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Prevalence of selected infectious disease agents in cats from Arizona

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The objective of this study was to use polymerase chain reaction (PCR) assays to determine the prevalence of *Ehrlichia* species, *Anaplasma phagocytophilum*, *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum' and *Bartonella* species from feral and relinquished cats in Phoenix and Nogales, Arizona. DNA from one or more of the organisms was amplified from 31 of 112 blood samples (27.7%). DNA consistent with *Bartonella clarridgeiae* 15 (13.4%), *Bartonella henselae* 14 (12.5%), '*Candidatus* M haemominutum' 9 (8.0%), and *M haemofelis* 5 (4.5%) were detected. DNA of *Ehrlichia* species, *Neorickettsia risticii*, or *A phagocytophilum* was not amplified. Failure to amplify DNA of *A phagocytophilum* may relate to the absence of appropriate tick vectors. Failure to amplify *Ehrlichia* species DNA suggests that cats were not exposed, exposed but not infected, or infected but the DNA was not detected by the PCR assay used in this study. The *Bartonella* species and hemoplasma results suggest flea control should be maintained.

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A number of different infectious diseases cause morbidity and mortality in the domestic feline population with some infectious agents being of zoonotic concern (Brown et al 2003). Flea and tick-associated diseases can be very important because the vectors may result in infection of other cats or people. *Ctenocephalides felis* commonly ingest *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum' (Woods et al 2005, Lappin et al, in press), *Bartonella henselae*, and *Bartonella clarridgeae* when taking a blood meal from infected cats (Chomel et al 1996, Foley et al 1998, Finkelstein et al 2002). Clinical signs in cats have been associated with both hemoplasmas (Tasker and Lappin 2002) and *B henselae* (O'Reilly et al 1996, Mikolajczyk and O'Reilly 2000). Additionally, fleas have been shown to transmit *B henselae* between cats and may be linked directly or indirectly with a number of human illnesses, including cat scratch disease (Higgins et al 1996, Foley et al 1998). Overall, blood from only small numbers of cats in the United States have been evaluated for hemoplasma DNA (Jensen et al 2001, Lappin et al 2004) and *Bartonella* species DNA (Jensen et al 2000) by PCR assay. Further information is needed concerning the prevalence of these infections in different regions of the United States.

Ticks have been associated with *Ehrlichia* species (Peavy et al 1997, Bjoersdorff et al 1999) and *Anaplasma phagocytophilum* (Lewis et al 1975, Gardner et al 2003, Lappin et al 2004) infections of humans and cats. While *Ehrlichia canis*-like DNA has been amplified from the blood of some cats, it is currently unknown whether the infective species was *E canis* or another closely related organism. Additional prevalence studies are needed to further define the importance of these infections in cats and to obtain DNA for further genetic characterization of the infective organisms. Overall, very few cats in the United States have been screened for *Ehrlichia* species, *N risticii* or *A phagocytophilum* DNA in blood by PCR assay (Breitschwert et al 2002, Lappin et al 2004, Luria et al 2004). Arizona has ideal conditions for the *Rhipicephalus sanguineous* tick vector of *E canis*; the seroprevalence rate in dogs was

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estimated at 12% in one study (Stephenson and Ristic 1978). Thus, we hypothesized that this would be a good region from which to identify additional cases of feline ehrlichiosis.

Most feral and relinquished cats either live or have access to the outdoors, eat varying diets, have exposure to ticks and fleas, have no arthropod prevention, and so may be more likely to be exposed to some infectious agents than pet cats (Luria et al 2004, Nutter et al 2004). For example, in a recent serologic study in North Carolina, feral cats were more likely than pet cats to be positive for *B henselae* and *Toxoplasma gondii* antibodies (Nutter et al 2004). Thus, we hypothesized that this group of cats would be optimal for identification of *Ehrlichia* species infections. In addition, further information concerning prevalence of infectious agents in feral cats is needed to assess the potential effects on human health and the domestic cat population. The objective of this study was to use PCR assays to determine the prevalence of *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', *Bartonella* species, *Ehrlichia* species, *N risticii*, and *A phagocytophilum* DNA in blood from feral and relinquished cats in Arizona.

