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Prevalence of selected infectious agents in cats in Ireland

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Vector-borne bacterial and rickettsial agents and *Toxoplasma gondii*, are common organisms in cats. Some are potentially zoonotic or may be transmitted via blood transfusion. The current study investigated the prevalence of these agents in cats from Dublin, Ireland, for which no published data exists. Whole blood ($n = 116$) and sera ($n = 83$) samples were obtained from 121 cats. DNA was extracted from blood and assayed using polymerase chain reaction techniques for *Anaplasma* species, *Bartonella* species, *Ehrlichia* species, *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', '*Candidatus* Mycoplasma turicensis' and *Rickettsia* species. IgG and *T gondii* IgG and IgM serum antibodies were detected by enzyme-linked immunosorbent assay. DNA consistent with *B henselae* (3.4%), *B clarridgeiae* (0.8%), both *Bartonella* species (0.8%), *C* M haemominutum (12.9%), or *M haemofelis* (2.5%) was amplified from 24/116 blood samples (20.6%). Antibodies to *T gondii* and *Bartonella* species were detected in 28 (33.7%) and 22 (26.5%) of 83 sera, respectively.

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A number of infectious agents are important, not only because of the diseases they induce in cats but also because they may be potentially zoonotic or may be carried silently and transmitted to other cats through blood transfusion. The most important zoonotic agents in cats are the enteric coccidian parasite *Toxoplasma gondii*, the vector-borne bacteria *Bartonella henselae* and *clarridgeiae* and the emergent vector-borne *Rickettsia felis*.[1] Recommendations from the United States suggest that blood for transfusion in cats should be screened for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) and haemoplasmas (*Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', and '*Candidatus* Mycoplasma turicensis') by polymerase chain reaction (PCR).[2–4] For *Bartonella* species, *Cytauxzoon felis* and select rickettsial agents (*Ehrlichia* species, *Anaplasma* species, and *Neorickettsia helminthoeca*), testing is considered conditional but no recommendations for *R felis* are given.[2–4]

Cats are the definitive hosts for *T gondii* and toxoplasmosis is a zoonotic disease in people. Toxoplasmosis can cause significant disease in both species. Although transmission can occur following ingestion of undercooked meat, oocysts passed into the environment from infected cats are a significant source of infection for humans.

R felis is a spotted fever organism known to infect cats and induce seroconversion.[75] The organism is common in *Ctenocephalides felis* and has been detected in fleas from neighbouring United Kingdom.[5,6] It is unknown if this agent infects cats in Ireland.

Cats and *C felis* are also considered important for transmission of *B clarridgeiae* and *B henselae* to humans.[7] The latter is the more pathogenic of the two and causes a variety of human illnesses ranging from cat scratch disease to death in immunocompromised patients. It has a worldwide distribution in domestic cats but with varying antibody (5–80%) and infection (up to 50%) prevalence rates according to geographical location, status (pet or stray), and the assays used.[8]

Healthy carriage of *Bartonella* species is common in cats, although experimentally, infection has been associated with a variety of mild and transient signs and occasionally myalgia, central nervous system disease, chronic and acute uveitis and reproductive failure.[9,10]

In cats, *M haemofelis* is considered a significant primary pathogen with a prevalence of between 1.4 and 14.5% depending on geographic location, cat population, and assays used.[11–16] Usually, *C* M haemominutum is a complicating factor of infection with

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retroviruses and other debilitating diseases and has a prevalence up to 50% in cats co-infected with FIV and FeLV.[17] However, some clinically ill cats with *C* M haemominutum infection have had no identifiable cofactors.[18] Of intermediate pathogenicity compared to the other two, *C* M turincensis is known to occur in some parts of Europe, the USA, South Africa, Australia,[19–21] and Japan.[22]

Ehrlichia canis and *E canis*-like organisms has been amplified from the blood of cats in the USA albeit rarely.[23–25] There have also been cases confirmed by PCR or the presence of morulae in mononuclear cells in other countries.[26–29] In Western Europe, antibodies that react to *E canis* morulae have been detected in France,[26] Italy[30] and Spain.[31–34]

Anaplasma phagocytophilum (previously *Ehrlichia equi*) has similarly been documented in cats from the USA,[25] the UK,[35] Sweden,[36] Ireland,[37] Switzerland,[38] and Italy.[39] Other studies in Western Europe have shown seroprevalence rates between 7.7 and 13.5%.[32,40] Routes of transmission for naturally infected cats are largely unknown but thought to be similar to dogs.[41] The vectors (*Ixodes* species) are common in Europe.

