9%); and DNA of a *Bartonella* species was amplified from five of 89 cats with anemia (5.6%) and seven of 87 healthy cats (8.0%). None of these differences were significantly different. After the results were stratified by flea risk, presence or absence of anemia, and type of anemia, numerical differences between some groupings were noted but statistical differences did not exist (Table 1).

DNA of *Ehrlichia* species or *A phagocytophilum* was not amplified from the blood of any cat with anemia or the healthy cats.

Discussion

While there are many known causes of anemia in cats, in many cases a diagnosis is not easily made. In this study, we reviewed the laboratory submission forms and medical records to attempt to eliminate cats with known causes of anemia including neoplasia, FeLV, FIV, suspected FIP, blood loss, chronic renal failure, bone marrow disease, other chronic diseases (eg, neoplasia), endocrinopathies, and previous cytologic evidence of hemoplasmosis to allow for selection of cases that were likely to have anemia from previously unrecognized infectious diseases or primary immune-mediated anemia.

There are a number of potential limitations to this retrospective study. Because recent vaccination has been associated with immune-mediated hemolytic disease in some dogs (Duval and Giger 1996) and antibiotic therapy can result in falsely negative PCR assay results for some infectious agents, we intended to exclude these cases. While none of the anemic cats were excluded for these criteria, we cannot be certain that failure to find the information in the medical records provided proves that the treatments did not occur.

Table 1. Distribution of *Mycoplasma haemofelis*, 'Candidatus M haemominutum', and *Bartonella* species PCR assay results from 89 cats with anemia and 87 healthy cats in the United States*

| | All anemia (total = 89) | Reg anemia (total = 27) | Non-reg anemia (total = 27) | Anemia class unknown (total = 35) | Healthy (total = 87) |
|-----------------------|----------------------------|----------------------------|--------------------------------|--------------------------------------|-------------------------|
| Low flea states | <i>n</i> = 44 | <i>n</i> = 20 | <i>n</i> = 14 | <i>n</i> = 10 | <i>n</i> = 16 |
| Any agent | 8 (18.2) | 5 (26.3) | 2 (14.3) | 1 (10.0) | 1 (6.3) |
| Any hemoplasma | 7 (15.9) | 4 (21.1) | 2 (14.3) | 1 (10.0) | 1 (6.3) |
| Any <i>Bartonella</i> | 1 (2.3) | 1 (5.3) | 0 (0.0) | 0 (0.0) | 0 (0) |
| Subgroups | | | | | |
| Mhm alone | 3 (6.8) | 3 (15.8) | 0 (0.0) | 0 (0.0) | 1 (6.3) |
| Mhf alone | 3 (6.8) | 1 (5.3) | 1 (7.1) | 1 (10.0) | 0 (0.0) |
| Mhf and Mhm | 1 (2.3) | 0 (0.0) | 1 (7.1) | 0 (0.0) | 0 (0.0) |
| Mhm and Bh | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Bh alone | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Bc alone | 1 (2.3) | 1 (5.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| High flea states | <i>n</i> = 45 | <i>n</i> = 7 | <i>n</i> = 13 | <i>n</i> = 25 | <i>n</i> = 71 |
| Any agent | 14 (31.1) | 6 (85.7) | 4 (30.8) | 4 (16.0) | 19 (26.8) |
| Any hemoplasma | 11 (24.4) | 6 (85.7) | 3 (23.1) | 2 (8.0) | 12 (16.9) |
| Any <i>Bartonella</i> | 4 (8.9) | 1 (14.3) | 1 (7.7) | 2 (8.0) | 8 (11.3) |
| Subgroups | | | | | |
| Mhm alone | 4 (8.9) | 1 (14.3) | 3 (23.1) | 0 (0.0) | 6 (8.5) |
| Mhf alone | 4 (8.9) | 4 (57.1) | 0 (0.0) | 0 (0.0) | 1 (1.4) |
| Mhf and Mhm | 2 (4.4) | 0 (0.0) | 0 (0.0) | 2 (8.0) | 4 (5.6) |
| Mhm and Bh | 1 (2.2) | 1 (14.3) | 0 (0.0) | 0 (0.0) | 1 (1.4) |
| Bh alone | 2 (4.4) | 0 (0.0) | 1 (7.7) | 1 (4.0) | 5 (7.0) |
| Bc alone | 1 (2.2) | 0 (0.0) | 0 (0.0) | 1 (4.0) | 0 (0.0) |
| Bh and Bc | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (2.8) |

*Results listed as number positive in each category (% positive). Information was not available for each cat in each category and so denominators vary between assessment categories. Mhf = *Mycoplasma haemofelis*; Mhm = 'Candidatus M haemominutum'; Bh = *Bartonella henselae*; Bc = *Bartonella clarridgeiae*.