Materials and methods

Blood (1.5 ml) was collected from feral and relinquished cats by veterinarians in Arizona, placed into 1.5 ml EDTA tubes, and stored at −20°C. Samples were collected between March 2004 and July 2004, times when environmental tick and flea loads are expected to be high in this region. Cats were not fully examined for the presence of ticks or fleas and so rates of ectoparasitism were not recorded. The samples were batched until shipped on cold packs by overnight express to Colorado State University. On arrival, the samples were stored at −20°C until assayed.

Samples were thawed to room temperature and prepared individually for PCR assays as previously described (Lappin et al 2004, Woods et al 2005). A multiplex PCR assay that amplifies the DNA of *Ehrlichia* species, *A phagocytophilum*, *Neorickettsia risticii*, *M haemofelis*, and '*Candidatus* Mycoplasma haemominutum' (Jensen et al 2001, Brewer et al 2005) and a separate PCR assay that amplifies the DNA of seven *Bartonella* species were used (Jensen et al 2000). The different hemoplasmas and *Bartonella* species give a different band size and so the results were also used to determine which species is present in the sample.

Results

Blood was submitted from 112 cats that were presumed by the participating veterinarians to be feral or relinquished. Fifty-seven were from a humane shelter in Nogales, five were from a veterinary clinic providing humane society support in Phoenix, and 50 samples were from a feral trap-neuter-return program in Phoenix. DNA from one or more of the organisms was amplified from 31 of 112 blood samples (27.7%). DNA consistent with *Bartonella clarridgeiae* (13.4%), *B henselae* (12.5%), '*Candidatus* M haemominutum' (8.0%), and *M haemofelis* (4.5%) were detected (Table 1). DNA of *Ehrlichia* species, *N risticii*, or *A phagocytophilum* was not amplified from the blood of any cat.

Discussion

Even though *E canis* is considered to be endemic in dogs in these regions of Arizona (Stephenson and Ristic 1978), we failed to amplify *Ehrlichia* species DNA from any of the cats in this study. This result may simply relate to the small sample size. However, other studies attempting to amplify *Ehrlichia* species DNA from cats in *E canis* endemic areas have had similar results (Luria et al 2004, Lappin et al 2005). For example, in a study of dogs and cats from the Quintana Roo region of Mexico, DNA was amplified and confirmed to be *E canis* through genetic sequencing in 26 of 92 dogs (28.3%) but 0 of 32 cats (Lappin et al 2005). We believe that these results suggest that cats are more resistant to *E canis* infections than dogs or interact differently with the tick vector. Most cats rapidly remove ticks when infested and so the minimal 24–48 h tick attachment time suspected to be required for transmission of most tick-transmitted infections may not be achieved (Kidd and Breitschwerdt 2003). It is also possible that cats have lower copy numbers of circulating *E canis* DNA than dogs resulting in false negative results. In a previous study, three cats with clinical ehrlichiosis had DNA consistent with *E canis* amplified by two different laboratories, but antibodies against *E canis* were not detected in either laboratory (Breitschwert et al 2002). These results suggested that there may be other organisms that were closely related to *E canis* that had their genes amplified, but are distinct enough to not induce cross-reacting antibodies. It is also possible that there are other closely related *Ehrlichia* species that infect cats that are not amplified by the PCR assay used here.

Table 1. Distribution of *Bartonella* species, *Mycoplasma haemofelis*, and '*Candidatus* Mycoplasma haemominutum' PCR assay results from the blood of feral or relinquished cats from Nogales and Phoenix Arizona

PCR assay result combinations	Cat ($n = 112$) # pos (%)
Mhf negative, Mhm negative, Bh negative, Bc negative	81 (72.3)
Any positive test result	31 (27.7)
Mhf negative, Mhm negative, Bh positive, Bc negative	7 (6.3)
Mhf negative, Mhm negative, Bh negative, Bc positive	8 (7.1)
Mhf negative, Mhm positive, Bh negative, Bc negative	5 (4.5)
Mhf negative, Mhm negative, Bh positive, Bc positive	4 (3.6)
Mhf negative, Mhm positive, Bh positive, Bc negative	0 (0)
Mhf positive, Mhm negative, Bh negative, Bc negative	1 (0.9)
Mhf positive, Mhm positive, Bh negative, Bc negative	2 (1.8)
Mhf positive, Mhm negative, Bh positive, Bc negative	1 (0.9)
Mhf positive, Mhm positive, Bh negative, Bc positive	1 (0.9)
Mhf positive, Mhm positive, Bh positive, Bc negative	0 (0)
Mhf negative, Mhm positive, Bh negative, Bc positive	1 (0.9)
Mhf negative, Mhm positive, Bh positive, Bc positive	1 (0.9)
Mhf positive, Mhm positive, Bh positive, Bc positive	0
Mhf positive, Mhm negative, Bh positive, Bc negative	0

Mhf = *Mycoplasma haemofelis*; Mhm = '*Candidatus* Mycoplasma haemominutum'; Bh = *Bartonella henselae*; Bc = *Bartonella clarridgeiae*.