While some of these infections are thought to be rare or unknown in Ireland, the sample sizes screened have been small and there is widespread concern that reduced restrictions on pet movement into Ireland increases the risk of such infections. In addition, with advances in therapeutics, administration of blood/blood products is becoming more common. As a consequence, data on prevalence of these organisms in cats would provide useful information on the likelihood of infection in patients presenting with clinical signs consistent with the disease and on the risks associated with blood donation or transmission to humans. The purpose of this prospective study was to investigate the prevalence of a selected group of vector-borne organisms and *T gondii* in cats from Dublin, Ireland, a region for which no published data yet exists.

Materials and methods

The sample population was cats living in or within 50 km of Dublin between January and May 2008. The cats were being bled for routine investigation of clinical disease, as part of a programme for elective blood typing or in the case of stray cats from charity organisations, for health screening purposes prior to re-homing. Informed owner consent was obtained in all cases. 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 Ethylene diamine tetraacetic acid (EDTA) KE/1.3-containing plastic tubes.

Within 24 h of sample collection, a complete blood cell count was performed for each cat using an ADVIA 2120 system (Siemens Medical solutions Diagnostic/Germany). Blood and, following separation, serum was stored at −20°C until shipped on dry ice to Colorado State University. On arrival samples were stored at −20°C until assayed. After being thawed at room temperature, blood was prepared for PCR assays as previously described.[42] PCR assays that amplify the DNA of *Ehrlichia*, *Neorickettsia*, and *Anaplasma* species,[25] *Rickettsia* species,[43] *M haemofelis*, *C* M haemominutum, and *C* M turicensis[44–46] and a PCR assay that amplifies the DNA of six *Bartonella* species[47] were used. The differing size of the PCR product from each bartonella assay was used to determine the species present in the sample. Both *M haemofelis* and *C* M turicensis are amplified within the same band size and when sufficient DNA was available these amplicons were sequenced to determine the infective species. Appropriate positive and negative controls were included in all assays.

For *T gondii* serology, IgM and IgG antibodies were determined by microtitre plate enzyme-linked immunosorbent assay (ELISA) as previously described.[48] For both IgM and IgG, antibody titres equal to or greater than 1:64 were considered seroreactive. *Bartonella* species IgG antibody titres were determined by ELISA as previously described.[49] Titres equal to or greater than 1:64 were considered seroreactive.

All statistical analyses were performed by computerised software (GraphPad Prism version 5.02) with significance defined as $P < 0.05$. Data was analysed for normality using Kolmogorov–Smirnov test. Age differences were assessed by Mann Whitney testing. Categorical data were analysed by χ^2 or Fisher's exact test as appropriate.

Results

Samples were collected from 121 cats including 116 whole blood samples from 41 client-owned and 75 stray cats and 83 serum samples from 22 client-owned and 61 stray cats. For 80 cats both whole blood and serum were available. The group consisted of 54 males (53 neutered, one entire) and 67 females (56 neutered and 11 entire). The median age was 4.0 years (range 0.3–15.0 years).

Of the 121 cats, 21 (17.4%) lived indoors and 100 (82.6%) had free outdoor access. The majority ($n = 106$, 87.6%) was clinically healthy while the remainder ($n = 15$, 12.4%) presented with various diseases (hyperthyroidism ($n = 4$), neurological disease ($n = 2$), respiratory disease ($n = 2$), and one of each of vitamin D-dependent rickets type II, feline lower urinary tract disease (FLUTD), liver disease, anaemia, oesophagitis, feline infectious peritonitis and a neoplastic intestinal mass).

Results from samples evaluated for DNA of *Ehrlichia* species, *R felis*, haemoplasmas and *Bartonella* species are presented in Table 1. DNA from an infectious agent was amplified from 24 (20.6%) of 116 samples. The median (range) age of the positive cats was 4.8 (2.0–13.0) years, which was not significantly

Table 1. Distribution of PCR assay results for DNA of haemoplasma and *Bartonella* species collected from 116 cats in Ireland.

