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Prevalence of *Anaplasma phagocytophilum* infection in feral cats in Massachusetts

Erin R Galemore, Mary A Labato and Elizabeth O'Neil

Abstract

Objectives The primary objective of this study was to determine the prevalence of *Anaplasma phagocytophilum* infection and exposure in adult feral cats in Massachusetts, an endemic area for *A phagocytophilum* and its tick vector *Ixodes scapularis*. The secondary objective was to determine if there were correlations between *A phagocytophilum* infection and the presence of anemia and thrombocytopenia.

Methods Blood samples were collected between June and December 2015 from 175 apparently healthy adult feral cats that were presented to trap and release spay/neuter centers in Massachusetts. Complete blood count, blood smear evaluation, SNAP 4Dx Plus test (IDEXX) and *A phagocytophilum* PCR were performed on all samples to document acute infection (PCR-positive and/or inclusions observed on blood smear) and exposure to *A phagocytophilum* (SNAP 4Dx Plus-positive for *A phagocytophilum* antibodies).

Results The prevalence of exposure to *A phagocytophilum* in feral cats in Massachusetts was 9.7%, whereas the prevalence of acute infection was 6.9%. All blood smears were negative for *Anaplasma* species inclusions; therefore, acute infection was defined as testing positive on PCR analysis. No statistically significant correlations were identified for cats that were positive for *A phagocytophilum* on PCR analysis or SNAP 4Dx Plus test and the presence of anemia or thrombocytopenia.

Conclusions and relevance The prevalence of *A phagocytophilum* exposure in feral cats approaches 10% and is higher than the previously reported national average prevalence of 4.3% in the USA. *A phagocytophilum* infection may be an emerging infectious disease in cats. Further research is needed to determine the prevalence of clinical illness associated with *A phagocytophilum* infection in cats living in endemic areas.

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Introduction

Anaplasma phagocytophilum is a bacterium transmitted to mammals via a tick vector, most commonly *Ixodes scapularis* or *Ixodes pacificus*.[1] Clinical signs of infection include fever, lethargy, joint pain and lymphadenopathy.[1–3] Common clinicopathologic abnormalities include anemia, thrombocytopenia, leukocytosis, elevated liver enzymes and proteinuria.[1–3] *Anaplasma* species infection has been commonly associated with clinical disease in humans and dogs.[1–3] However, *Anaplasma* species infection in cats has not routinely been associated with clinical illness in cats.

Recent reports have shown that *Anaplasma* species infection can cause significant disease in cats.[4–9] The first report of clinical illness associated with *Anaplasma* species infection was published in 1999 and reports of clinical disease caused by *Anaplasma* species are becoming more frequent.[4–11] A 2013 case series by Adaszek et al[8] described three cases of anaplasmosis in cats in Poland. Infection was confirmed with a PCR assay and visualization of morulae within neutrophils of these cats

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(2/3 cats).[8] Clinical signs reported included fever, pale mucous membranes, inappetence, lethargy, lameness, swollen and painful joints, and epistaxis.[8] Hematologic evaluation showed anemia and thrombocytopenia in all cats.[8] A case series of 16 cats from the northeastern USA with acute *A phagocytophilum* infection was published in 2016.[7] All cats in this study tested positive for *A phagocytophilum* via PCR analysis with 3/16 cats also having morulae present within neutrophils.[7] The most common clinical signs found were lethargy, fever and anorexia.[7] These studies demonstrate that *A phagocytophilum* is a causative agent of clinical disease in feline patients.

The prevalence of *Anaplasma* species infection in humans has grown tremendously in recent years. *A phagocytophilum* infection became a reportable human infectious disease in 1999.[12] The Centers for Disease Control report that the number of human cases in the USA more than doubled from 2007 to 2010.[12] Also, a recent report on the prevalence of tick-borne diseases in dogs noted an increase in prevalence of *Anaplasma* species infection in the northeastern USA as compared with previous reports.[13] In 2015, the year the current study was performed, the Companion Animal Parasite Council reported an 11.4% seroprevalence of *Anaplasma* species infection in dogs in Massachusetts.[14] A study performed in 2007 by Billeter et al[15] looked at the average prevalence of *Anaplasma* species infection in cats in the USA. Positive antibody titers were found in 4.3% of the cats and all cats were PCR-negative for *Anaplasma* species.[15] Given the marked increase in human and canine cases of anaplasmosis in recent years, it is possible that the prevalence of feline anaplasmosis has also increased significantly; however, there are no previously published data available on the prevalence of feline anaplasmosis in the state of Massachusetts.[12]

The primary objective of our study was to determine the prevalence of acute *A phagocytophilum* infection and *A phagocytophilum* exposure in feral cats living in Massachusetts, an area that has one of the highest incidences of *Anaplasma* species infection in humans and dogs.[12,14] A secondary objective was to evaluate abnormalities in complete blood count (CBC) analyses to determine if there were correlations between *A phagocytophilum* infection, exposure, and the presence of anemia and thrombocytopenia.