Cats can be experimentally infected with *A phagocytophilum* and infection has been confirmed in client-owned cats by use of both molecular and serological assays (Lewis et al 1975, Lappin et al 2004). The failure to detect *A phagocytophilum* DNA in the cats described here likely relates to the small sample size or absence of the vector in the test region. The tick vector for *A phagocytophilum*, *Ixodes pacificus*, has been found on vegetation and lizards in the Hualapai Mountain Park, Mohave County, Arizona (Olson et al 1992). However, that region of Arizona is several hundred miles from the collection sites used in the current study and the cats tested were unlikely to have traveled. To our knowledge, *I pacificus* is not considered endemic to regions from which samples were collected in the current study. However, as discussed for *E canis*, it is also possible that cats remove the ticks before adequate time for transmission occurs. Experimental data suggest that ticks removed before 36 h of feeding time did not allow transmission of *A phagocytophilum* (previously known as the human granulocytic ehrlichial agent) to mice (Katavolos et al 1998, Des Vignes et al 2001).

The significance of *N risticii* in naturally infected cats has yet to be determined. *N risticii* morule were detected in two out of six cats intravenously inoculated in one study (Dawson et al 1998). One cat developed intermittent diarrhea and the other cat developed lymphadenopathy, acute depression, and anorexia. Currently, the mode of transmission for *N risticii* is unknown. However, DNA has been isolated in *Juga* species snails in both Ohio and California (Reubel et al 1998, Barlough et al 1998). It is unknown whether our failure to amplify *N ristcii* DNA was related to lack of exposure, resistance of cats to the infection, or the sensitivity of the PCR assay used.

Bartonella species PCR assays that use the 16S-23S rRNA intergenic region can also amplify *Mesorhizobium* species DNA resulting in a 420-bp amplicon (Maggi and Breitschwerdt 2005). None of the amplicons in this study were of that size and so we believe the results document presence of *Bartonella* species DNA in the positive samples. In a previous study of cats and fleas completed in our laboratory, all amplicons in the size range of *B henselae* or *B clarridgeiae* were confirmed to be the expected species on genetic sequencing (Lappin et al, in press). The DNA amplification rates for *B clarridgeiae* (13.4%), *B henselae* (12.5%), '*Candidatus* M haemominutum' (8.0%), and *M haemofelis* (4.5%) in the cats of this study were similar to other studies of feral cats. In feral cats in Florida, the DNA amplication rates for '*Candidatus* M haemominutum' and *M haemofelis* were 12.2% and 8.3%, respectively (Luria et al 2004). In feral cats from Mexico, the DNA amplification rates for *B henselae*, *B clarridgeiae*, '*Candidatus* M haemominutum', and *M haemofelis*, were 25%, 12.5%, 12.5%,

and 6.3%, respectively (Lappin et al 2005). However, each of these infections is also common in client-owned cats. For example, in a recent study of client-owned cats with fleas, the DNA amplification rates for *B henselae*, *B clarridgeiae*, '*Candidatus* M haemominutum', and *M haemofelis*, were 34.8%, 20.7%, 21.7%, and 7.6%, respectively (Lappin et al, in press). Unfortunately, there have been few studies to directly compare prevalence rates of these infectious agents in feral cats and client-owned cats in the same environment. In a recent serological study, seroprevalence rates for *B henselae* antibodies were greater in feral (93%) than in client-owned cats (75%) (Nutter et al 2004). However, it cannot be determined from the results of these studies whether presence of feral cats impacts the health of client-owned cats or people and it is likely that prevalence rates vary based on the effects of environment on flea biology. *C felis* are very susceptible to environmental factors such as a relative humidity below <50% and temperatures over 95°C (Silverman et al 1981). Because each of these organisms is flea-associated, flea control should be administered to as many cats as possible.

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