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Prevalence of *Bartonella* species, haemoplasmas and *Toxoplasma gondii* in cats in Scotland

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The objective of this study was to determine the prevalence rates for select infectious agents of cats presented to the Royal (Dick) School of Veterinary Studies at the University of Edinburgh, Scotland. Whole blood, serum, and oral mucosal and nail bed swabs were collected. While *Ehrlichia* species, *Anaplasma* species or *Rickettsia felis* DNA were not amplified from any cat, 44.2% of the cats had evidence of infection or exposure to either a *Bartonella* species (15.3% were seropositive and 5.8% polymerase chain reaction (PCR) positive), a haemoplasma (28.6% PCR positive), and/or *Toxoplasma gondii* (19.2% seropositive). No *Bartonella* species DNA was amplified from the nail or oral mucosal swabs despite a 5.8% amplification rate from the blood samples. This finding likely reflects the absence of *Ctenocephalides felis* infection from our study population, as this organism is a key component for *Bartonella* species translocation in cats. The results from this study support the use of flea control products to lessen exposure of cats (and people) to *Bartonella* species and support discouraging the feeding of raw meat to cats and preventing them from hunting to lessen *T gondii* infection.

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There are many infectious agents that can cause morbidity and mortality in cats. Several organisms that infect cats are also zoonotic, for example, the flea-borne pathogens *Bartonella* species and *Rickettsia felis* and the coccidian parasite *Toxoplasma gondii*. In addition, the feline haemoplasmas, *Ehrlichia* species, and *Anaplasma phagocytophilum* are known to infect cats and are potentially important causes of disease in this species.[1]

Cats are the only known definitive host for *T gondii* and they perpetuate the organism in the environment by shedding oocysts. Transplacental infection of human fetuses as well as systemic infection of immunocompromised people can result in significant illness in humans. While *T gondii* seropositive cats have been detected in the United Kingdom,[2,3] the current seroprevalence rates in cats in Scotland are unknown.

Ctenocephalides felis carry haemoplasmas, several *Bartonella* species and *R felis*.[4] Cats are the reservoir for several *Bartonella* species that are associated with illness in people including *Bartonella henselae*, *Bartonella clarridgeaie* and *Bartonella koehlerae*.[5] Disease manifestations in people are diverse and include cat scratch disease, bacillary peliosis, bacillary angiomatosis and a variety of other chronic illnesses.[6,7] *Rickettsia felis* can induce a mild spotted fever syndrome in humans.[8] The haemotropic mycoplasmas (*Mycoplasma haemofelis*, '*Candidatus* M haemominutum', and '*Candidatus* M turicensis') have been detected in cats of the UK.[9] While *C felis* is known to exist in the UK and has been shown to carry *R felis* and *B henselae*,[4] it is currently unknown how many cats in Scotland have been exposed to or are carriers of these agents.

The tick-borne infectious agents, *Ehrlichia* species, *A phagocytophilum* and *R felis* have been documented in cats from other countries in western Europe.[10] *Ixodes* species ticks are the vectors of *A phagocytophilum*, and these ticks have been documented in the UK; in addition, the vole may be a competent reservoir for *A phagocytophilum*.[10] Whether these agents are common in cats in Scotland is currently unknown.

Determination of prevalence rates for these infectious agents in cats living in Scotland would be useful for predicting the likelihood of infection in cats presenting with appropriate clinical signs as well as for assessing for potential zoonotic risks. Furthermore, there is little information available concerning numbers of naturally exposed cats that have the DNA of these organisms residing on the nail beds and gingival

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mucosa. Data on this are important as these are likely means of transmission of infectious disease between cats and to the human population. Lastly, with the increased use of therapeutic blood products in cats, determining the regional occurrence of these blood-borne agents could be useful in the development of optimal screening protocols.[11–13] The aim of this prospective study was to determine the prevalence of *Bartonella* species, *R felis,* haemoplasmas, *Ehrlichia* species, *A phagocytophilum* and *T gondii* from companion and shelter cats residing in Scotland.

Materials and methods

Cats

Samples were collected from cats presenting to the Hospital for Small Animals at the Royal (Dick) School of Veterinary Studies (RDSVS) between June and August 2009. Blood was being collected for routine investigation of clinical disease or, in the case of stray cats from charity organisations, for health screening prior to re-homing. Information regarding the use of a flea and tick preventative was gathered from each owner, or the shelter supervisor, prior to sample collection. Informed owner consent was obtained in all cases and the study was approved by the Veterinary Ethics and Research Committee of the RDSVS.