Materials and methods

The study population was comprised of adult feral cats presenting to two trap and release spay/neuter clinics in Massachusetts: the Lerner Clinic at Tufts Cummings School of Veterinary Medicine in North Grafton, MA, and Dakin Humane Society's Spay/Neuter Clinic in Springfield, MA. All cats were trapped in the state of Massachusetts. Adult age was assessed based on the presence of adult dentition. All samples were collected while the cats were under anesthesia for routine castration or ovariohysterectomy. This study was approved by and conducted in accordance with the Institutional Animal Care and Use Committee at Tufts University.

Blood samples were collected from June to December 2015 and placed in sodium EDTA and serum separator tubes. After allowing time to clot, serum separator tubes were centrifuged for 10 mins to separate serum. Blood samples were stored on ice during transport from clinic to laboratory. CBC and blood smear preparation were performed on sodium EDTA whole-blood samples within 24 h of sample collection. CBC analyses were performed by IDEXX Laboratories and cats were considered to have anemia or thrombocytopenia when the values were below the laboratory reference interval. To document acute infection, blood smears were evaluated for the presence of morulae within neutrophils and whole-blood samples were submitted for PCR analysis. Blood smears were submitted to the clinical pathology laboratory at Tufts Cummings School of Veterinary Medicine for pathologist review. For consistency, a single clinical pathologist evaluated all blood smears (EO'N). Sodium EDTA whole-blood samples were submitted for *A phagocytophilum* PCR analysis to IDEXX Laboratories. To document previous *Anaplasma* species exposure, serum samples were evaluated for *A phagocytophilum* antibodies using a SNAP 4Dx Plus test (IDEXX) according to manufacturers' guidelines. The *Anaplasma* portion of the SNAP 4Dx Plus test is not species-specific (unpublished data, personal communication with IDEXX Medical Affairs Manager, September 2014). The SNAP 4Dx Plus test is currently only approved for use in dogs; however, this test has been successfully used to document antibodies to *A phagocytophilum* and *Borrelia burgdorferi* in several other species, such as domestic cats, horses and sheep (only *A phagocytophilum* antibodies have been studied in sheep using this test).[16–24]

Statistical analysis

Power analysis was performed and revealed that 141 samples were needed to achieve a statistical power of 0.8, whereas 188 samples were required to reach a statistical power of 0.9. Data were analyzed using SAS statistical software and VassarStats online statistical software (http://vassarstats.net). The prevalence of *Anaplasma* species exposure (positive antibody response) and infection (presence of organism in blood sample on blood smear or via PCR) were determined for the study population. Fisher's exact test was used to compare categorical variables between groups, ie, presence of anemia and *Anaplasma* species antibodies. A χ^2 test was used to compare the proportions of males and females testing positive for *Anaplasma* species exposure and acute infection. Data were considered statistically significant for P values ≤ 0.05.

Table 1 Positive sample results for *Anaplasma phagocytophilum* (*Ap*) PCR, *Ap* antibody ELISA and *Borrelia burgdorferi* (*Bb*) antibody ELISA in the study population of feral cats presenting to spay and neuter clinics in Massachusetts

	Positive samples	*B burgdorferi* co-exposure
Ap antibody (n = 175)	17 (9.7)	4 (2.3)
Ap PCR (n = 173)	12 (6.9)	4 (2.3)
Ap PCR and antibody (n = 173)	7 (4.0)	2 (1.2)
Bb antibody (n = 175)	42 (24.0)	–

Data are n (%)

Table 2 Complete blood count (CBC) results for feral cats surveyed at spay and neuter clinics in Massachusetts

CBC parameter (RI)	RI	Cats PCR positive for *A phagocytophilum*	Cats antibody positive for *A phagocytophilum*	Cats negative for *A phagocytophilum* antibodies and PCR	Total population
Hematocrit (%)	28.2–52.7	31.9 (23.3–45.0)	31.9 (23.5–45.0)	31.1 (17.1–45.0)	31.1 (17.1–45.0)
WBC count (K/µl)	3.9–19.0	7.608 (4.8–12.7)	10.406 (4.90–19.70)	11.239 (1.6–37.4)	11.335 (1.6–37.4)
Neutrophil count (K/µl)	2.620–15.170	4.614 (2.778–9.995)	6.893 (2.014–15.682)	7.829 (0.526–33.286)	7.935 (0.526–33.286)
Lymphocyte count (K/µl)	0.850–5.850	2.256 (1.215–4.284)	2.565 (0.888–13.002)	2.448 (0.449–17.280)	2.562 (0.449–17.280)
Monocyte count (K/µl)	0.040–0.530	0.179 (0–0.300)	0.210 (0–0.394)	0.269 (0–1.122)	0.272 (0–1.122)
Eosinophil count (K/µl)	0.090–2.180	0.551 (0.084–1.335)	0.753 (0–2.243)	0.640 (0–2.654)	0.648 (0–2.654)
Platelet count (K/µl)	155–641	400 (136–598)	448 (221–807)	448 (73–2677)	459 (73–2677)

