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Prevalence of selected infectious disease agents in stray cats in Catalonia, Spain

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Abstract

Objectives The objective of the current study was to investigate the prevalence rates of the following infectious agents in 116 stray cats in the Barcelona area of Spain: *Anaplasma phagocytophilum, Bartonella* species, *Borrelia burgdorferi, Chlamydia felis, Dirofilaria immitis, Ehrlichia* species, feline calicivirus (FCV), feline herpesvirus-1 (FHV-1), feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), haemoplasmas, *Mycoplasma* species and *Rickettsia* species.

Methods Serum antibodies were used to estimate the prevalence of exposure to *A phagocytophilum, Bartonella* species, *B burgdorferi, Ehrlichia* species and FIV; serum antigens were used to assess for infection by *D immitis* and FeLV; and molecular assays were used to amplify nucleic acids of *Anaplasma* species, *Bartonella* species, *C felis, D immitis, Ehrlichia* species, FCV, FHV-1, haemoplasmas, *Mycoplasma* species and *Rickettsia* species from blood and nasal or oral swabs.

Results Of the 116 cats, 63 (54.3%) had evidence of infection by *Bartonella* species, FeLV, FIV or a haemoplasma. *Anaplasma* species, *Ehrlichia* species or *Rickettsia* species DNA was not amplified from these cats. A total of 18/116 cats (15.5%) were positive for FCV RNA (six cats), *Mycoplasma* species DNA (six cats), FHV-1 DNA (three cats) or *C felis* DNA (three cats).

Conclusions and relevance This study documents that shelter cats in Catalonia are exposed to many infectious agents with clinical and zoonotic significance, and that flea control is indicated for cats in the region.

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Introduction

There are many infectious agents of clinical importance to cats and some have the potential for zoonotic significance to people. Stray cats often have high prevalence rates for most infectious agents that are associated with direct contact with other cats, including feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), the haemoplasmas, feline herpesvirus-1 (FHV-1), feline calicivirus (FCV), *Mycoplasma* species and *Chlamydia felis*. In addition, agents vectored by fleas (*Bartonella* species, the haemoplasmas) or ticks (*Anaplasma phagocytophilum, Borrelia burgdorferi;*) can also be very common in stray cats because parasite control products are usually not used in these cats.

In-clinic tests are available for detection of FeLV antigens and FIV antibodies in serum. Recently, it was shown that an in-clinic assay designed for detection of antibodies against *A phagocytophilum, B burgdorferi, Ehrlichia canis* and *Dirofilaria immitis* antigens in canine sera can be used with feline sera to detect *A phagocytophilum* and *B burgdorferi* antibodies.[1] Molecular assays for amplification of the RNA or DNA of multiple bloodborne infectious agents are now widely available. While several studies have evaluated prevalence rates for vector-borne agents in cats in Spain, no study has combined the use of

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molecular assays with the commercially available kit that can detect *A phagocytophilum* and *B burgdorferi* antibodies.[2–8]

The respiratory disease-causing agents FHV-1, FCV, *Mycoplasma* species, *C felis* and *Bordetella bronchiseptica* are generally common in cats housed in shelters, but, to our knowledge, no study has evaluated prevalence rates for these agents in cats in Spain.[9–12] Additionally, minimal studies exist that have genotyped respiratory *Mycoplasma* species from cats with and without clinical signs of disease, to evaluate for the presence of novel *Mycoplasma* species.[13] Thus, the objectives of this part of the study were to use samples from cats housed in shelters in Catalonia to determine the prevalence of selected vector-borne agents using molecular assays, to determine the *B burgdorferi* and *A phagocytophilum* antibody prevalence rates using a commercially available assay, to determine the prevalence of selected respiratory disease agents using molecular assays, and to genetically characterise respiratory *Mycoplasma* species.