Samples

Blood samples were obtained by jugular venepuncture and once aliquots were removed for their primary analyses, any remaining blood was placed in serum separator and/or EDTA KE/2-containing plastic tubes. Samples from the oral mucosa and nail beds were collected using sterile cotton-tipped bacterial culture swabs. The swabs were soaked in 200 µl of phosphate buffered saline solution before collection. Swabs were rubbed vigorously on the base of the right maxillary canine tooth and from the nail beds of digits 3 and 4 of the right forepaw.[5] All samples were stored at −20°C until being shipped on ice packs to Colorado State University. Upon arrival the samples were stored at −70°C until assayed.

Assays

After being thawed at room temperature, total DNA was extracted from swabs and blood samples and prepared for polymerase chain reaction (PCR) assays using commercially available reagents as previously described.[5] PCR assays that amplify the DNA of *Ehrlichia*, *Neorickettsia*, and *Anaplasma* species, *Rickettsia* species, haemoplasmas, the DNA of six *Bartonella* species, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (DNA control) were performed on each extract.[1,13–16] Samples negative for GAPDH were excluded from the analysis. Positive and negative controls were assessed with all PCR assays using the previously published assays and standard operating procedures of the Center for Companion Animal Studies (http://csuvets.colostate.edu/companion/index.htm).

Most of the *Bartonella* species give a different amplicon size and these sizes are used to determine the species present in the sample. However, *B henselae* and *B koehlerae* DNA are within the same amplicon and so all positive samples were confirmed by genetic sequencing using a commercially available service (Macromolecular Resources, Colorado State University, Fort Collins, CO 80523). In the haemoplasma PCR assay, DNA of both *M haemofelis* and '*Candidatus* M turicensis' are within the same amplicon and so when sufficient DNA was available these amplicons were sequenced to determine the infective species.

Bartonella species antibody titres were determined by enzyme-linked immunosorbent assay (ELISA) as previously described.[5] Titres equal to or greater than 1:64 were considered seroreactive. For *T gondii*, IgM and IgG antibodies were determined by ELISA as previously described.[17] For both IgM and IgG, antibody titres equal to or greater than 1:64 were considered seroreactive.

Statistical analysis

Fisher's exact test was used to compare prevalence rates between some groups of results. Statistical significance was defined as $P < 0.05$.

Results

A total of 78 cats were entered into the study. Of the cats, 61 were client-owned and 17 were from rescue centres; 40 were clinically ill and 38 were considered healthy. Disease manifestations varied and were not specific for any infectious agent associated syndrome. The age ranges for the cats were <1 (three cats); 1−3 years (22 cats), 4−6 years (14 cats), 7−9 (12 cats), 10−12 (11 cats), >13 (16 cats). The study population included intact male cats (three), neutered male cats (42), spayed female cats (27), and intact female cats (six). Of the cats, 23 (29.5%) were currently on a flea or tick preventative at the time of sample collection. Fleas or flea dirt were not found on any of the cats on physical examination. All samples were not available from all sites in every case. This varied based on whether remnant blood samples were available (blood and serum) and on the temperament of the cat (mouth and nail bed swabs).

DNA of *Ehrlichia* species, *A phagocytophilum* or *R felis* were not amplified from any sample.

Bartonella species antibodies were detected in 8/52 sera (15.3%) and *Bartonella* species DNA was amplified from 3/52 blood samples (5.8%). All *Bartonella* species PCR positive cats were seropositive and each of the species was *B henselae*. None of the PCR positive or seropositive cats in this group were on a flea preventative. *Bartonella* species DNA was not amplified from oral

mucosal ($n = 71$) or nail bed swabs ($n = 71$) from any of the cats in the study, including the three cats that were positive for *Bartonella henselae* DNA in blood.

The haemoplasma PCR assay was performed on 63 oral mucosal swabs, nail bed swabs and blood samples. None of the nail bed swabs were positive for haemoplasma DNA. DNA of one or more haemoplasmas was amplified from 9/63 (14.3%) of the blood samples. '*Candidatus* M haemominutum' DNA was the only haemoplasma amplified from the blood of six cats. DNA of '*Candidatus* M haemominutum' and *M haemofelis*/*Candidatus* M turicensis were amplified concurrently from the blood of two cats; one of these amplicons was confirmed as *M haemofelis* by sequencing and the other amplicon could not be sequenced.