For some cases, the duration of illness could not be determined which may have resulted in the classification of some cases into the non-regenerative anemia category when in fact the cases may have just been too acute to have developed a regenerative response. The blood samples were not assessed for all known infectious causes of anemia with the most sensitive techniques that are available. For example, while none of the veterinarians or complete blood count reports noted organisms consistent with *C felis* or *Babesia* species, it is possible some cats could have been cytology negative but PCR assay positive (Baneth et al 2004, Birkenheuer et al 2006a,b). Lastly, because of the sample size of some groupings, our ability to perform statistical analyses was limited and so some conclusions may be affected by type II error.

Regardless of these limitations, we believe the study results provide new information concerning anemia in cats. For example, infection by a hemoplasma species is thought to be one of the most common causes of anemia in cats and the majority of the samples assessed in this study were suspected to have hemoplasmosis by the referring veterinarian as evidenced by the submission for hemoplasma testing. However, hemoplasma DNA was amplified from only 20.2% of the cats with anemia and there was no significant difference in hemoplasma prevalence rates between healthy and anemic cats, regardless of flea risk. While both hemoplasmas are associated with anemia in some cats, not all infected cats develop anemia, most infected cats survive regardless of antibiotic treatment, and infection can persist for months in cats with or without

antibiotic therapy (Tasker and Lappin 2002). These facts probably explain our failure to detect differences in prevalence rates between cats with anemia and healthy cats. However, the fact that a historical control group was used in this study versus an age-matched sample drawn from a healthy cat on the same day from the same clinic as anemic samples might also account for the lack of a difference. In addition, these results also document that amplification of hemoplasma DNA from the blood of a cat does not directly prove the anemia was a result of the infection. Failure to document hemoplasma infection in 79.8% of suspect cases supports our hypothesis that other causes of anemia in cats exist and may be common. In future studies, PCR assays that amplify *C felis*, *Babesia* species, and '*Candidatus M turicensis*' should also be used.

Because *Bartonella* species can reside within erythrocytes of cats (Kordick and Breitschwerdt 1995, Guptill 2005) and because some *Bartonella* species of people and dogs have been associated with hemolytic anemia (Spach and Koehler 1998, Van Audenhove et al 2001, Breitschwerdt et al 2004), we hypothesized that *Bartonella* species infection may be a differential diagnosis for cats with unexplained anemia. However, results of this study fail to link *Bartonella* species to presence of anemia, regardless of flea risk. In a separate study of sick cats in North Carolina, cats with *B henselae* antibodies in their serum were no more likely to be anemic than cats that were *B henselae* seronegative (Breitschwerdt et al 2005). We believe that the results of these two studies suggest *B henselae* and *B clarridgeiae* are not common causes of anemia in cats. There are several potential limitations to prevalence studies based on PCR assay results. While the primers utilized in this study occasionally amplify non-*Bartonella* species DNA, all amplicons detected in this study were of the appropriate size and so we do not believe false positive results occurred (Maggi and Breitschwerdt 2005a). As mentioned, if antibiotics had been previously administered and not recorded in the medical record, some cases may have had falsely negative results. In addition, while PCR assays are very sensitive, blood culture is still considered the most sensitive and specific test to prove *Bartonella* species bacteremia (Guptill 2005, Maggi et al 2005b). Future prospective studies should include blood culture, serology, and PCR assay results.

The absence of isolation of *A phagocytophilum* and *Ehrlichia* species DNA from any healthy or

anemic cat suggests that those organisms are an uncommon cause of anemia in cats. However, both of these infections are also thought to be rare in cats and can be geographically defined. For example, while *E canis* infections are common in dogs in the south-eastern United States, *E canis* DNA was not amplified from feral cats in Florida (Luria et al 2004). In addition, *A phagocytophilum* infection is transmitted by *Ixodes* species ticks and so infection is only documented in some parts of the United States (Lappin et al 2004).

Primary immune-mediated disease is thought to be the most common cause of hemolytic anemia in dogs (Dodds 1977, Klay et al 1993, Day 1999). Primary immune-mediated anemia can also be non-regenerative if the immune reaction is directed toward bone marrow precursors (Stokol and Blue 1999, Weiss 2002). In cats, anemia has frequently been attributed to infectious and other diseases with primary immune-mediated disease thought to be less common (Werner and Gorman 1984). However, in the anemic cats described here, approximately 75% of cats with regenerative or non-regenerative anemia had no identifiable infectious cause and 60% of cats with regenerative anemia had no obvious sign of blood loss or identifiable infectious cause; however, we did not test for some known infectious causes of anemia in cats including *C felis*, *Babesia* species, and '*Candidatus M turicensis*'. The data presented and recent work from Germany (Kohn et al 2006) suggest that cats are more predisposed to primary immune-mediated anemia than previously thought or may have another unidentified infectious cause of hemolytic anemia. The possibility that primary hemolytic anemia may be more common in cats than previously recognized warrants further prospective study.

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