Materials and methods

Case selection

All cats were housed at local animal shelters in Barcelona (CAAC Barcelona, CAAC Mataró and CAAD Argentona) or had been admitted to a clinic (Bitxos, Hospitalet) outside Barcelona from different shelters or colonies, and permission to collect the samples was granted to each attending veterinarian. Performance of the study was approved by the Clinical Review Board of the Department of Clinical Sciences, Colorado State University. Cats were chosen solely on whether samples could be collected (September–November 2012) without causing undue stress.

Clinical evaluation

Prior history was not available for the cats. For all cats, age was estimated by a veterinarian and stratified into kittens up to 16 weeks of age or adults. To our knowledge, all the cats had outdoor access and had previous contact with other cats. Vaccines containing FHV-1, FCV and feline panleukopenia were administered to each of the cats at the shelters at the time of admission but not repeated regularly. Owing to the feral nature of most cats, a complete clinical evaluation was not possible, but cats were classified as healthy or unhealthy depending on the clinical history when available and presence of evident clinical signs.

Sample collection

Blood (1.5 ml) was collected from the jugular or cephalic vein and 0.5 ml placed into EDTA and 1 ml allowed to clot for serum separation. Oral or nasal swabs were collected by inserting the tip of a sterile dry cotton urethral swab just inside the nares or between the cheek and gum, and gently rotated. All samples were stored at –80°C on the day of collection at the Autonomous University of Barcelona (Spain) until being shipped on ice packs to the Colorado State University (USA) and then stored at 4°C until assayed.

Serological assays

The 116 sera were tested for FeLV antigen and antibodies against FIV using a commercially available kit (SNAP Combo FeLV/FIV test; IDEXX) and for antibodies against *A phagocytophilum, B burgdorferi, E canis* and *D immitis* antigens using a different commercially available kit (SNAP 4Dx; IDEXX). IgG antibodies against *Bartonella* species were detected by ELISA using *Bartonella henselae* as the antigen source.[14]

Molecular assays

Positive and negative controls were assessed with all PCR assays using the previously published assays and standard operating procedures (Center for Companion Animal Studies, Colorado State University, Fort Collins, CO, USA). Total DNA was extracted from 200 µl of the 116 blood samples in EDTA and assayed using PCR assays that amplify the DNA of *Anaplasma* species, *Bartonella* species, *Ehrlichia* species, haemoplasmas and *Rickettsia* species with genetic sequencing used to confirm the results.[15–18] Total DNA and RNA were extracted from the 116 nasal or oral swab samples, and conventional PCR assays that amplify the RNA of FCV, and the DNA of FHV-1, *C felis* and *Mycoplasma* species were performed on each extract.[19–23] *Mycoplasma* species-positive samples were sequenced to determine the species and only cases confirmed by sequencing were considered positive.[13]

Statistical analysis

As detailed information was not available for most cats and case selection was based on convenience sampling, the majority of the results are presented descriptively. Fisher's exact test was used to compare the percentage of positive results between groups for some parameters, with significance defined as $P < 0.05$.

Results

Bloodborne agents

There were 12 kittens aged between 5 and 16 weeks of age; the other 104 animals were estimated to be adult cats. Of the 116 cats, 63 (54.3%) had evidence of infection by *Bartonella* species, FeLV, FIV or a haemoplasma (Table 1). One healthy 3-month-old kitten was positive for *B burgdorferi* antibodies in serum. *Anaplasma* species, *Ehrlichia* species or *Rickettsia* species DNA was not amplified from these cats.