From oral mucosal swabs, five cats were positive for the DNA of one or more haemoplasma. DNA of *M haemofelis* alone was amplified from one cat and DNA of both *M haemofelis* and '*Candidatus* M haemominutum' were amplified from three cats. Sequencing from one sample that was PCR positive for both revealed *M haemofelis* 16S rRNA gene and '*Candidatus* M haemominutum' strain IT23 with 80% and 82% homology, respectively. There was insufficient DNA available for sequencing the remaining four PCR positive samples. Of the five oral swabs that were PCR positive for either *M haemofelis* or '*Candidatus* M haemominutum', PCR on the blood was found to be positive (two cats), negative (two cats) or not available (one cat).

The proportions of cats positive for haemoplasma DNA were compared between client-owned and shelter cats (Table 1) and between normal and clinically ill cats (Table 2). Shelter cats were more likely than client-owned cats to have the DNA of any haemoplasma in blood ($P = 0.028$), *M haemofelis* DNA alone in the blood ($P = 0.023$), and DNA of both *M haemofelis* and '*Candidatus* M haemominutum' from oral swab samples ($P = 0.044$). Healthy cats were more likely than clinically ill cats to have DNA of any haemoplasma species in the blood ($P = 0.031$).

Overall, 10/52 cats (19.2%) were seropositive for *T gondii* species in serum. IgG alone (five cats), IgM alone (four cats), or both antibodies (one cat) were detected.

Discussion

All serological tests and PCR assays were performed on blood or serum samples from 52 cats, of which 23 (44.2%) were positive in one or more infectious agents. The high (44%) prevalence of one or more infectious agents indicate that haemoplasmas, *Bartonella* species, and *T gondii* infections should be on the differential list for cats with appropriate clinical signs of disease. While we compared prevalence rates between cats with and without clinical illness, these results should be interpreted cautiously as the sample set is very small and the clinical diseases reported were not necessarily consistent with those associated with haemoplasmas, *Bartonella* species and *T gondii*. The failure to amplify *Ehrlichia* species, *Anaplasma* species or *R felis* DNA from samples from these cats may merely reflect the small sample size and larger numbers of samples from cats with appropriate clinical findings (fever, thrombocytopenia, polyarthritis) should be performed.

While *Bartonella* species DNA has previously been amplified from *C felis* collected in the UK,[4] this is the first report of *Bartonella* species infections in cats from Scotland. *Bartonella henselae* DNA was the only *Bartonella* species amplified from the three positive blood samples. However, additional cats were seropositive (15.3%) and the *Bartonella* species ELISA used can cross react with *B clarridgeaie* and *B koehlerae* antibodies. Further samples will need to be assessed by PCR to determine whether other *Bartonella* species exist in cats in Scotland.

Table 1. Select infectious agent test results stratified by the source of cats in Scotland.

	Total positive	Shelter	Client	*P* value
Any *Bartonella* species test positive	8/52 (15.4%)	2/10 (20%)	6/42 (14.3%)	0.6415
Bartonella species serology	8/52 (15.4%)	2/10 (20%)	6/42 (14.3%)	0.6415
B henselae PCR	3/52 (5.8%)	0/10 (0%)	3/42 (7.1%)	0.6226
T gondii serology	10/52 (19.2%)	2/10 (20%)	8/42 (19.0%)	1
Any haemoplasma in blood	9/63 (14.3%)	4/10 (40%)	5/53 (9.4%)	0.0288
Mhm alone	7/63 (11.1%)	2/10 (20%)	5/53 (9.4%)	0.3065
Mhf alone	2/63 (3.2%)	2/10 (20%)	0/53 (0%)	0.023
Any haemoplasma swab	5/60 (7.0%)	3/15 (20%)	2/45 (4.4%)	0.0943
Mhf alone	1/60 (1.4%)	0/15 (0%)	1/45 (2.2%)	1
Mhf and Mhm	4/60 (5.6%)	3/15 (20%)	1/45 (2.2%)	0.0448

Mhf = *Mycoplasma haemofelis*; Mhm = '*Candidatus* M haemominutum'; swab = DNA collected from the base of the canine tooth or claw bed.