DNA amplification	All cats ($n = 116$)	Subgroup of cats	
		Stray ($n = 75$)	Client owned ($n = 41$)
Either of the haemoplasmas	19 (16.4%)	13 (17.3%)	6 (14.6%)
Either of *Bartonella* species	6 (5.2%)	6 (8%)	0
Subgroups*			
M haemofelis alone*	1 (0.9%)	1 (1.3%)	0
M haemofelis or *C* M turicensis	2 (1.7%)	1 (1.3%)	1 (2.4%)
C M haemominutum alone	15 (12.9%)	10 (13.3%)	5 (12.2%)
C M haemominutum and *B henselae*	1 (0.9%)	1 (1.3%)	0
B henselae alone	3 (2.6%)	3 (4%)	0
B clarridgeiae alone	1 (0.9%)	1 (1.3%)	0
B henselae and *B clarridgeiae*	1(0.9%)	1 (1.3%)	0

*Adequate DNA for sequencing was only available for one cat with *M haemofelis*/*C* M turicensis DNA and it was *M haemofelis*.

different from the median age of the negative cats (4.0 (0.3−15.0) years). Infection prevalence was significantly higher ($P = 0.04$) in male (15/50, 30%) compared with female (9/66, 13.6%) cats. All cats from which *Bartonella* species DNA was amplified were strays. Adequate DNA for sequencing was only available for one cat with an amplicon consistent with *M haemofelis* or *C* M turicensis and for that cat the agent was *M haemofelis*. The prevalence rates of *M haemofelis*/*C* M turicensis and *C* M haemominutum were not significantly different in client-owned cats (respectively, 1/41, 2.4% and 5/41, 12.2%) compared with stray cats (respectively, 2/75, 2.7% and 10/75, 13.3%). Haemoplasmas were isolated more frequently in male cats than in female cats (11/19, 57.9% and 8/19, 42.1%, respectively). *C* M haemominutum in particular was more frequent in male cats than female cats (11/16, 68.8% and 5/16, 31.2%). However, these differences were not statistically significant. One cat positive for *C* M haemominutum had a packed cell volume of 0.17 l/l but the anaemia was non-regenerative and the cat also suffered from a neoplastic pleural effusion. *Ehrlichia* species, *Anaplasma* species, *Neorickettsia* species or *Rickettsia* species was not amplified. The mean haematocrit standard deviation (SD) of the cats positive for either mycoplasma species (0.40 l/l (0.061)) was not statistically different from the haematocrit of those that were negative (0.41 (0.065)).

Results for samples evaluated for *T gondii* and *Bartonella* species serological assays are presented in Table 2. Antibodies to *T gondii* and/or *Bartonella* species were detected in 40 (48.2%) of 83 samples analysed. The median (range) age of the seropositive cats was 4.0 (0.7−13.0) years, which was not statistically different than the seronegative cats (4.0 (0.3−15) years). The prevalence of seropositive cats was not significantly different in male (14/35, 40%) compared with female cats (26/48, 54.2%). Prevalence of antibodies to any agent of interest was not significantly different between stray (33/61, 54%) and client-owned cats (7/22, 31.8%).

There was no statistical difference in the prevalence of *T gondii* seroreactivity between client-owned (6/22, 27.2%) and stray (22/61, 36.1%) cats. In indoor cats ($n = 8$) and those with restricted garden access ($n = 8$) the prevalence of *T gondii* (2/16, 12.5%) was lower than in cats with free outdoor access (26/67, 38.8%). However, this was not statistically significant ($P = 0.07$). There was no statistical difference in age or sex between positive and negative cats.

The prevalence of *Bartonella* species seroreactivity was significantly higher ($P = 0.04$) in stray (20/61, 32.8%) compared with client-owned cats (2/22, 9%). There was no statistical difference in age or sex between stray and client-owned cats seropositive for *Bartonella* species. Prevalence of DNA of infectious agents was significantly higher ($P = 0.03$) in sick (6/13, 46.1%) than in healthy cats (18/103, 17.4%). Positive sick cats suffered from various diseases (two hyperthyroid, two with neurological disease and one each of FLUTD and respiratory disease). The highest prevalence of *T gondii* was found in sick (4/7, 57.1%) versus healthy (24/76, 31.6%) cats. However, this was not statistically significant. Only one of the cats seropositive for *Bartonella* species was sick.