Overall, 51 cats (44%) had evidence of exposure to or infection by *Bartonella* species; *B henselae* and *Bartonella*

Table 1 Prevalence of selected bloodborne agents in 116 shelter cats from the Barcelona area

Result	Positive
Any test positive	63 (54.3)
Any *Bartonella* positive (IgG or PCR)	51 (44.0)
Bartonella IgG	41 (35.3)
Bartonella PCR	26 (22.4)
B henselae	17 (14.7)
B clarridgeaie	10 (8.6)
Any haemoplasma	9 (7.8)
'*Candidatus* Mycoplasma hemominutum'	8 (6.9)
Mycoplasma hemofelis	1 (0.9)
FeLV	7 (6.0)
FIV	3 (2.6)

Data are n (%)
FeLV = feline leukaemia virus; FIV = feline immunodeficiency virus

clarridgeaie were both detected individually and one cat had dual infection. Evidence of a *Bartonella* species exposure or infection was more common in adults (48/104 cats; 46.2%) than kittens (3/12 kittens; 25.0%), but the difference was not significant. Nine cats were *Bartonella* species PCR-positive but IgG negative. The majority of the cats with evidence of infection or exposure to *Bartonella* species were apparently healthy. DNA of '*Candidatus* Mycoplasma haemominutum' (eight cats) or *Mycoplasma hemofelis* (one cat) was amplified from the blood of 7.8% of cats; five cats had concurrent evidence of infection or exposure to a species of *Bartonella*, but dual infections with both haemoplasmas were not detected. None of the haemoplasma cats had physical examination evidence of haemolytic anaemia. Haemoplasma DNA was not amplified from the blood of any kitten.

FeLV antigen was detected in the serum of seven cats (6.0%) (Table 1); six cats were positive for FeLV alone. FeLV antigen was detected in a higher percentage of kittens (2/12; 16.7%) than adults (5/104; 4.8%), but the difference was not significant. Of the cats that were positive for FeLV alone, five were healthy and one had respiratory distress. Three cats (2.6%) were positive for FIV antibodies and all were clinically ill. One cat positive for FIV antibodies, FeLV antigen and '*Candidatus* M haemominutum' DNA had gingivitis. One cat with FIV antibodies alone had haemolytic anaemia. The third FIV antibody-positive cat had gingivitis, *Bartonella* species IgG, and DNA of *B clarridgeiae* and '*Candidatus* M haemominutum'.

Upper respiratory disease agents

None of the 12 kittens sampled in this study had clinical signs of upper respiratory disease and all the kittens were negative for the selected agents. Eighteen of the 116 cats (15.5%) were positive for FCV RNA (six cats), *Mycoplasma* species DNA (six cats), FHV-1 DNA (three cats) or *C felis* DNA (three cats). Of the 116 cats, 18 (15.5%) had clinical signs of upper respiratory infections (12.9%). Five cats had clinical signs of rhinitis alone, five cats had gingivitis alone, four cats had conjunctivitis alone, two cats had rhinitis and conjunctivitis, and two cats had respiratory distress. However, of the 18 cats with clinical signs of upper respiratory infections, only five cats were positive for any of the targeted infectious agents.

Of the *Mycoplasma* species, genetic sequencing showed three to be *Mycoplasma arginini*, two to be *Mycoplasma gateae* and one to be *Mycoplasma feliminutum*. One cat had FCV RNA, FHV-1 DNA and respiratory distress. One other FCV RNA-positive cat had conjunctivitis and the other four cats had no signs of upper respiratory infection, including a cat that was positive for FCV RNA and *M arginini* DNA. The other two cats with FHV-1 DNA had conjunctivitis or had no signs of upper respiratory infection, respectively. Of the six *Mycoplasma* species DNA-positive cats, five had no signs of upper respiratory infection and one cat with *M arginini* DNA had signs of conjunctivitis and rhinitis. Of the *C felis* DNA-positive cats, two had conjunctivitis and one had no signs of upper respiratory infection. None of the cats with gingivitis were positive for FCV RNA (Table 2).