Table 2. Select infectious agent test results stratified by the clinical status of the cats evaluated in Scotland.

	Total positive	Healthy	Clinically ill	*P* value
Any *Bartonella* species test positive	8/52 (15.4%)	3/26 (11.5%)	5/26 (19.2%)	0.703
Bartonella species serology	8/52 (15.4%)	3/26 (11.5%)	5/26 (19.2%)	0.703
B henselae PCR	3/52 (5.8%)	0/26 (0%)	3/26 (11.5%)	0.238
T gondii serology	10/52 (19.2%)	5/26 (19.2%)	5/26 (19.2%)	1
Any haemoplasma in blood	9/63 (14.3%)	7/27 (25.9%)	2/36 (5.5%)	0.0312
Mhm alone	7/63 (11.1%)	5/27 (18.5%)	2/36 (5.5%)	0.1279
Mhf alone	2/63 (3.2%)	2/27 (7.4%)	0/36 (0%)	0.1797
Any haemoplasma swab	5/60 (7.0%)	5/35 (14.2%)	0/25 (0%)	0.1474
Mhf alone	1/60 (1.4%)	1/35 (2.8%)	0/25 (0%)	1
Mhf and Mhm	4/60 (5.6%)	4/35 (11.4%)	0/25 (0%)	0.1333

Mhf = *Mycoplasma haemofelis*; Mhm = '*Candidatus* M haemominutum'; swab = DNA collected from the base of the canine tooth or claw bed.

Bartonella species DNA was not amplified from the nail bed or oral mucosa swabs from the cats described in this study. This finding may reflect the use of flea control products in some of the cats (29.5%) and the lack of evidence of current flea infestation in all cats. In contrast, a similar study evaluating feral cats residing in high *C felis* infestation areas in the United States, showed that 17.6% of 51 cats sampled had *Bartonella* species DNA amplified from nail bed or oral mucosal samples.[5] *Bartonella* species has been documented in *C felis* flea dirt and survives for days after passage from the flea.[18,19] *Bartonella* species DNA was not amplified from the oral mucosa of any cat in the current study which is likely to reflect the absence of oral cavity disease and the lack of fleas or flea dirt which can contaminate the oral cavity during grooming. It was recently shown that administration of imidacloprid blocks transmission of *B henselae* amongst cats.[20] As cats without fleas are unlikely to be sources of *Bartonella* species infections for humans, the Centers for Disease Control recommends avoiding cats bites and scratches and encourages the use of flea control products rather than testing or treating healthy cats for *Bartonella* species infections.[21] The *Bartonella* species prevalence rates described here suggest that use of flea control products is indicated in cats in Scotland.

The prevalence rates for haemoplasma species DNA in blood were similar to other studies in the region.[22] The failure to amplify '*Candidatus* M turicensis' DNA may merely reflect the small sample size. In previous studies of experimentally infected or naturally exposed cats, haemoplasma species DNA has been amplified from fleas, flea dirt, oral mucosal swabs, saliva, and nail bed swabs.[1,5,23–26] It has been documented that some haemoplasmas can be transmitted directly between cats.[25] Amplification of haemoplasma DNA from the oral mucosa of some cats described here supports the findings of that study. The failure to amplify haemoplasma DNA from nail beds in the cats in the current study probably reflects the absence of fleas or flea dirt as discussed for *Bartonella* species.

With increasing frequency, blood transfusions are given to anaemic and bleeding feline patients.[11,13,27] Overall, 3/52 (5.8%) and 9/63 (14.3%) of cats in this study were positive in blood for *Bartonella* species or haemoplasma species DNA, respectively. These results suggest that cats used as blood donors in Scotland should be maintained on flea control products and should be screened for haemoplasma species and *Bartonella* species infections by PCR before use.[12]

Toxoplasma gondii infections have previously been reported in cats living in Scotland.[2,28] Prevalence rates for *T gondii* antibodies were similar to others that studied cats in similar climates.[17] In accordance with public health guidelines, limiting hunting and the feeding of raw meat to cats may indirectly lessen the human risk of exposure to this infectious agent.[21]

The results from this study support the use of flea control products to lessen exposure of cats (and potentially, people) to *Bartonella* species and they support the recommendations to discourage the feeding of raw meat to cats and to prevent them from hunting so that the risk of them becoming infected with *T gondii* is reduced.

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