Discussion

Results of this study show that the DNA of *M haemofelis*, *C* M haemominutum, *B henselae* and *B clarridgeiae* can be amplified from the blood of both stray and client-owned cats in the Dublin area of Ireland. By contrast, DNA from *Ehrlichia*, *Anaplasma* species, *Neorickettsia* species or *Rickettsia* species was not amplified from any cat in the present study.

Table 2. Results of the *Toxoplasma gondii* and *Bartonella* species serologies.

Antibodies	All cats ($n = 83$)	Subgroup of cats	
		Stray ($n = 61$)	Client owned ($n = 22$)
T gondii IgM or IgG	28 (33.7%)	22 (36.1%)	6 (27.3%)
T gondii IgM	10 (12%)	9 (14.8%)	1 (4.5%)
T gondii IgG	12 (14.5%)	8 (13.1%)	4 (18.2%)
T gondii IgG and IgM	6 (7.2%)	5 (8.2%)	1 (4.5%)
Bartonella species	22 (26.5%)	20 (32.8%)	2 (9.1%)
T gondii and *Bartonella* species	10 (12%)	9 (14.8%)	1 (4.5%)

The prevalence of *M haemofelis* and *C* M haemominutum was similar to that previously reported in other areas: 8.3% and 12.2% in Florida,[14] 4.5% and 8% in Arizona,[50] 3.4% and 7.6% in a population of cats used as blood donors in the USA,[2] 4.1% and 23.1% in Australia[51] and 2.8% and 11.2% in a UK laboratory survey,[16] respectively. The prevalence of haemoplasmas was similar regardless of the status of the animal (stray or client owned) and was higher in male than in female cats, although this was not statistically significant. This finding is in accordance with previous studies showing both male cats and those with a history of cat bite abscess had a higher prevalence of infection.[13,14,20,21] Direct transmission from cat to cat is now thought likely[52] as *C* M haemominutum has been isolated in salivary tissue and saliva from cats.[53] Previously, blood sucking arthropods were implicated in the transmission based on multiple reports demonstrating the presence of haemoplasmas in ticks or fleas.[6,15,42] However, other studies have failed to demonstrate transmission via this route.[42,54] Other modes of transmission are from queens to their newborn offspring in utero, during parturition or nursing and via blood transfusion. To the authors' knowledge, none of the cats in the present study had received a blood transfusion. Only one of the cats carrying DNA of haemoplasmas was anaemic. However, in this cat the anaemia was non-regenerative and thought to be a result of another underlying disease process. Therefore, all of the cats in this study were considered asymptomatic carriers of haemoplasmas. The frequency with which it was diagnosed suggests that all feline blood donors in Dublin and, therefore, presumably Ireland should be tested for haemoplasmas. In Ireland, *C* M turicensis has not been reported and was not amplified from the cats in the present study. However, DNA from only 1/3 cats positive for *M haemofelis* could be sequenced to confirm the species. Further studies are required to determine the prevalence of *C* M turicensis in Ireland.

The prevalence of *Bartonella* species antibodies was comparable to other studies conducted in Spain (29.6%)[55] or in the Netherlands (50%).[56] Prevalence can be variable as it is higher in warm humid regions such as South East of the USA (54.6%), Hawaii (47.4%) than in cold dry regions, such as Alaska (5.0%), the Rocky Mountain region (3.7%)[57] or Norway (0%).[58] Serum antibodies against *Bartonella* species can be present for months to years after bacteraemia has resolved, therefore, positive test results do not necessarily prove current infection, explaining the discrepancy between the seroprevalence of *B henselae* and/or *clarridgeiae* detected by PCR and the antibody prevalence demonstrated by the serological test. The prevalence of *Bartonella* species bacteraemia was low (5.2%) and *Bartonella* species was not amplified by PCR from any client-owned cats. The antibody prevalence to *Bartonella* species was significantly higher in stray than in client-owned cats as previously reported.[59] This may relate to an increased likelihood of stray cats being exposed to fleas. The relatively low prevalence in cats suggests that the risk of human infection associated with cat ownership in Ireland is minimal. However, it remains present and, therefore, worthy of further consideration. Flea prevention is important in limiting transmission between cats and from cats to humans via flea faeces. It was recently documented that the monthly application of imidacloprid—moxidectin blocked flea-borne transmission of *B henselae* amongst experimentally inoculated cats.[60] Although *Bartonella* species infection usually causes mild and self-limiting signs in healthy immunocompetent cats, the effect of transfusing bacteraemic blood to a diseased, already weakened cat has not been studied. Therefore, caution should be exerted and testing cats in Ireland for *Bartonella* species infection prior to blood donation should be considered, particularly if the cat was known to be flea infested, until further data is available.