Discussion

Overall, 54.3% of the 116 cats studied had evidence of exposure to a bloodborne agent. Consistent use of flea and tick preventives in the recent past in the cats described here was considered unlikely, which probably explains the *Bartonella* species prevalence rate of 44%, as many *Bartonella* species are transmitted by fleas. Both *B henselae* and *B clarridgeaie* are human pathogens and can be associated with illness in cats, and so the results suggest that flea control is indicated for cats in the region. The results of this study are similar to others in Spain that have shown *Bartonella* species seroprevalence rates of 23.8% (Madrid), 29.6% (Catalonia) and 71.4% (Catalonia and Mallorca Island).[4,8,24] Other studies from Spain have documented *B henselae* and *B clarridgeiae* DNA in the blood of cats; however, the prevalence of 22.4% reported here is much higher than other studies that ranged from 0.3–17%.[4,5,8] The differences between studies likely relate to the source of the cats, with higher risk being associated with shelter cats because of flea exposure. Alternatively, the assays used were different among the studies, which could also explain the differences.

The haemoplasma prevalence rates in this study (7.8%) were similar to another in Catalonia, which reported DNA from all three major species in the blood of cats; the prevalence rates were 3.7% for *M haemofelis*, 9.9% for '*Candidatus* M haemominutum' and 0.5% for '*Candidatus* Mycoplasma turicensis'.[7] In another

Table 2 Prevalence of selected respiratory pathogens in 116 shelter cats from the Barcelona area

Result	Positive*	Cats with URTD signs (n)	Cats with URTD signs over the number of positive to the test
Any test positive	18 (15.5)	5	27.8
FCV RNA	6 (5.2)	2	33.3
FHV-1 DNA	3 (2.6)	2	66.7
Chlamydia felis DNA	3 (2.6)	2	66.7
Mycoplasma species DNA	9 (7.8)	1	11.1
M arginini	3 (2.6)	1	33.3
M gateae	2 (1.7)	0	0
M feliminutum	1 (0.9)	0	0

*Data are n (%)
URTD = upper respiratory tract disease; FCV = feline calicivirus; FHV-1 = feline herpesvirus-1

previous study of domestic cats in different Spanish provinces, haemoplasma DNA was amplified from the blood of 30% of the cats tested.[4] Both studies were on domestic cats. No data are available for stray animals. Haemoplasmas have several routes of transmission, including vectors and direct contact; thus, stray cats may be at a greater risk of exposure to the infections.[25]

In this study, antibodies against *E canis* or *Anaplasma* species were not detected in the commercially available kit, and DNA of *Anaplasma* species, *Ehrlichia* species, *Neorickettsia* species or *Rickettsia* species were not amplified. The PCR assays have been validated for use with cat blood and so the negative results likely indicate low levels of exposure to these cats and are similar to other PCR-based studies in Spain, which also failed to amplify DNA of these genera from cat blood.[4,5] In contrast, antibodies against *Rickettsia conori* were detected in 44% of cats tested in Catalonia and Mallorca island,[6] 1–2.4% of cats tested had antibodies against *Neorickettsia risticii*,[2,8] and, in separate studies, antibodies against *E canis* have been detected in between 3.8% and 17.9% of cats.[2–4,6,8] The commercial antibody test kit used has been shown to detect *A phagocytophilum* antibodies in the serum of cats, but has not been validated to detect *Ehrlichia* species antibodies in serum.[1] Thus, *Ehrlichia* species antibody results of this study cannot be directly compared with the previous studies. However, as *Ehrlichia* species are known to induce clinical disease in cats, the previous seroprevalence studies suggest these agents and *R conori* should continue to be assessed as the cause of clinical illness in cats in Spain.

In the present study, one kitten was positive for *B burgdorferi*. There are no previous data about this infection in cats in Catalonia, which has never been considered an area at risk for Lyme disease. However, *B burgorferi* was found to have a low prevalence in dogs in central Spain and in people in northeast Spain.[26,27] The commercial kit utilised here is known to detect *B burgdorferi* antibodies in cats,[1] and so the result is likely a true positive. However, the kitten was young and so the positive test likely reflects maternal antibodies from the queen.