Values for all parameters were found to be similar between groups, regardless of *Anaplasma phagocytophilum* infection or exposure status. No statistically significant correlations were identified for cats that were positive for *A phagocytophilum* on PCR analysis or SNAP 4Dx Plus, and the presence of anemia, thrombocytopenia, neutropenia or neutrophilia (*P* values ranged from 0.34–0.99 for these variables). Results expressed as mean (range) for each group
RI = reference interval; WBC = white blood cell

Results

Blood samples were collected from 175 adult feral cats. Blood samples were inadequate to perform all laboratory tests for six cats, four of which only had a PCR analysis and SNAP 4Dx Plus test (IDEXX) performed. One cat had a CBC, blood smear evaluation and SNAP 4Dx Plus test performed but no PCR analysis. Another cat had a SNAP 4Dx Plus test performed but no CBC, blood smear evaluation or PCR analysis. Cats with missing data were excluded from analyses of variables for which there were missing data. There were 74 females and 84 males included in the study. There was no statistically significant difference between the proportions of males and females testing positive for anaplasmosis via PCR and/or positive antibody response ($P = 0.888$). Seventeen cats had no sex recorded at the time of sampling. The majority of cats were domestic shorthair cats (n = 117). Fifteen domestic longhair cats and seven domestic mediumhair cats were included, as well as one Siamese cat. The breed was not recorded at the time of sampling for 35 individuals.

Twelve cats tested PCR positive for *A phagocytophilum*, whereas 17 individuals were antibody positive for *A phagocytophilum* (Table 1). Seven of these cats were both PCR and antibody positive. Blood smear evaluation showed no evidence of *Anaplasma* species morulae within neutrophils in any sample. The prevalence of *Anaplasma* species exposure (antibody positive) was 9.7%, whereas the prevalence of acute infection, as indicated by a positive PCR assay, was 6.9%. CBC results are shown in Table 2. No correlations were found between cats being positive for *Anaplasma* species antibodies and the presence of anemia ($P = 0.415$) or thrombocytopenia ($P > 0.999$). Similarly, no correlations were found between being PCR positive for *A phagocytophilum* and the presence of anemia ($P = 0.524$) or thrombocytopenia ($P > 0.999$).

B burgdorferi exposure was common with 24% of cats (42/175) testing positive for *B burgdorferi* antibodies (Table 1). Co-exposure was also identified with four individuals testing positive for *B burgdorferi* and *A phagocytophilum* antibodies. An additional four cats were positive on *A phagocytophilum* PCR analysis and tested positive for *B burgdorferi* antibodies. Two individuals tested positive on *A phagocytophilum* PCR analysis and tested positive for *B burgdorferi* and *A phagocytophilum* antibodies.

Discussion

The results of this study show that the prevalence of *A phagocytophilum* exposure and infection in feral cats in Massachusetts is higher than the previously published average seroprevalence of feline anaplasmosis in the USA.[15] Massachusetts is an endemic area for both *A phagocytophilum* and its tick vector, *I scapularis*.[1,12–14] Therefore, a higher prevalence would be expected in this area, which was confirmed by the results of the current study.

In this study, we included only feral cats in an effort to gauge the prevalence of *A phagocytophilum* infection and exposure in a population of cats that lived exclusively outdoors and were not treated with flea and tick preventatives. Our goal was to determine the true prevalence in free-ranging cats, in an effort to determine the risk for cats living in this region. Extrapolation of this information to cats that do not live exclusively outdoors and are treated with flea and tick preventatives may not be possible. However, the knowledge that the prevalence of *A phagocytophilum* infection and exposure in cats is higher than previously thought may help to guide diagnostic and therapeutic plans for cats in this area. A recent study by Hoyt et al[16] evaluated antibodies to *A phagocytophilum* and *B burgdorferi* in healthy and sick cats in Maine using a SNAP 4Dx Plus test (IDEXX).[16] Nineteen percent of the cats in this study tested positive for antibodies to *Anaplasma* species.[16] We now know that anaplasmosis can cause significant disease in cats and lead to lethargy, fever, decreased appetite, anemia, thrombocytopenia and polyarthropathy.[4–11] The current study and the report from Maine show that there is a higher prevalence of anaplasmosis in cats in New England.[16] This information should encourage clinicians in endemic areas to perform testing for *Anaplasma* species more readily in cats with clinical signs consistent with anaplasmosis. Furthermore, earlier detection of infection will allow for earlier treatment of these cats, which could improve clinical outcome.