The prevalence of *T gondii* was higher in animals with free access to outdoors. Although it was not significant, such a trend may reflect that these animals are more likely to hunt and ingest intermediate hosts and is in agreement with previously published studies.[61,62] It would have been interesting to study whether the prevalence was linked to being fed raw meat by the owners. Unfortunately, this data was not available for the present study. The prevalence reported in this study is similar to previous studies ranging from 31.6% in the USA,[63] 25.2% in China,[64] 27% in Belgium,[65] 45% in Spain,[66] to 47.6% in

Hungary.[67] In Japan,[68] the prevalence reported was low at 5.4% in 2003. This contrasted with a prevalence of 73% in 1972. No explanations were proposed for these discrepancies. The results presented here emphasise that *T gondii* infection in cats is relatively common in Ireland and that people should take appropriate care when exposed to feline faeces (gardening) as well as undercooked meats.[69]

There were several reasons for attempting to amplify DNA of *Ehrlichia* species and *Anaplasma* species in cats from Ireland. Firstly, clinical disease has been recently reported in cats elsewhere in the world.[23,24,28,32,33,38] Although canine and feline ehrlichiosis has never previously been considered endemic in Ireland, climate changes and abolition of quarantine potentially increase the risk of vector-borne diseases in countries previously exempt. The transmission mode of *Ehrlichia* species to cats is unknown, although it is suspected to be, as in other species, tick-borne.[41] In cats infected with *A phagocytophilum*, ticks of the genus *Ixodes* have been implicated.[31,32,40,41,70] Vectors of most *Rickettsia* species such as *Ixodes* species or *Rhipicephalus sanguineus*, are widely present in the UK and Ireland[71] and *A phagocytophilum* (the human agent of granulocytic ehrlichiosis) has been isolated from ticks in the UK.[72] *Ehrlichia*, *Anaplasma* or *Neorickettsia* species were not detected in the present study. This could suggest that the agents are not present in the region of study, the prevalence is very low, the organisms were not in the blood, or the organisms were present at levels below the detectable limits of the assays. However, the small sample size limits any firm conclusion. Larger studies including a greater number, and a more rural selection of cats, is warranted. Assessing the presence of *Ehrlichia* species and *Anaplasma* species in ticks would also provide more evidence regarding the mode of contamination.

The newly recognised pathogen *R felis* has not previously been investigated in Ireland. It is thought to be transmitted from cats to cats via the flea *C felis* and its presence in fleas from the UK has already been established.[6] *R felis* DNA was not amplified from the blood of any cat in this study which may indicate that the organism does not exist in Irish cats. It is also possible that the negative results relate to other factors as discussed above for *Ehrlichia* and *Anaplasma* species. Cats from countries with fleas have been occasionally reported as reservoirs for the disease.[73] However, despite experimental studies showing that cats can be naturally infected by *R felis* but have a very short bacteraemia,[74] several studies have failed to demonstrate its presence in cat blood.[43,45,75,76] Potentially *R felis* could be sequestered in tissues other than blood and bacteraemia may only be intermittent and missed when only one sample is assessed. Recently, *R felis* DNA was amplified from the skin of a cat in the USA. Alternatively, the small sample size may influence the results which may not be representative of the global Irish cat population. Assessing the prevalence of the parasite in Irish fleas would be interesting and, if the parasite is detected, matching cats' blood samples to their fleas would provide additional information regarding the mode of transmission.

In conclusion, these results suggest that all cats used as blood donors in the Dublin area should be assessed for haemoplasmas in addition to the previously recommended infectious agents (FIV/FeLV). Although the prevalence of *Bartonella* species infection was relatively low, it should also be considered as a risk factor for blood transfusion. Infection with *Ehrlichia* and *Rickettsia* species was not detected in this study. However, further larger studies are required to confirm their absence or true prevalence and whether blood donors should be screened for these infectious agents. The prevalence of the potentially zoonotic agents in cats described in this study was not unexpected and while suggesting that risks associated with cat contact is not unduly high, appropriate care with handling cat faeces and regular flea control remain important strategies. Additional studies are required to extend this regional data to the broader Irish cat population.

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