The prevalence rates for FeLV and FIV (6.0% and 2.6%, respectively) were slightly lower than reported in a previous study in Catalonia and Mallorca island, where the prevalence rates were 8.5% for FeLV and 7.4% for FIV.[4] One cat positive for FeLV was presented to the clinic in respiratory distress, but unfortunately no diagnostic tests were carried out to confirm or rule out a possible mediastinal lymphoma or other disease potentially related to FeLV. A few cats that presented with gingivitis had multiple infections: a FeLV-positive cat was also positive for FIV antibodies and '*Candidatus* M haemominutum' DNA. Another, which was positive for FIV, was also positive for *Bartonella* species IgG, and DNA of *B clarridgeiae* and '*Candidatus* M haemominutum'. Multiple infections are common in cats affected by FIV and most clinical signs are a consequence of secondary diseases.

The molecular assays used to amplify nucleic acids of FCV, FHV-1, *C felis* and *Mycoplasma* species were positive in 18/116 cats (15.5%) and each agent was amplified from more than one cat. To our knowledge, this is the first time that these agents have been documented in shelter cats in Spain. The prevalence rates compared with shelter studies in the USA are low.[11] Similar to other studies, positive test results did not always correlate to the presence of clinical signs of disease. This is likely related, in part, to the use of modified live FHV-1- and FCV-containing vaccines: the persistence of these strains may explain how it was possible to obtain positive results for PCR assays on samples obtained from apparently healthy cats.[11] In addition, FHV-1, FCV, *C felis* and *Mycoplasma* species can also be carried by healthy cats. The pathogenicity of *Mycoplasma* species has been questioned in previous studies because it is frequently detected in samples obtained from clinically normal cats.[28] It has been suggested that *Mycoplasma* species is pathogenic only when it is associated with other organisms.

In this study two cats had positive results for both *Mycoplasma* species and FCV, but only one of them had conjunctivitis. A study carried out in Germany in 2010 found a much higher prevalence of mycoplasmas in cats with conjunctivitis; 20/41 cats (48.8%) were positive.[13] However, in the present study the previous history of these cats was mostly unknown, so it is possible that some of these animals had episodes of conjunctivitis or rhinitis as kittens that were not observed at the shelters; therefore, subclinical carriage of organisms such as FHV-1 and FCV is another possible explanation.

There were many cats in the study with conjunctivitis and/or rhinitis from which DNA of an infectious agent was not amplified. These cats may have had another unrecognised cause of upper respiratory tract disease, such as *B bronchiseptica* infection. Moreover, specific information on antimicrobial treatment of cats was not available. It is possible that some cats in the present study were being treated with antimicrobials, which could have reduced the detection rates of *C felis* and *Mycoplasma* species in cats with conjunctivitis. However, this would be unlikely in the healthy cats. In future prevalence studies, it should be verified that samples are collected before administration of antimicrobial treatments. It is also possible that the PCR assay results were falsely negative because of shipping. The samples were shipped from Spain to Colorado with ice packs, but arrived to the laboratory with melted ice and at ambient temperature. However, in another study results of FHV-1 DNA PCR assays did not differ for identical samples sent overnight on ice or mailed at ambient daytime temperatures during a period of several days, indicating that shipping temperature should not be a major limiting factor in DNA assays, but it may be regarding FCV RNA.[29] Another explanation for the low rate of positives could be the sampling location, which included both nasal and gingival swabs. Previous studies have shown that oropharyngeal samples could be ideal to obtain DNA for FHV-1 and RNA for FCV and respiratory pathogens.[30,31]

Conclusions

Clinical information from shelter cats like those reported herein is often limited. Thus, it is difficult to assess accurately for disease associations in these studies. However, this work documents that shelter cats in Catalonia are exposed to many infectious agents with clinical and zoonotic significance. The *Bartonella* species prevalence rates suggest that flea control is indicated for cats in the region, as reported in previous studies in domestic cats.

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