The SNAP 4Dx Plus test (IDEXX) has been successfully used to document antibody response in cats experimentally infected, as well as those naturally infected with *A phagocytophilum*, though this test is not currently approved for use in this species.[16–20] Further research into the use of the SNAP 4Dx Plus test is needed before this test can be recommended for routine use as a screening test in feline patients. Immunofluorescent antibody testing and PCR testing are currently available from commercial laboratories and are recommended for use at this time to document exposure and/or active infection in cats. Serologic testing is useful as a screening tool in asymptomatic patients to document exposure to the organism and may also be useful in patients with clinical disease. However, antibody testing may be negative early in the course of disease, prior to development of an antibody response. Also, a positive serologic test merely shows that the patient was exposed to the organism and does not prove current infection. PCR testing for *A phagocytophilum* is useful in patients with clinical illness and can be used as a confirmatory test for anaplasmosis. A limitation of PCR testing is that the test recognizes DNA and cannot confirm that the organism is living and causing disease. In addition, accuracy of PCR testing relies on strict laboratory protocols, as sample contamination can lead to false-positive results. Despite these limitations, PCR testing is the current recommended diagnostic to confirm anaplasmosis in feline patients.[18,25,26] Alternatively, paired antibody titers showing a four-fold increase in titer performed 4 weeks after the initial titer may be used to confirm a diagnosis.[4,9,10,18,25,26]

Twenty-four percent (42/175) of the cats in this study had antibodies to *B burgdorferi*. Twenty-four percent (10/42) of these cats also tested positive for antibodies to *A phagocytophilum* and/or were PCR positive for *A phagocytophilum*. This highlights the fact that co-infections are common. Massachusetts and the New England region are highly endemic areas for *A phagocytophilum* and *B burgdorferi*, as well as their shared tick vector, *I scapularis*.[12–14] As cats living in these endemic areas can harbor infection with both *A phagocytophilum* and *B burgdorferi*, they can act as silent hosts or carriers that allow these infections to persist in the environment. In addition, infected companion cats could harbor infected ticks and pose a risk to their human caretakers.

The Companion Animal Parasite Council reports prevalence information on *A phagocytophilum* infection in dogs in the USA.[14] These data are listed by state, as well as by county. The prevalence of *A phagocytophilum* infection varies greatly from county to county in Massachusetts.[14] We sought to compare the prevalence of feline anaplasmosis between counties in this study. The trappers confirmed that all cats participating in the study were trapped in the state of Massachusetts. However, we did not receive thorough information on the specific locations where the cats were trapped and were unable to report on this information. This would be an interesting area to investigate further in future studies.

We found no correlations between the presence of anemia or thrombocytopenia and being antibody or PCR positive for *A phagocytophilum*. This is in contrast to

previous reports of clinically ill cats exhibiting anemia and/or thrombocytopenia.[7,8] However, the cats included in this study were apparently healthy and not showing signs of illness. We suspect that cats with clinical disease are more likely to exhibit these changes, whereas those with subclinical infections may not be afflicted with anemia and thrombocytopenia. However, further research is needed to evaluate cats with clinical and subclinical infection to better characterize the clinicopathologic abnormalities.

This study has several limitations. First, no previous medical history was available for any of the cats in this study, as they were feral cats trapped for castration or ovariohysterectomy. Also, there was limited knowledge about the behavior and roaming patterns of these cats. Second, it is possible that some of the cats trapped could have been pets from the community and not truly feral or stray cats. Therefore, it is possible that they may have previously been treated with flea and tick preventatives and may have been housed indoors at some time in the past. If any of the cats in this study were previously treated with flea and tick preventatives and/or lived indoors, this would have decreased the likelihood of exposure to ticks and tick bites, which could have falsely decreased the prevalence of *A phagocytophilum* infection and exposure in this study. Last, we were not able to get complete sample collections on all cats in this study, which was mostly likely due to peripheral vasoconstriction associated with the intramuscular sedation given prior to venipuncture. We were unable to perform all planned diagnostics on six cats in this study owing to short sampling or blood clots present in the sodium EDTA tubes.

Conclusions

This study shows that the prevalence of *A phagocytophilum* infection and exposure in feral cats in Massachusetts are higher than the previously reported average seroprevalence of *A phagocytophilum* in cats in the USA.[15] Recent studies have shown that anaplasmosis can cause clinical disease in cats and *A phagocytophilum* infection may be an emerging infectious disease in cats.[4–11] Further research is needed to determine the prevalence of clinical illness associated with *A phagocytophilum* infection in cats living in endemic areas.

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